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Recommendation Report

Abstract
Creatine is one of the largest supplements sold, mainly because it is proven to be effective. However, creatine has low bioavailability, and is not completely stable, thus larger doses are needed in order to achieve desired plasma creatine concentrations. Many types of creatines currently exist all being marketed as solving these problems. With so much hype and marketing material it becomes increasingly difficult for supplement companies, sports nutritionist, trainers and athletes to know what is truly effective. Even if it were possible to evaluate and make recommendations on every current creatine supplement on the market, the report would be quickly outdated. Therefore, this article will not only make specific recommendations on which creatines to use and which ones to avoid, but also provide reasons and principles that the reader can employ, to evaluate future creatines. This article will be of use from the sports nutritionist and supplement company down to the trainer and everyday athlete.

Executive Summary
The properties of creatine and its transporter are discussed. Intestinal absorption of creatine is close to 100%. However, that does not mean that 100% of the creatine is up-taken by the muscles. Creatine is transported into the muscles against a concentration gradient via a sodium chloride transporter. During creatine supplementation the creatine transportation activity is down regulated. This occurs by negative feedback signaled by intramuscular non-phosphorylated creatine. Muscles have a finite capacity to store creatine and after muscle saturation levels are reached creatine dosages should be reduced proportionately. Optimal creatine loading is two to three days of 0.25 g/Kg of lean body mass per day and optimal maintenance consist of 0.0625 g/Kg of lean body mass per day.

It requires around a 20mmol creatine concentration increase in muscle tissue, in order to realize significant strength gains. By the same token, the more you can increase muscle creatine concentrations, the greater potential benefits you will experience by creatine supplementation. 20-30% of the population is unable to achieve these levels with normal creatine monohydrate supplementation. Various methods that aid at increasing creatine concentrations are discussed. It is observed that insulin aids in up-regulating creatine transportation. Therefore consuming creatine with carbohydrates and protein—which induce an insulin response—can significantly increase muscle creatine saturation. Consuming creatine close to exercise can further enhances total creatine concentrations. It is recommended to consume creatine with protein and carbohydrates before or as close to exercise as possible.

The physicochemical properties of creatine are also discussed. Creatine normally exhibits low solubility and thus bioavailability. Higher doses of creatine are therefore necessary to give the desired plasma...
concentrations. The more soluble the creatine the more potent will be its effects and thus a smaller dosage which will in turn be easier on the body’s organs. It is thus recommend to ensure that creatine is completely dissolved before consumption, and to avoid lozenges or suspensions as they have limited bioavailability. The stability of creatine is elucidated upon, because creatine does degrade to the byproduct creatinine, conditions such as pH and saturation levels are important, to further increase relative bioavailability. Therefore specific creatines are recommended as they exhibit certain physicochemical properties; which not only prevents degradation, but also increases relative bioavailability and uptake into the muscle. Likewise specific creatines are recommended to avoid as they degrade faster, and have a limited uptake.

In regards to creatine muscle retention, other recommendations to both utilize and avoid specific ingredients are also given.

Introduction

Properties of Creatine

Creatine absorption
Once ingested, creatine must make its way to the target tissues. The digestion and absorption of creatine is important to understand. Creatine transporters have been identified throughout the intestinal wall, including the ileum Jejunum, the apical, and the basolateral membranes of enterocytes. The latter study suggested that creatine, like many other hydrophilic drugs and peptides, may exhibit a paracellular movement by solvent drag as a possible mechanistic pathway of absorption. However, like MCCALL and Persky explained, using the Caco-2 monolayer as a model of intestinal absorption, creatine showed poor apical to basolateral movement, suggesting that the contribution of the paracellular route to the transcellular route is minimal and that the Caco-2 cell may not express creatine transporters.

A study conducted over 85 years ago demonstrated that intestinal absorption of creatine is close to 100%. Many have misinterpreted the above study to mean that nearly 100% of the creatine is absorbed or taken up by the muscles, whereas in fact, 33-77% of the ingested creatine (8-21g/d) is excreted in the urine. The creatine arterial bioavailability is close to the dose administered, but is significantly less at the venous level and even less actually gets absorbed into the skeletal muscles.

Creatine transporter and Clearance
It is important to note that in order for creatine to exert a beneficial effect on performance and metabolism, an increase in total creatine content of at least 20 mmol/kg dm is required. However, various studies have revealed that 20 to 30% of individuals do not respond to creatine supplementation; i.e., they demonstrate less than a 10 mmol/kg dm (8%) increase in muscle total creatine following 5 days of 20 g/day oral creatine supplementation. The exact reasons for this anomaly are currently unknown; however, there are some plausible ideas that may prove effective in helping this population. First, an understanding of the fate of creatine once absorbed by the intestines helps us understand why creatine does not exhibit 100% bioavailability; this will allow us to suggest methods to increase creatine
retention. In order for creatine to reach the target tissues, it must be up taken by a SLC6 transporter in the cells. This transporter is known as the creatine 1 transporter (cret1)—there are other creatine transporters, but these are not pertinent to this article—. Many studies have shown that the CRT is not only Na\(^+\), but also Cl\(^-\) dependent\(^8\)—which is important because not all SLC6 transporters are Cl\(^-\) dependent. It requires at least 2 sodium cations and one chloride anion to transport one creatine molecule into the cell. It is known that inwards-directed Na\(^+\) and Cl\(^-\) gradients coupled with outwards-directed K\(^+\) gradients serve as a driving force for the transport of biogenic amines such as serotonin. NaCl gradient is inwards directed, thus it would drive creatine into the cell from the serosal side. In other words, creatine uptake stimulation is not exerted by the Na\(^+\), K\(^+\)-pump, but rather by the presence of NaCl in the extravesicular solution. Over 90% of creatine uptake occurs via this Na\(^+\)/Cl\(^-\) CreT against a concentration gradient.\(^9\)

Some discussion on the pharmacokinetic implications is warranted. In humans, the K\(\text{m}\) for plasma membrane transport is on the order of 20 to 100 μM. Under normal conditions plasma concentrations are 25 to 50 μM, which is in the range of the K\(\text{m}\) of the transporter. The kinetics of the system keep interstitial concentrations at approximately 40% of plasma concentrations.\(^10\) Once in the cell, creatine may also be taken up into mitochondria through a creatine transporter, although the evidence for this is limited.\(^11\) Finally, the creatine kinase reaction is at equilibrium under normal conditions with approximately 65% of the total creatine pool in the phosphorylated form.\(^12\)

Clearance signifies the clearing of the blood, in the case of creatine this is accomplished by organs and tissues.

In agreement with earlier work, it has also been demonstrated that the majority of tissue creatine uptake occurs during the initial days of supplementation, with close to 30% of the administered dose being retained during the initial 2 days of supplementation, compared with 15% from days 2 to 4.\(^13\)

The decrease in creatine retention is principally attributed to the fact that the ability of skeletal muscle to accumulate creatine is finite; that is, it is possible to saturate muscle stores of creatine.\(^14\) Similar to the uptake of glucose and its stored form glycogen, there is variability among individuals, but the saturation level is still finite. Therefore, long term daily ingestion of large amounts of creatine results in large amounts of creatine/creatinine excretion in the urine, which may potentially induce renal overload.\(^15\) Exactly how much the kidneys and muscle contribute to creatine clearance is probably dependent on dose and dose frequency.\(^16\) As a person continues to take creatine, muscle stores become saturated and clearance shifts from muscle uptake to primarily renal elimination. Therefore, because creatine pharmacokinetics change over time, so should the dosage; for example, during the loading phase consuming 5 g three times a day (15 g/d) will maintain concentrations well above the K\(\text{m}\) for the plasma membrane transporter throughout the day. After a few days of the loading phase, muscle stores will approach saturation, resulting in an increased fraction of the dose being lost in the urine. Thus, after a short loading phase, doses can be reduced to 3 to 5 g/d to compensate for the reduction in clearance of creatine and to compensate for the loss of creatine via creatinine formation which is on average 2 g/d.\(^16\)
The ranges of creatine accumulation in the muscle vary from person to person with a range somewhere between 100 to as high as 200mmol/kg dry muscle, which seems to be the limit. As was mentioned earlier, it requires around a 20mmol muscle creatine concentration increase, in order to realize significant benefits. Therefore, hypothetically speaking, the more you can increase muscle creatine concentrations, the greater potential benefits you will experience by creatine supplementation. For example, increasing muscle creatine concentrations from 100mmol to 120mmol is sufficient to experience significant benefits; however, if you could further increase the concentration from 120mmols to around 200mmols than theoretically you would experience even further gains.

**Creatine transporter regulation**

Now that we have reviewed how creatine reaches its target tissues upon ingestion, we must understand what controls the creatine transporter. Some have reported that because creatine is concentration dependent, it invokes Le Chatelier’s principle in that if the creatine concentration on the outside of the cell is greater than on the inside, then creatine transport into the muscle will be expedited and augmented. It is true to a certain extent that the extracellular creatine content regulates the transport of creatine into cells, because the reaction rate increases with increasing substrate concentration \([S]\), asymptotically approaching the maximum rate \(V_{\text{max}}\). However, this may only be the case for the first few days of chronic ingestion, where high extracellular creatine concentrations may increase the \(V_0\) (current reaction rate), but not change the \(K_m\) by saturating the creatine transporter according to the following equation.

\[
v_0 = \frac{v_{\text{max}} [S]}{K_M + [S]}
\]

To be clear, \(K_m\) is the inverse of enzyme affinity: the smaller the \(K_m\) the greater the affinity. \(V_{\text{max}}\) is the maximum conversion of substrate to product when all the enzyme is bound to the substrate. \([S]\) is substrate concentration.

Because the \(K_m\) does not change despite elevated plasma creatine concentrations, chronic creatine loading could potentially increase plasma concentrations of the substrate, creatine, before the allosterically regulated enzyme is communicated via negative feedback to decrease the \(V_0\), and thus supersaturate the muscles with creatine. However, other studies show that you can reach the same muscle creatine concentrations by taking significantly smaller doses for a few weeks, but whether that is true for everyone is yet to be investigated.

It was once believed that it was principally extracellular creatine that down-regulated the creatine transporter, so that creatine uptake is eventually inhibited with prolonged exposure to high plasma creatine levels. The referenced study found that cultured myocytes exposed to high extracellular Cr concentrations displayed a significant decrement in Cr uptake. This was not related to a decrease in the affinity of the transporter for creatine, but by a two- to threefold loss in maximal transporter activity. However, it was later found that cells incubated in a sodium-free solution with the same Cr concentration did not demonstrate reduced Cr uptake. This suggests that creatine uptake is actually dependent on intracellular creatine concentrations, and not extracellular creatine concentrations.
Creatine must enter the cell to exert this regulatory activity on creatine transporters. It appears that elevated plasma creatine levels promote an initial rise in creatine uptake and resultant intracellular creatine concentration, which may in and of itself begin to inhibit uptake by negative feedback. However, it is possible that in vivo prolonged exposure to high creatine concentrations may also alter the number of creatine transporter proteins expressed by the cell. Once inside creatine cannot undergo passive diffusive efflux because of its cationic character at physiological pH, and its high molecular weight.

In short, the rate-limiting step in muscle creatine uptake is obviously not substrate concentration, but again intracellular creatine content. The $K_m$ values determined for CreTs are lower than the concentration of creatine in the blood, meaning that the transporter normally works close to saturation and that creatine uptake rate is already close to its maximal capacity—which alone shows that regulation of the level of CreT in the plasma membrane would alter creatine uptake. Because of these finding, there is sufficient evidence to suggest that chronic creatine ingestion and resultant chronic elevations in creatine plasma levels will not increase creatine uptake into the muscles, and if elevated too high for an excessive period of time it may actually inhibit creatine uptake, and place unnecessary strain on the kidney.

Along the same lines, it was demonstrated that intracellular phosphocreatine stores do not play a significant role in regulating creatine transporters. It appears that the main regulator is intracellular unphosphorylated creatine, which comprises about one-third of the creatine pool. The down-regulation of creatine uptake may be due to an inhibitory protein. Indeed, it was demonstrated that cultured cells exposed to cycloheximide, a protein synthesis inhibitor, lost the down-regulation of Cr uptake. And in a later study, the presence of cycloheximide allowed for a 2.4 fold increase in intracellular creatine. This suggests that high intracellular concentrations may induce the expression of a protein that may either directly inhibit the CrT protein, or functionally remove them from the plasma membrane. This regulatory action may be a safety mechanism to prevent an unfavorable metabolic situation. If left unregulated, ATP stores may significantly decline causing serious issues; therefore, down-regulation of creatine transporters would certainly prevent the excess accumulation of intracellular creatine concentrations.

If only the non- phosphorylated intracellular creatine levels significantly regulate this system, and the ratio of phosphorylated to non-phosphorylated is 1:3, then it leads one to think that if you could increase creatine kinase activity and therefore phosphorylate more creatine, then you could also store more creatine, maintaining that same ratio but increasing total muscle creatine concentrations. Therefore, the kinetics of the intracellular system may dictate which individuals or groups respond to creatine and how well.
Creatine Retention

Results

Effect of exercise on muscle creatine concentration
It is important to note that in order for creatine to exert a beneficial effect on performance and metabolism, an increase in total creatine content of at least 20 mmol/kg dm is required. However, various studies have revealed that 20 to 30% of individuals do not respond to creatine supplementation; i.e., they demonstrate less than a 10 mmol/kg dm (8%) increase in muscle total creatine following 5 days of 20 g/day oral creatine supplementation. The exact reasons for this anomaly are currently unknown; however, there are various methods employed, to increase creatine concentration, that are effective. Some of these insights were first seen with the elderly during creatine consumption. Interestingly, after creatine supplementation in the elderly, there was no increase in intramuscular phosphocreatine levels, despite similar plasma exposure and urinary excretion. These findings may very well be due to the lack of conversion of creatine to phosphocreatine in either the cytosol or mitochondria. A later study demonstrated that when the elderly participated in resistance training their phosphocreatine did increase. It is known that exercise increases mitochondrial and creatine kinase content, thus in the absence of exercise, elderly muscles may not respond to creatine supplementation due to lower creatine kinase activity or mitochondrial function. Indeed these findings substantiate the idea that increased creatine kinase activity, would result in higher muscle creatine concentrations.

This same increase is also seen with young healthy subjects. Namely that if exercised, that muscle can increase creatine content by a further 10% compared to its non-exercised counterpart, from what it was after supplementation. More research also shows this effect even in the same person, by exercising one leg, but not the other. The data is clear that consuming creatine immediately after exercise, significantly increases creatine stores, for the exercised muscles, significantly more than the unexercised muscles. Another reason why exercise may augment creatine content is that exercise may increase activation of the sarcolemmal sodium-potassium pump, as well as blood flow, which in turn potentiates the delivery and uptake of creatine.

Effects of insulin in creatine retention
Exercise not only increases the creatine kinase activity, but also, similar to insulin, translocates GLUT-4 to the muscle membrane, allowing the muscle to uptake glucose in the absence of insulin’s signal, via the AMPK pathway. If insulin has the same effect as exercise on GLUT 4, insulin could possibly have an effect on the creatine transporter, just as it does on the GLUT-4. Indeed, it was demonstrated that the presence of insulin (at supraphysiological levels) increased muscle creatine accumulation in humans. This improved cellular creatine retention from insulin is attributed to insulin’s ability to indirectly stimulate sodium-potassium pump activity, which enhances creatine transport.

Simple carbohydrates—having a high glycemic index—evoke a high insulin response. Because of this, it would make sense that creatine with carbohydrates would increase muscle creatine retention. Indeed Green and colleagues tested this idea with excellent success: not only a reduction in urinary creatine, but also a 60% increase in muscle creatine accretion. This latter study used 93g of glucose, 4 x 5g-day,
for two days compared to the same dose of creatine alone. In another study,\textsuperscript{35} using lower doses of carbohydrates (18g of glucose) and 5grams of creatine, (4 x-day for 3 days) resulted in significantly higher creatine retention than an equivalent dose of creatine monohydrate. Stout et al.\textsuperscript{36} reported that a smaller dose of carbohydrates (35g glucose) with each 5g dose of creatine monohydrate is also effective. The combination of creatine monohydrate with whey protein has been reported to result in similar benefits in terms of strength gains.\textsuperscript{37} Another study reported that the combination of protein and carbohydrates with creatine\textsuperscript{38} seems to reduce the individual variations in muscle creatine accumulation previously reported. This type of combination used close to exercise (immediately before and/or after) appears to further improve total creatine accumulation in muscle.\textsuperscript{39-40}

High fructose beverages should be avoided because fructose does not induce a significant rise in insulin, so its addition would not result in enhanced muscle creatine retention;\textsuperscript{58} maltodextrins or dextrose are good choices. Some have suggested that taking creatine with protein and/or amino acids and/or BCAA’s would inhibit creatines absorption by competing for the same transporter. However, it has been reported that muscle creatine uptake is not affected by cellular concentrations of various amino acids such as glycine, glutamine, alanine, arginine, leucine and isoleucine or the sulfur-containing amino acids methionine and cysteine.\textsuperscript{59-60}

**Effects of Creatine on Water Retention**

Creatine is an osmotically active compound, such that as creatine enters the cell it also brings with it water, thus increases total body weight. Virtually every study on creatine supplementation shows an increase in body weight in the first few days.\textsuperscript{41} This rapid change in bodyweight is attributed to water retention, in fact within the first three days in the loading period there is a significant reduction in urine output.\textsuperscript{42}

This phenomenon is related to the osmotic load caused by creatine, and perhaps solvent drag in the fluid absorbed through the paracellular pathway. Regardless of the exact mechanism the water is not only retained extracellularly where it would alter fluid distribution, leading to health risks, but is also observed extracellularly and thus able to maintain normal osmotic and oncotic pressure.\textsuperscript{43}

In fact, creatine induced water retention has been shown beneficial. Increases in cell volume appear to be a proliferative, anabolic signal that may enhance protein synthesis\textsuperscript{44-45} which suggests a method by which extended creatine supplementation may promote muscular hypertrophy water retention.\textsuperscript{46}

**Effects of creatine on muscle anabolism**

Besides the most obvious benefit of creatine, in that it is significantly able to maintain ATP stores and thus enhance muscular output.\textsuperscript{46-47} More recently it is being shown that the increased strength and size after creatine supplementation is not only due to a better anaerobic workout, but also there exists a few mechanistic reasoning for the resulting hypertrophy, strength and power by creatine supplementation.

As was mentioned earlier, creatine increases water retention\textsuperscript{41}, causing increased cell volume which has been suggested to stimulate protein synthesis.\textsuperscript{43-45,48}
Myostatin, a member of the TGF-β superfamily, is a skeletal muscle growth inhibitor. The main target of these cells are primarily satellite cells whose proliferation and differentiation are inhibited by myostatin.

Creatine supplementation in conjunction with resistance training significantly decreased serum myostatin levels compared to control, by turning on IGF-1 signalling in muscles. Furthermore; creatine enhances both gene expression and satellite cell activation resulting in a hypertrophic response.

Creatine was also shown to stimulate selectively the rate of synthesis of two major contractile proteins, actin and myosin heavy chain.

As well as facilitate muscle anabolism by increasing the expression of growth factors and the phosphorylation of anabolic signaling molecules, involving IGF and 4E-BP1. As well as increases satellite cell mitotic activity during compensatory hypertrophy.

**Conclusion**

As a person continues to take creatine, muscle stores become saturated and clearance shifts from muscle uptake to primarily renal elimination. Therefore, because creatine pharmacokinetics change over time, so should the dosage; for example, during the loading phase, consuming 5 g three times a day (15 g/d) will maintain concentrations well above the \( K_m \) for the plasma membrane transporter throughout the day. After a few days of the loading phase, muscle stores will approach saturation, resulting in an increased fraction of the dose being lost in the urine. Thus, after a short loading phase, doses can be reduced to 3 to 5 g/d to compensate for the reduction in clearance of creatine and to compensate for the loss of creatine via creatinine formation, which is on average 2 g/d.

Because of these findings, there is sufficient evidence to suggest that chronic creatine ingestion and resultant chronic elevations in creatine plasma levels will not increase creatine uptake into the muscles, and if elevated too high for an excessive period of time it may actually inhibit creatine uptake and place unnecessary strain on the kidney.

Doses should be based on lean body mass. It was determined by dual-energy x-ray absorptiometer the loading dose and the maintenance dose should consist of 0.25 and 0.0625 g/Kg of lean body mass per day, respectively. This will optimize intramuscular creatine content and whole body creatine retention.

In order to benefit from creatine supplementation, an increase of at least 20 mmol/kg dm is required. That amount is needed in order to significantly attenuate the drop in ATP levels during intense exercise bouts, as well as to elicit a proliferative anabolic signal and cell mitotic activity, which is partly attributed to cell volumization via induced water retention. Around 20-30% of the population isn’t able to achieve these levels and thus experiences no benefits. It has been demonstrated that exercise not only helps non responders, (e.g. elderly and others) but also aids in further increasing intramuscular creatine content. Another method is to invoke the aid of insulin by consuming creatine with carbohydrates alone, protein alone, or both. One should not be concerned that protein will inhibit creatine absorption or uptake. In fact, consuming a carbohydrate/protein/creatine blend may be the most effective at
increasing muscle creatine content, while also providing the added benefit of the protein: further aid in recovery and protein synthesis. This combination may be particularly useful for those desiring to decrease an excessive carbohydrate intake. Moreover, high fructose beverages do not offer any benefit at increasing muscle creatine concentration, and therefore should be avoided.

Supplement timing was also seen to have an important effect. Taking creatine before exercise may be the best at increasing total muscle creatine stores, because it takes around 30 minutes to an hour (depending on dose) to reach peak plasma concentrations. Most workouts take about that same amount of time to complete. Taking it before allows muscles to immediately use the available creatine not only to replenish its stores, but to further increase muscle creatine accumulation. If taken after, the creatine transporter and creatine kinase activity may have already significantly decreased by the time plasma creatine concentrations reach their peak, about 30min to an hour later, and the plasma creatine concentrations would be even further decreased if taken with carbohydrates and protein due to reduced intestinal transit time. This may even further decrease the affect of exercise on creatine transporter activity. Taking the creatine before, alone or with carbohydrates and/or protein, eliminates this problem. However, some people may experience some gastrointestinal distress if they exercise immediately after consuming food or drink. The most important action is to consume the creatine as close to exercise as possible.

**Recommendation**
- Optimal creatine loading is two to three days of 0.25 g/Kg of lean body mass per day
- Optimal maintenance consist of 0.0625 g/Kg of lean body mass per day
- Consume creatine with carbohydrates and protein
- Don’t be concerned about inhibiting creatine uptake by concomitantly consuming protein
- Ingest supplement as close to exercise as possible, preferably before if it can be tolerated
- Don’t use fructose for carbohydrates, maltodextrins and/or dextrose are good choices

**Physicochemical Properties**

**Results**

**Structure**
Before we can understand solubility, absorbability and stability we must first understand the creatine structure.

The following structures are different forms of creatine at different units of pH 1-12.

![Protonated Creatine](image1.png)
![Zwitterion Creatine](image2.png)
![Neutral Creatine](image3.png)
![Anion Creatine](image4.png)
As is noted by the images the structural changes vary with pH\(^{1*}\), these changes directly affect the solubility—and thus the bioavailability, and stability of the molecule.

**Solubility**

Solubility is a key aspect relative to, bioavailability, absorption and the rate of action. In order to exert their full potential, pharmaceuticals must exhibit high solubility and dissolution properties so that they can completely and effectively reach the target tissues. Generally the more soluble the compound is the easier and quicker it can be absorbed. When a compound is added to water there are a few things that can happen to it. It can form a homogeneous mixture known as a solution in which each individual particle, ion or solute, is solvated by water molecules forming a hydration shell. It can also form a colloid in which a few particles are collectively hydrated by water, or a suspension in which even more particles are collectively suspended in the solvent and will eventually settle.

To better understand how this works a brief note concerning enthalpy of solution (\(\Delta H_{\text{soln}}\)) is warranted. For a compound to enter into solution the process must be favorable, that is it must in some way, maximize entropy and \(\Delta G\) (Gibbs free energy change) must be negative. There are three main steps involved in the dissolution process; the first two are endothermic which are separating the solute into individual particles and overcoming solvent-solvent attractions. The last one, called solvation—hydrating the solute, is exothermic.

The individual creatine molecule has a diameter less than 0.01 micrometers, the creatine powder exists as tiny crystal conglomerates of many creatine molecules with a diameter of 300-500 microns, and when placed in water many of these crystals don’t disintegrate into the individual creatine molecules because of the energy required to break the solute-solute interactions. Therefore getting creatine into solution is challenging. The creatine crystals that don’t undergo disintegration will by hydrated by water and exit in the form of a suspension. Under normal conditions creatine exhibits low aqueous solubility of approximately 10-16 mg/ml. At a pH range of 3-12, creatine is principally in its zwitterionic form, (i.e. exhibits both a negative and a positive charge) this leads to intermolecular induced dipole/induced dipole interactions with each other, causing the creatine molecules to aggregate together; which

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\(^{1*}\) The protonated form shown above has two positive charges on the guanidino group. This structure is given only for demonstrative purposes. In reality such a structure is not likely because the guanidino group has resonance, and if protonated the conjugation in that pi system would be lost. Also when a species gets one positive charge, it becomes orders of magnitude more difficult to add another proton due to proton-proton repulsion. At a low pH the real structure would resemble more closely the images shown below. These would exhibit similar properties as the one shown previously, but it is easier understand the characteristics of the protonated form when analyzing the above structure.
prevents individual creatine molecules to be solvated by water. Consequently the amount of creatine that enters in solution is reduced, thus more of the creatine is only suspended in water. Solubility and pH and are inversely proportional, that is, as the pH increases the solubility decreases, and conversely as the pH decreases the solubility significantly increases. This is because at a pH <3 creatine is predominantly in its cationic form, and will therefore repeal other positively charged creatine molecules away allowing each creatine molecule to be hydrated by water. In terms of only solubility, an acidic environment is favored; however, creatine is not completely stable under acidic conditions. (discussed later)

Another important factor influencing solubility is temperature. Increasing the temperature of the water increases the overall energy of the system making it easier to overcome the activation energy required for the first two endothermic steps of dissolution (i.e. break the lattice structure and the solvents intermolecular interactions—hydrogen bonding). This will help the overall $\Delta H_{\text{soln}}$ be zero assuring maximal amount of creatine in solution. On the other hand; the colder the water, the less energy available for those first two steps and therefore less creatine in solution and more in suspension. In terms of solubility only hot water is favored; however, heat also increases the rate of creatine degradation. In short, mixing creatine with water slightly above room temperature and drinking it immediately, to prevent degradation, would be effective.

If creatine is completely dissolved in solution it will not only reach peak plasma concentrations faster and give higher peak concentrations, but the AUC will also be greater. This observation, makes sense because before being able to be absorbed, the molecule is required to be in solution. Solid dosage forms (e.g. meat, lozenge) must undergo disintegration and dissolution while suspensions require dissolution of the suspended particles. Because solutions remove these steps they exhibit a more rapid rate of absorption.

It is also important to note that with a solution, the solvation process is favorable; this is because of the displacement of water molecules which maximizes entropy. However, the presence of other electrolytes (hydrated ions) increases the ionic strength of the solution which may negatively affect the amount of creatine that can be dissolved. Similarly as the solutions solute concentration increases, the ability of creatine to enter into solution may also decrease. This is because some water molecules are already occupied in hydrating other particles. Not only would it require more energy to hydrate the newly added particles, but entropy for the solution would not increase. Therefore the newly added particles would not be able to enter into solution, and if they did they would displace already solvated particles forcing them to precipitate out of solution. These phenomena are related to both the common ion effect and the salting out effect. A simple example of this is adding salt to a glass of water, you can only add so much salt to a glass of water before it becomes saturated and starts to precipitate out of solution and settle to the bottom of the glass. By the same token the presence of other particles, (e.g. proteins, amino acids, sugars, etc) may decrease the amount of creatine that enters into solution and thus the absolute bioavailability. Another way to look at is, that the addition of more particles (solute, ions, etc) may decrease the amount of particles in solution and move them to colloids, as more particles are added the zeta potential (which stabilizes the colloids) approaches zero until the amount of particles in colloidal form begin to only be suspended in water, and eventually the suspended particles will
precipitate out and settle to the bottom. Taken together, it may be wisest to mix creatine in water alone. In other words, not mixing it directly with protein, carbohydrates, milk or other solutes—and specifically those compounds that would give a charge upon its dissociation.

**Bioavailability**

One of the principal pharmacokinetics of drugs is there bioavailability, which by definition, is 100% when medication is administered intravenously. However, when administered non-intravenously (i.e. oral, sublingual, transdermal, etc.) the bioavailability is decreased, due to incomplete absorption or the first-pass metabolism effect. Absolute bioavailability is measured by dividing the area under the curve (AUC) of the non-intravenously administered dose, by the AUC when same dose is administered intravenously.

In order to achieve sufficient creatine uptake into the muscles the plasma creatine concentrations need to only be elevated above the $K_m$ which is between 50 and 100 μmol$^2$. Though this can easily be done by consuming 2 grams (15.252mmols) of creatine which will give a peak plasma concentration of 180-390 μmol,$^6$ these levels, however, came back down to baseline in a few hours giving a total AUC of 386±87μmol. Because saturation of the transporter occurs at around 100 μmol, higher creatine plasma levels will not increase cellular creatine uptake any more than levels at saturation levels, (e.g. 200μm is as effective as 2200μmols) so with this view point in mind a 1 gram dose is as effective as a 20 gram dose, but only for the short period of time that the one gram dose has plasma creatine concentrations greater than the $K_m$. Because the plasma creatine levels return back to baseline so quickly (2-3 hours), it may not give the muscles sufficient time to uptake the creatine. In the long run 20 grams is more effective because it won’t return to baseline until around ten hours or more, and also gives an AUC of around 4 mmols, which will therefore result in significantly greater intramuscular creatine accumulation than the 1 gram dose. However, this equates to less than 3%$^2$ of the administered dose appearing in the AUC.

We see that the total administered dose doesn’t reach systemic circulation,$^5$ and therefore a larger dose is needed in order to significantly increase intramuscular creatine concentrations. We also see that 2 grams of creatine only result in less than 400 μmols AUC, which is also less than 3%. Therefore taking 1 gram 15-20 times a day, as opposed to ingesting 20 grams at once, may not result in a significantly greater bioavailability, but will however, decrease excessive strain on the body’s organs due to extremely high creatine plasma levels (2200μmols), and decrease the risk of down regulating creatine transportation activity. If one could increase the relative bioavailability of creatine smaller doses could be used with greater effectiveness than 20 gram doses, without the adverse side effects like gastrointestinal distress, bloating, excessive swelling, and strain on the kidney, liver and other organs.

Again solubility directly correlates to a solutes absorbability, because incomplete dissolution of creatine, results in a decreased bioavailability, for only dissolved creatine will be absorbed, and thus if creatine does not enter solution it will not be absorbed.$^2$

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$^2$ This is only looking at AUC which was measured at 30min intervals; therefore amount taken up by the muscles is not seen. (e.g. as more creatine enters the muscles, the lower the AUC will get until it is back to baseline) By taking into account how much of the administered dose ended up in the muscles and in the urine reveals about a 35% uptake, from some of the studies done by Harris et al.
One study compared the relative plasma concentrations of creatine ingested as a meat, solution, suspension and lozenge.\textsuperscript{61} (Note that the solution was prepared by mixing creatine in warm water and the suspension by mixing creatine in ice cold water.) It was demonstrated that creatine given in solution resulted in not only the highest peak plasma concentration, but also reached its peak the fastest. The suspension, lozenge and meat where similar in peak plasma concentrations and amount of time to reach their peaks. However, meat maintained its plasma concentration longer and returned to baseline significantly slower.

Both the creatine delivered in solution and ingested in meat had similar area under the curve (AUC) to one another. However, the AUC for the lozenge and suspension was significantly lower. The authors suggest that this is indicative of lower bioavailability of lozenge or suspension compared to solution. They also suggest that in order for a suspension or lozenge to give an AUC similar to creatine monohydrate, you would need to ingest 20% more of that form.

However, another study testing creatine in solution, protein and beta glucan\textsuperscript{62} showed no significant differences of altered bioavailability relative to AUC. They along with Persky\textsuperscript{63} suggest the reason for this discrepancy is that in the latter study the AUC was integrated from 0 to 6 h, whereas integration to infinity is necessary to assess the significance of bioequivalence.

However, examining the graph of the AUC for the suspension and lozenge after peak concentration, it is clearly seen that they quickly declined, and by 6 hours creatine plasma levels were similar to baseline. The meat was still semi-elevated above baseline, but the difference would most likely not be significant, this is further evidenced by the fact that both the meat and the creatine in solution contained approximately the same amount of creatine, and after 6 hours exhibited similar AUC.

Because virtually all the creatine of the meat was accounted for within those 6 hours, and because meat requires significantly longer time to digest than other foodstuffs, especially suspensions and lozenges, then it would require a stretch to believe that if the AUC was continued to infinity for the those two groups, that the creatine plasma concentrations would sporadically rise and maintain plasma concentrations above control and then go back down to baseline. This would unreasonably suggest that meat not only digested faster, but also the creatine in the suspension somehow avoided intestinal absorption until at least 6 hours later.

Interestingly the AUC for the solution at six hours appears to be slightly above baseline, and consequently also above the suspension and lozenge group. This observation suggests that if the AUC was carried out to infinity for all the groups, the only differences that would be noted are greater creatine plasma concentrations for the solution and meat, but no difference for the lozenge or suspension.

**Stability**

It is important to understand that creatine is not a completely stable molecule and is non-enzymatically degraded into the byproduct creatinine. The conversion rate is increased as creatine approaches its saturation level.\textsuperscript{72} The pH also effects the equilibrium of creatine to creatinine in solution, under acidic conditions the reaction is spontaneous. The literature contains numerous references to the conversion
of creatine into creatinine in the presence of acid catalysts under these conditions creatine is practically quantitatively converted to creatinine. On the other hand, creatinine is at least partially converted into creatine in alkaline solution, the reaction having served as a method of preparation of creatine from creatinine. In neutral and alkaline solutions, a condition of equilibrium is reached, in which appreciable quantities of both substances are present; in acid solution the position of equilibrium must lie so far on the right (this would be creatinine) as to escape measurement. In fact the Merck Index says the following about creatine “...aqueous alkaline solutions contain an equilibrium mixture of creatine and creatinine, while in acid solution the formation of creatinine is complete...creatine is usually prepared from commercial creatine by treatment with HCl.”

The proposed mechanism for creatines non-enzymatic degradation is that the lone pairs from the nitrogen attack the electrophilic carbonyl in a nucleophilic type manner leading to cyclization, according to the following reaction:

In order to deal with this undesired reaction, a number of products on the market have attempted to solve these problems, and are marketed as the favored choice over regular creatine monohydrate. The first product to attempt to solve this problem was introduced to the market in 2002 and is known as Kre-Alkalyn, which claims to be completely stable. As was mentioned earlier, creatine degrades into creatinine spontaneously in an acidic environment. As the name implies, Kre-Alkalyn is very basic. They use normal creatine monohydrate and then add alkali-buffering salts (principally magnesium glycerol phosphate and carbonate) to the creatine powder. This raises the pH to around 12, which prevents any degradation. A number of studies from this company show that when normal creatine monohydrate is mixed with water or protein, it instantly converts to creatinine, and total conversion is complete at 8.4 minutes. This same study also proclaims that when creatine is taken with fruit juice, fruit flavored drink mixes, effervescent mixes or sports drinks, 100% of the creatine is degraded to creatinine is 43 seconds. They also allege that if the creatine hasn’t completely converted to creatinine before you ingest it, once it reaches the low pH of the stomach it will instantly convert to the byproduct creatinine. To confirm this they present another study, in which they simulated the conditions of the stomach by lowering the pH with a hydrochloric acid solution to a pH 3. They showed that where 1.5

* A reaction is said to be spontaneous if Gibbs free energy (ΔG) is negative according to the equation: ΔG=ΔH-TΔS. Spontaneity revels noting about the rate, but only the direction of the reaction that occurs, without external intervention, and is determined by entropy. e.g. Diamonds spontaneously convert to graphite.
grams of Kre-Alkalyn had zero percent conversion to creatinine, the same dose of creatine monohydrate was completely degraded, showing the inability of creatine to withstand the harsh conditions of the stomach.  

Superficially, this product truly does seem to be the magical formula; however, as good as these studies or theories sound on the surface, they are not backed by peer reviewed scientific literature and they contradict basic logic. For example, the creatine in meat, although subject to the acidic conditions of the stomach, showed negligible creatinine conversion and significantly increased creatine plasma levels. The present article has also clearly demonstrated that creatine monohydrate significantly increases muscle creatine concentration, which would be impossible if all the ingested creatine really did degrade.

One study published American Association of Pharmaceutical Scientists evaluated the stability of creatine prepared from effervescent creatine formulations. It was shown that creatine monohydrate, di-creatine citrate and effervescent creatine did indeed degrade to creatinine but the conversion was very slow, total conversion never happened even after 45 days of being in solution.

At the 4th Annual International Society of Sports Nutrition Conference, Tallon and child presented a poster entitled, “Kre-alkalyn® supplementation has no beneficial effect on creatine-to-creatinine conversion rates” this study compared the stability of creatine monohydrate to Kre-Alkalyn when added to a 1 pH aqueous solution. They reported that not only was creatine monohydrate very stable under these acidic conditions that replicate the stomach with less than 1% degradation, but that Kre-Alkalyn actually resulted in a 35% greater degradation.

This poster became a matter of intense public attention and resulted in many letters written back and forth between the attorney of All American Pharmaceuticals (inventors of Kre-Alkalyn) and Dr.s Tallon and Child. The subject of these letters was that All American Pharmaceuticals demanded an immediate retraction from the former publication concerning the statement that kre-alkalyns has no beneficial effects on the creatine to creatinine conversion rate, and furthermore if they did not take immediate action they would be sued. Tallon responded that he would not retract it, because he asserts it was the case and he has other data from other laboratories confirming those same findings and that they are currently working on the manuscript and plan to get it published in a peer reviewed journal. This type of communication continued for some years, but in the end Tallon and Child never did publish the manuscript and action was taken against them. For a full review on this matter see report.

Later Tallon published an article on creatine monohydrate and the truth about its stability. Using peer reviewed articles, he clearly points out that although creatine does degrade into creatinine it is not near as unstable as some make it appear. He also references articles that show the negligibility of creatines degradation upon consumption.

The proposed mechanism for creatines non-enzymatic degradation is that the lone pairs from the nitrogen attack the electrophilic carbonyl in a nucleophilic type manner leading to cyclization according to the following reaction:
In basic conditions this 1st step of the reaction is very quick because of the greater quantity of the nucleophilic negative alkaline species. However, the next step, dehydration, is the rate limiting step because of a lack of protons required to protonate the hydroxyl group. Research has shown that at pH ≥12 dehydration does not occur, thus preventing cyclization to creatinine.

In an acidic environment the 1st step in the reaction becomes the slow step, because there is less of the reactive alkaline nitrogen species present to attack the carbonyl; however, once the attack is made, and cyclization has occurred, the 2nd step, dehydration, is instantaneous producing the waste product creatinine and H₂O according to the above equation.

This degradative type reaction occurs most favorably and quickly in a pH of 3.4; however, if it were true that creatine is completely degraded to creatinine in an acidic environment, then how is it we still get creatine from eating meat, which digestion in the stomach produces a very acidic pH environment? It turns out that under extreme acidic conditions (i.e. stomach) creatine is observed to be very stable, i.e. it does not undergo the intramolecular cyclization to creatinine that other creatines would in acidic solutions. The reason why it is stable, according to previous research and our proposed mechanism, is because at a pH <2 the nitrogen involved in the nucleophilic attack gains an additional proton and thus has no lone pairs to donate. See below:

Thus it is still possible to get creatine from animal sources, as was mentioned earlier. Where it was observed that although creatine in aqueous solution gave a higher peak plasma concentration, the area under the curve was similar to that of meat containing the same creatine content.⁶¹

Therefore once creatine is ingested, the rate of conversion is quite slow.⁷⁸ The half-lives of degradation are 55, 7.5 and 40.5 days at pH values 1.4, 3.7 and 6.8, respectively.⁷⁹ Thus, considering a pH of stomach near to 2 and a dose of 5 g, only 0.1g of this amount would be converted to creatinine within the first hour after ingestion. This rate of creatine degradation is in the same range in the intestinal tract (pH = 6–7). As creatine remains a longer period of time in the intestine than in the stomach, it is likely to be
degraded in a larger proportion at this level. However, the stable plasma creatinine concentration following ingestion of creatine, in addition to its undetectable amount in feces, corroborate the hypothesis that the conversion of creatine to creatinine remains negligible in the gastrointestinal tract. 79

In regards to stability on storage, the shelf life of a product, according to the FDA, is the time it takes for 10% of the starting material to degrade into the byproduct or inactive form. In that respect creatine monohydrate has a shelf-life of about 7 days when stored in aqueous solution.

Conclusion
It has been demonstrated that bioavailability is dependent upon the compounds ability to dissolve into solution. Thus solubility is indicative of bioavailability; creatine has low aqueous solubility and thus low bioavailability, its low bioavailability may partly be attributed to the first-pass metabolism effect.

Higher doses of creatine are needed in order to reach and maintain the necessary plasma creatine concentrations that will lead to an increased muscle creatine accumulation. If the bioavailability of creatine is increased the dosage may be decreased, while still giving the same plasma creatine concentrations. This would be useful in not placing excessive strain on the body or its organs, and simply being able to take less would save on cost to the consumer as well.

There is evidence to suggest that creatine in the form of a lozenge or suspension will have diminished plasma uptake due to there the low solubility. One method to increase the amount of creatine that enters solution is by mixing it with warm water alone, as opposed to mixing it with the workout drink.

It has also been shown that as creatine reaches its saturation point the rate of degradation is increased. This can be avoided if one uses plenty of solution to dissolve the creatine.

It has also been demonstrated that creatine degrades into creatinine, and the conversion is favored under acidic conditions. Though this is an important property to be aware of, the degradation rate is so slow that the conversion is thought to be negligible. Kre-Alkalyn purports that this degradation is basically instantaneous, but such an idea is not backed up by the scientific literature and in fact it contradicts with what is known. Furthermore, Kre-Alkalyn may or may not be a better product than creatine monohydrate, but because no 3rd party studies published in peer reviewed journals have validated their claims, and because said claims contradict current knowledge, one must be very careful on how much belief they put on other benefits that they assert.

Recommendations
- Before purchasing a certain type of creatine, ensure solubility is high
- Prior to consumption allow creatine to fully dissolve in solution
- Mix in water slightly above room temperature and drink immediately
- To maximize the amount of creatine in solution, mix the creatine in water, as opposed to mixing it with the work out drink or in milk
- Avoid lozenges and suspensions due to their diminished solubility and thus bioavailability
- Once creatine supplement is prepared, immediately consume it, avoid letting it sit for prolonged periods of time
• Disregard claims that creatine instantly converts into creatinine
• If companies make false claims, be wary of how much confidence you put in further claims

Various types of creatines

Results

Creatine HCl

CreatineHCl or creatine hydrochloride known as CON-CRET is a creatineHCl salt, manufactured by Promera Health. It exhibits an acidic pH around 2, not only does this significantly increase its solubility above creatine monohydrate, but also the stability is significantly greater because the nitrogen is protonated and thus can’t make the nucleophilic attack on the carbonyl. In fact a number of their studies—used on for their patent—using H-NMR show that where creatine monohydrate has a shelf-life of one week, CrHCl has a shelf life of 45 to 90 days. Once ingested the creatine moves from the stomach into the intestines to be absorbed into the blood. The company has a number of studies showing not only its stability and solubility above monohydrate, but also creatine uptake is also significantly higher. According to one of the patents for this creatine, the solubility for CrHCl is in the range of at least 250mg/ml to as high as 1000mg/ml, compared to monohydrate at only 10-16mg/ml. Therefore the bioavailability of this creatine is significantly greater than creatine monohydrate. According to three studies, they use in there patent from various universities, the bioavailability of CrHCl is 50-70% greater than creatine monohydrate. Thus they claim that you only need approximately 0.8 grams per 100lbs as opposed to creatine monohydrate which is about 3 grams per 100lbs.

Creatine Nitrate

Creatine Nitrate also known as creatrate exhibits an acidic pH, thus preventing the nitrogen from attacking the carbonyl carbon. The counter ion to the cationic creatine is nitrate. Nitrate has recently been extensively studied for its vasodilating properties. Many studies show increased time to exhaustion, increased $V_{O_2}$, 19% more energy-dense ATP molecules per oxygen molecule consumed, and many more. This company also has studies that demonstrate the increased, solubility, stability and
dissolution properties over creatine monohydrate and buffered creatine. The results show that compared to creatine nitrate, the degradation rates in acidic environment were 58.67% and 44.00% higher for creatine monohydrate and buffered creatine respectively.\textsuperscript{81}

It has been reported by some of these non-peer reviewed studies that a buffered creatine is not very stable once placed in conditions replicating that of the stomach. This may actually make sense in our proposed mechanistic model. Because the 1\textsuperscript{st} step, cyclization, occurs rapidly in basic conditions but the 2\textsuperscript{nd} step, dehydration won’t ever occur under these conditions. But as soon as we add acid to the basic conditions the dehydration takes place instantaneously, because cyclization already occurred. Theoretically Kre-Alkalyn should be able to buffer the stomach sufficiently so that no dehydration occurs, but the amount of base you actually consume may be insignificant relative to the amount of acid in your stomach. i.e. you can’t neutralize a liter of stomach acid with a few milligrams of base. Therefore by analyzing the mechanism one could conclude that the most effective scenario for total creatine to creatinine conversion would consist of placing the creatine in a basic solution and allowing complete cyclization of the molecules to take place, and then adding it to acidic conditions so that dehydration can rapidly occur on all the cyclized creatine molecules assuring 100% creatinine conversion.

Conversely creatrate and CON-CRET start in acidic conditions so that the first step never occurs, as it moves into the intestines and the blood the pH is going up, which prevents any dehydration which may have occurred, thus assuring 100% creatine plasma bioavailability from the dose administered.

**Micronized Creatine**

Micronized creatine is creatine that has passed through some type of micronization process, at least milled to 200 mesh or more. As mentioned earlier, the individual creatine molecule has a diameter less than 0.01 micrometers, and the creatine powder exists as tiny crystal conglomerates of many creatine molecules with a diameter of 300-500 microns.\textsuperscript{4}\textsuperscript{*} After the micronization process the particles size are reduced to 45-75 microns.\textsuperscript{5}\textsuperscript{*} These smaller creatine crystals have a greater propensity to disintegrate allowing the creatine to enter into solution. Therefore not only will more creatine be in solution, but the creatine that didn’t quite make it into solution, will stay in suspension significantly longer. Some companies claim to have micronized creatine, but the creatine particles are only 180 microns in diameter (basically only an 80 mesh.) Therefore, before purchasing a micronized creatine make sure that they used a 200-300 mesh.

\textsuperscript{4}\textsuperscript{*} This is what I have found; however, companies claim that a 200 mesh makes creatine particles 20 times smaller. If a 200 mesh corresponds to 74 microns then that would suggest that the normal creatine particles are closer to 1480 microns. Some of the creatine crystals may be that large but on the average they are probably closer to 300-500 microns.

\textsuperscript{5}\textsuperscript{*} An example of the difference would be to compare sea salt, which are little rocks that need to be grinded, to refined table salt which is like micronized sea salt each grain being about 100 microns, as opposed to around 5000 microns for sea salt. Because each one has a large solubility product constant $K_{sp}$ both will eventually dissolve into solution, but the refined one will do it significantly faster.
Creatine ethyl ester

Creatine ethyl ester (CEE) was designed like many of the pharmaceuticals with an ester attached to it. Because creatine monohydrate is polar its absorption not only into the blood, but also into the muscle is dependent on transporters. This creatine is non-polar and lipophilic which would theoretically allow it to bypass the creatine transporter and therefore increase skeletal muscle uptake. Once in the muscle an esterase enzyme would cleave of the ester preventing its efflux back into plasma. In theory it sounds great, but studies show differently. Spilane\textsuperscript{82} and colleagues compared creatine monohydrate and CEE and found that CEE was not as effective at increasing serum and muscle creatine levels or in improving body composition, muscle mass, strength, and power. It did significantly increase the serum creatinine waste byproduct compared to the placebo ($p = 0.001$), however.

A later study investigated the hydrolysis of CEE and found using H-NMR that the half-life of CEE in blood is on the order of one minute, suggesting that CEE may hydrolyze too quickly to reach muscle cells in its ester form.\textsuperscript{83} Earlier it was also reported that Creatine ethyl ester rapidly degrades to creatinine in stomach acid.\textsuperscript{84} Giese demonstrated that when CEE enters aqueous conditions of a pH 7.4 the cyclization to creatinine becomes nearly instantaneous.\textsuperscript{85}

Attaching the ester increases the electrophilicity of the carbonyl carbon making it orders of magnitude more susceptible to hydrolysis and/or nucleophilic attack leading to its cyclization and complete degradation to creatinine as one product and toxic ethanol as the other.

These same principles apply to CEE derivatives; such as Creatine ethyl ester hydrochloride, creatine ethyl ester malate, creatine ethyl carbonate ester, etc.

Creatine anhydride

Creatine anhydride is simply two creatines bonded together by the oxygen. This was produced in attempts to stabilize the molecule from degradative reactions. However, acid anhydrides are even less stable and thus more reactive then the ester, making the carbonyls extremely electrophilic and highly increasing the rate of hydrolysis and/or intramolecular cyclization from the nucleophilic attack of the nitrogen.
Creatine pyruvate
There are a number of studies involving creatine pyruvate all of which suggest ergogenic effects similar to creatine monohydrate. However, because it is attached to pyruvate it may have an added benefit. This was demonstrated in a study comparing it to plain creatine citrate and found that creatine pyruvate significantly increased relaxation velocity and force during the last of multiple intervals. Thus creatine pyruvate may increase endurance, due to the enhanced activity of the aerobic metabolism. Another study found that creatine pyruvate compared to creatine monohydrate and tri-creatine citrate resulted in significantly greater mean peak concentrations and area under the curve. However, despite these higher elevations, there was no difference between the estimated velocity constants or elimination between the groups. Thus the small differences in kinetics most likely will not increase muscle creatine accumulation during periods of creatine loading.

Creatine taurinate
Creatine taurinate is simply creatine bound to the amino acid taurine. This compound does have a patent and some interesting ideas about its effectiveness; there is no reason to believe this would be less effective than creatine monohydrate. Taurine is able to stimulate insulin secretion and thus may increase muscle creatine retention, all other benefits that potentially exist can be attributed to the ergogenic effect of the amino acid taurine itself.

Creatine alpha-ketoglutarate
Creatine alpha-ketoglutarate is marked as being more effective in delivering the creatine to the muscles, as well as an energy benefit from its counter ion. One abstract showed that it improves power output physical performance, body composition, and some of characteristic homeostatic indices in elite athletes. However, another study reports that it remains experimentally unproven

Aqueous creatines
Aqueous creatines are those already in solution as opposed to powder; such as, creatine serum and liquid creatine. These are marketed as being better because they are already dissolved; however, the longer creatine is in solution the more of it will degrade, these would not be a very good option.

Other creatines
The following creatines may or may not be as effective as creatine monohydrate. Some have been researched, and others have not, but there is no reason why they wouldn’t be effective. creatine gluconate, creatine malate, dicreatine malate, tricreatine malate, creatine citrate, dicreatine citrate, tricreatine citrate, creatine phosphate, creatine anhydrous, creatine pyroglutamate, magnesium creatine chelate etc.

Conclusions
It has been demonstrated that creatine HCl and creatine Nitrate have greater solubility and stability than creatine monohydrate, and although this research was not peer-reviewed, it does exhibit certain connectivity with the current research and does fit our proposed mechanism fairly nicely. Creatine nitrate may have the same or greater bioavailability as creatine HCl due to its high solubility. However, creatine HCl is the one with the reported studies on enhanced plasma uptake and so this may or may not
be true. Creatine nitrate also has the added benefit from having the nitrate counter ion, which has been shown to be ergogenic itself. Micronized creatine would have an increased solubility compared to non-micronized thus increasing the absolute bioavailability. Creatine pyruvate though not as good, does have some studies that show the AUC is greater and it outperformed the creatine monohydrate when endurance was required. All of these creatines appear to be more effective than creatine monohydrate.

Kre-Alkalyn known as the buffered creatine, has some conflicting results and don’t look so good on the mechanistic side of things. It may be better than monohydrate if taken on an empty stomach with no food, carbs, or protein; so that it does not undergo dehydration quicker into creatinine, if it is true that it is already cyclized in the basic solution. However, if you are not able to take this creatine with carbs and/or protein you may lose some of the beneficial effects mentioned earlier. It is also hard to believe some of their studies on Olympic lifters, because of the appeared false data of rapid complete degradation of creatine into creatinine.

Creatine ethyl ester and creatine anhydride are extremely susceptible to nucleophilic attack, and thus degrade in around a minute to the waste product creatinine. These two creatines appear to not be as effective as creatine monohydrate.

It has also been stated of the following creatines, that they may or may not be as effective as creatine monohydrate. creatine gluconate, creatine malate, dicreatine malate, tricreatine malate, creatine citrate, dicreatine citrate, tricreatine citrate, creatine phosphate, creatine anhydrous, creatine pyroglutamate, magnesium creatine chelate, Creatine taurinate and Creatine alpha-ketoglutarate. Some have been researched, and others have not, but there is no current mechanistic reason why they wouldn’t at least as effective as creatine monohydrate.

As mentioned earlier mixing creatine with other solutes (proteins, carbs, etc) may decrease its overall solubility. This applies more specifically to the mixing of creatineHCl and creatine nitrate with protein. This is because protein will act as a buffer, raising the pH, and thus decrease the overall solubility. It would still most likely be more soluble that monohydrate; however, not as soluble as if it were mixed in water alone. The increase in pH would also deprotonate the creatine making it more susceptible to undergo cyclization. Therefore, if one needs to mix the creatine with their protein, (or maybe it is already mixed in the work out drink form from the manufacture) don’t mix the formula in liquid and let it sit out for an extended period of time.

Also though it is a common practice of many supplement companies and athletes to mix creatines hoping to get the best of each one. Mixing creatineHCl or Creatine Nitrate with any buffered creatine (e.g. Kre-Alkalyn) would significantly decrease the stability and thus the ergogenic effect of both types of creatines. Mixing CreHCl with CreNitrate would not pose a problem because they exhibit near the same pH.

**Recommendations**
- Creatine HCl known as CON-CRET is a preferred choice over creatine monohydrate
- Creatine nitrate known as creatrate is a preferred choice over creatine monohydrate
- Micronized creatine is a preferred choice over non-micronized creatine.
- Kre-Alkalyn may be as good or better as monohydrate if consumed on an empty stomach
- Creatine ethyl ester should be completely avoided, along with any product that contains it
- Creatine anhydride should be dealt with similarly to creatine ethyl ester
- Don’t mix CreHCl or CreNitrate with protein and let it sit out for an extended period of time.
- The following may be at least as effective as creatine monohydrate
  - Creatine taurinate
  - Creatine alpha-ketoglutarate
  - Creatine gluconate
  - Creatine malate
  - Dicreatine malate
  - Tricreatine malate
  - Creatine citrate
  - Dicreatine citrate
  - Tricreatine citrate
  - Creatine phosphate
  - Creatine anhydrous
  - Creatine pyroglutamate
  - Magnesium creatine chelate

**Creatine combinations**

Many creatines on the market contain various ingredients in hopes to increase muscle creatine accumulation. Some of these are shown to be effective, whereas others seem to have the opposite effect.

**Results**

**Creatine analogous**

Some products contain creatine analogous or precursors to creatine such as creatine alpha-amino butyrate, creatinol or creatinol-o-phosphate and Beta guanidinopropionic acid. Not much is known about the former two with respect to creatine supplementation. Because they are precursors some claim that they can be turned into creatine in the body. However, the latter is also a creatine analogue and it has been clearly demonstrated to up-regulate creatine transporters\(^ {90-91}\). On the surface this may sound like the key to increased muscle creatine stores; however, studies show that the reason why creatine transporters are up-regulated is because this analogue significantly decreases muscle creatine concentrations,\(^ {92-93}\) leading to an increase in mortality in rats after having a myocardial infarction due to substantial ATP depletion.\(^ {94}\)
**Beta-glucan bars**

These bars are polysaccharides which are very fibrous and drastically decrease the velocity of intestinal absorption. This may be desired to reduce the potential risks that larger doses may exert on the kidney. This would also lead to less urinary creatine excretion and thus a better retention of creatine. Indeed, one study found that a 2g dose of creatine consumed in the form of either aqueous solution or protein resulted in approximately 15% of the dosage being excreted into the urine within the first 24 hours; whereas, only 8% of the dose in the beta-glucan group. This study suggests that beta-glucan with creatine facilitates creatine retention by slowing down the absorption rate and its excretion into the urine. They also showed that the gastrointestinal absorption is complete without any creatine findings in fecal matter.62

**Alpha lipolic acid**

If insulin improves intramuscular creatine retention, then it would follow that supplements that potentiate the action of insulin may also be useful at increasing muscle creating levels. Alpha lipolic acid is known to improve glucose levels in patients with type 2 diabetes.95 This is done by increasing insulin sensitivity, via activation of the AMPK pathway in skeletal muscle.96

It appears to be rather effective; in fact the results from one study indicate that the ingestion of creatine and sucrose or creatine alone compared with co-ingestion of alpha-lipoic acid with creatine and a small amount of sucrose found the latter combination to be more effective at enhancing total muscle creatine.97 Similarly another study showed that alpha lipoic acid potentiated creatine uptake.98

**D-Pinitol**

Another compound D-pinitol (a plant extract) also has insulin sensitizing characteristics.99 Co-ingestion of D-pinitol and creatine monohydrate compared to creatine monohydrate alone for three days resulted in greater whole-body creatine retention. However, higher doses of D-pinitol, showed no greater creatine retention than in the group given creatine monohydrate alone.100 Because of these discrepancies regarding the higher versus low doses of D-pinitol, further investigation is warranted before a clear conclusion can be drawn.101

**Cinnulin**

Cinnamon is also known to increase insulin signaling to cells.102 Cinnulin (a cinnamon extract) mimics the effects of insulin. In one study it was co-ingested with creatine monohydrate during a seven-week resistance training program. At the end of the study, results showed that the Cinnulin and creatine group, compared to creatine alone and placebo, elicited greater mean increases in 1-RM leg press, thigh lean mass, body water and total Akt protein content. However, between the creatine with Cinnulin and creatine alone group the intramuscular creatine increases showed no significant differences.103

**Biocreat**

Biocreat is a proprietary patent pending molecule of INDUSBIOTECH that is hypothesized to enhance creatine uptake. It comes from a unique molecule extracted from Fenugreek (Trigonella Foenun
greacum) seeds. This herb has been used to help with glucose and insulin stability\textsuperscript{104} and to help lower glucose in non-insulin dependent diabetics.\textsuperscript{105} as well as its effective use at increasing lactation.\textsuperscript{106} This herb appears in a number of products on the market, its safety has not been fully elucidated upon; however, to date no serious side effects have been reported. One study was tested biocreat against 5 grams creatine monohydrate with 75 grams of dextrose for eight weeks. Results showed no significant differences for muscular endurance on bench press or leg press or in any clinical safety data including lipid panel, liver function, kidney function, and/or CBC panel (p > 0.05). However at 4 weeks’ time biocreat showed a significantly (p ≤ .05) greater Wingate peak power. These results suggest that this derivative of fenugreek may increase total body mass and strength similar to creatine and carbohydrate ingestion.\textsuperscript{107}

**Minerals**

Because minerals are needed to catalyze various reactions, and are involved in creatine uptake or insulin activity, there needs to be an adequate amount. However, above that there is no research which shows a heightened effect. Some of these needed minerals are magnesium, chromium, calcium iron, zinc, cobalt, selenium, indium etc.\textsuperscript{108}

**Conclusions**

It has been demonstrated that creatine analogs specifically beta guanidinopropionic acid, does indeed up-regulate the creatine transporter; however, the mechanism by which this is accomplished is unfavorable (i.e. it depletes creatine in the muscles as well as the levels of ATP.) Therefore, due to these findings it is concluded that creatine analogs are not a good choice to include with creatine supplementation.

We have also seen that beta-glucan bars are an effective way to deliver creatine and decrease urinary losses as well as excessively high plasma levels. We have also observed that alpha lipoic acid helps to increase muscle creatine retention similar to what creatine and sucrose would do. This is a major benefit for those who are slight insulin resistant, or who have a caloric restriction.

The same can be said for D-pinitol and cinnulin, but to a smaller extent because of the discrepancies in the former, and the effectiveness of the later at increasing max strength. Because only few studies have been done on these so far, it is difficult determine their actual effect. It would also be important to do longer studies using these additives, to verify an ergogenic effect. However, these compounds at worst have been shown not to work, and at best that they do work. (They may be an individual variation as well.) Moreover there is no current reason why consuming these compounds concomitantly with creatine would decrease the effectiveness of creatine supplementation; therefore, its consumption is neither recommended nor discouraged.

Biocreat does appear to be effective according to the one study that was done. Caution should be taken with it, as the study was funded by the producers; however, it does appear from the literature to be more effective than not taking it, and is similar to when creatine is co-ingested with carbs. It may even be beneficial to take this with carbs and protein as mentioned previously. However, the long term effects of its supplementation are currently unknown and thus care should be taken during its usage.
Consuming creatine with minerals may only be helpful if there is a dietary inadequacy, which is normally not likely. These minerals are indeed needed for creatines uptake and retention, because many of them are needed in the pathways were creatine is used, but as to whether or not there supplementation would increase creatine uptake has not been proven.

**Recommendations**

- Creatine should not be consumed concomitantly with any of its analogous, specifically guanidinopropionate.
- Beta-Glucan bars may be an effective method to increase muscle creatine retention and decrease creatine loss out the urine
- Alpha lipoic acid co-ingested with creatine will help overall total creatine retention
- D-pinitol and cinnulin will possibly assist at increasing creatine accumulation in muscles
- Biocreat is also effective; however, caution should be taken with long term consumption
- Specific minerals may increase effectiveness, but normally only if there is an inadequacy. There is no reason to believe that they are a necessity

**Master list of recommendations**

- Optimal creatine loading is two to three days of 0.25 g/Kg of lean body mass per day
- Optimal maintenance consist of 0.0625 g/Kg of lean body mass per day
- Consume creatine with carbohydrates and protein
- Don’t be concerned about inhibiting creatine uptake by concomitantly consuming protein
- Ingest supplement as close to exercise as possible, preferably before if it can be tolerated
- Don’t use fructose for carbohydrates, maltodextrins and/or dextrose are good choices
- Before purchasing a certain type of creatine, ensure solubility is high
- Prior to consumption allow creatine to fully dissolve in solution
- Mix in water slightly above room temperature and drink immediately
- To maximize the amount of creatine in solution, mix the creatine in water, as opposed to mixing it with the work out drink or in milk
- Avoid lozenges and suspensions due to their diminished solubility and thus bioavailability
- Once creatine supplement is prepared, immediately consume it, avoid letting it sit for prolonged periods of time
- Disregard claims that creatine instantly converts into creatinine
- If companies make false claims, be wary of how much confidence you put in further claims
- Creatine HCl known as CON-CRET is a preferred choice over creatine monohydrate
- Creatine nitrate known as creatrate is a preferred choice over creatine monohydrate
- Micronized creatine is a preferred choice over non-micronized creatine.
- Kre-Alkalyn may be as good or better as monohydrate if consumed on an empty stomach
- Creatine ethyl ester should be completely avoided, along with any product that contains it
- Creatine anhydride should be dealt with similarly to creatine ethyl ester
• Don’t mix CreHCl or CreNitrate with protein and let it sit out for an extended period of time.
• Creatine should not be consumed concomitantly with any of its analogous, specifically guanidinopropionate.
• Beta-Glucan bars may be an effective method to increase muscle creatine retention and decrease creatine loss out the urine
• Alpha lipoic acid co-ingested with creatine will help overall total creatine retention
• D-pinitol and cinnulin will possibly assist at increasing creatine accumulation in muscles
• Biocreat is also effective; however, caution should be taken with long term consumption
• Specific minerals may increase effectiveness, but normally only if there is an inadequacy. There is no reason to believe that they are a necessity
• The following may be at least as effective as creatine monohydrate
  o Creatine taurinate
  o Creatine alpha-ketoglutarate
  o Creatine gluconate
  o Creatine malate
  o Dicreatine malate
  o Tricreatine malate
  o Creatine citrate
  o Dicreatine citrate
  o Tricreatine citrate
  o Creatine phosphate
  o Creatine anhydrous
  o Creatine pyroglutamate
  o Magnesium creatine chelate

References


90. Shoubridge EA, Radda GK. A 31P-nuclear magnetic resonance study of skeletal muscle metabolism in rats depleted of creatine with the analogue beta-guanidinopropionic acid. Biochim Biophys Acta. 1984 Sep 14;805(1):79–88