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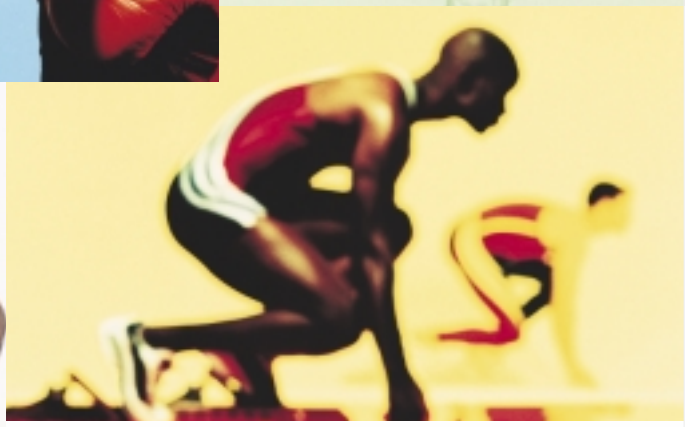
BioActives

The Use of ***Creatine Monohydrate*** *in Sports Nutrition*



CREAPURE

About Degussa BioActives

Degussa BioActives is specialized in Dietary Supplements, Functional Food and Special Nutrition. With our global network, superior quality and strong R&D activities, we are well prepared to meet the future challenges in the growing market of nature-based nutraceuticals and food ingredients.

We offer our customers best service through our sales offices in Europe, USA, and Asia. Our research centers are based in Freising/Germany, as well as in Waukesha/Wisconsin and Champaign/Illinois, USA.

Innovative Sports nutrition

As the market leader in Creatine products, we are intensifying our focus on sports nutrition. Building upon our strong scientific evidence for each of our products and brands, we will continue to invest in this growing industry and provide new and innovative systems for our customers.

Clinical studies, regulatory approval, and market support are among our key activities contributing to our customers' success.

This publication series will provide detailed information about our sports nutrition products and systems, such as:

➔ ***Creapure*** *(Creatine Monohydrate)*
the Power Supplement of the 90's

➔ ***Creapure Citrate*** *(Creatine Citrate)*
improved bioavailability, solubility and taste

➔ ***Creapure Purovate*** *(Creatine Pyruvate)*
the supplement for high intensity interval exercise

➔ ***Leci-PS*** *(Phosphatidylserine)*
for cortisol control

➔ ***Leci-Choline*** *(Phosphatidylcholine)*
giving endurance athletes a competetive edge

➔ ***Arthred*** *(Hydrolyzed Collagen)*
for healthy joints and to build muscle

➔ ***Alipure*** *(alpha-Lipoic Acid)*
the ideal, universal antioxidant involved in energy production

The use of Creatine Monoydrate in Sports Nutrition by Dr. Ralf Jäger

Degussa BioActives Publication Series on Sports Nutrition, © 2003, Freising, Germany

The history of Creatine

In 1832 the French scientist Chevreul discovered a new ingredient of meat to which he gave the name Creatine, according to the source from which it was extracted (Kreas: Greek for flesh). The German scientist Justus von Liebig confirmed that Creatine is a regular constituent of flesh. Creatine levels in wild animals were 10 times higher compared to captive animals suggesting that physical activity might have an influence on the amount of Creatine present in flesh. A meat extract (Liebigs Fleischextrakt) was the only source for Creatine supplementation over the next century.

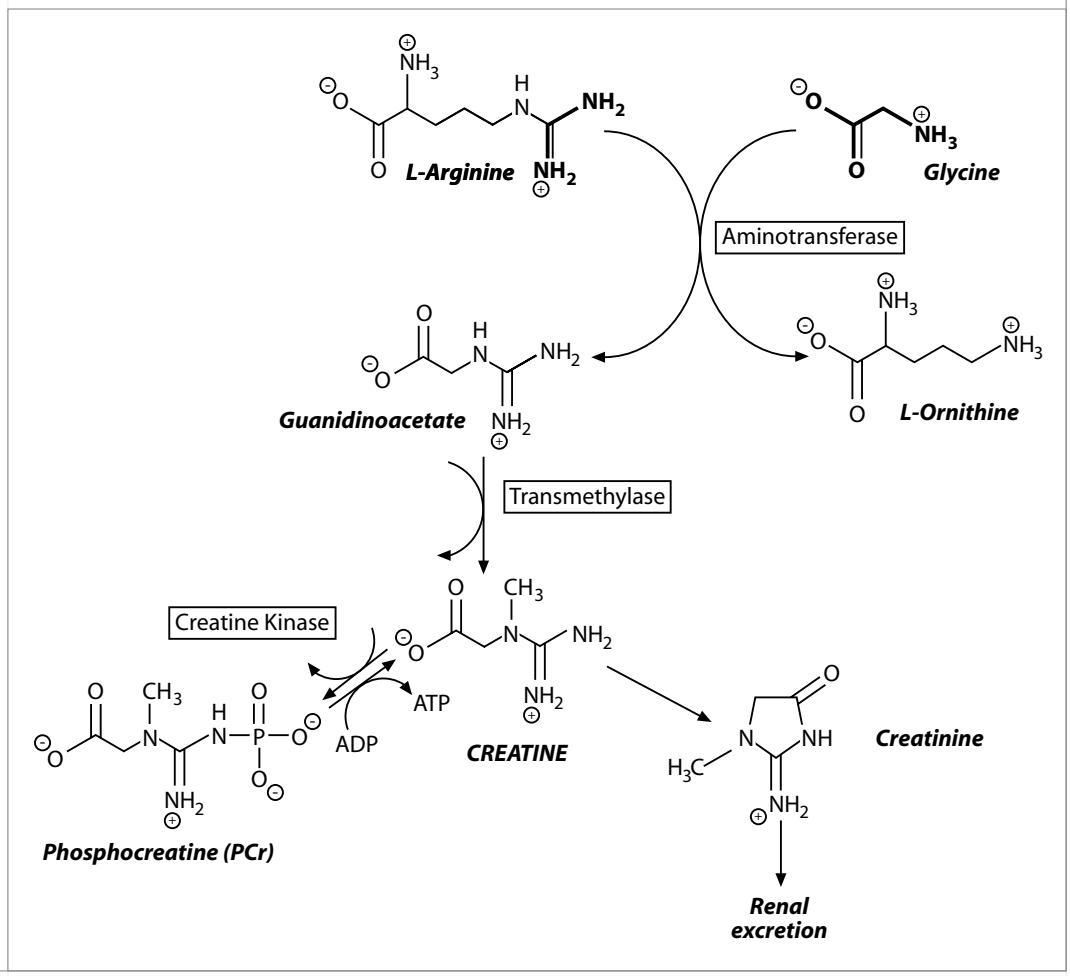
Anecdotal reports in the early 1990's suggested that Creatine supplementation might improve sport performance. British track and field 1992 Olympic champions Linford Christie (100 m dash) and Sally Gunnell (400 m hurdles) reportedly used Creatine, as did the Cambridge University rowing team in training for three months before defeating the heavily favored Oxford [16]. Numerous controlled clinical trials followed in the upcoming years proving the benefits of Creatine supplementation in different sports. Many celebrated professional athletes and Olympic champions acknowledge Creatine use and estimated 80 % of the athletes at the 1996 Summer Olympics in Atlanta used Creatine. Mark McGwire, one of major league baseball's greatest sluggers, used Creatine during the 1998 season and his legendary race to set the single season home run record, making Creatine the most popular sports nutrition in the US. Creatine supplementation has become a common practice among professional, elite, collegiate and amateur athletes to enhance exercise performance.

Today, Creatine is one of the best-studied supplements in the field of sports nutrition and its proven efficacy as an ergogenic substance was reviewed and accepted by numerous authorities [17].

Creatine sources and metabolism

Creatine [1] is naturally occurring in the human body and plays a major role in the bodies energy production. The total Creatine store in humans is approximately 120 g for the average-sized adult person (70 kg) [2] and the daily turnover rate of Creatine has been estimated to be about 1.6 % of the total Creatine pool (2 – 3 g per day) [3]. Body-own (endogenous) Creatine is synthesized from three different amino acids (Arginine, Glycine and Methionine) primarily in the liver and kidney. Creatine is transported via the bloodstream to various body tissues, including the heart, brain and testes; however, the vast majority (app. 95 %) is found in the skeletal muscle. Creatine is taken up from the blood against a large concentration

[Fig. 1] **Fig.1. Body-own Creatine synthesis and Creatine metabolism**



gradient by a sodium dependent Creatine transporter (CreaT) that spans the plasma membrane. Extracellular Creatine is sequestered into the cytosol, where rapid phosphorylation by the enzyme Creatine Kinase [4] takes place. About 60 – 70 % of the muscle total Creatine content is stored in the form of the high-energy molecule Phosphocreatine that is unable to pass trough membranes, thus trapping Creatine in the cell. Creatine is eventually being degraded to Creatinine in a spontaneous, non-enzymatic reaction and excreted by the kidneys.

Inborn errors of the human body's Creatine synthesis (guanidinoacetate methyltransferase (GAMT) deficiency [5] and arginine:glycine amidinotransferase (AGAT) deficiency) as well as defects of the Creatine transporters have been described. The resulting Creatine deficiency, especially in the brain and muscles, leads to serious physical and mental underdevelopment and even death. Creatine supplementation, however, can overcome this deficit and restore normal Creatine levels.

Only half of the daily Creatine requirement is synthesized by the body, the rest must be supplied from our daily diet. Dairy products contain only small quantities of Creatine (0.1 g / kg in milk), whereas high concentrations can be found in raw meat (e.g. beef and pork 5 g / kg) and fish (e.g. herring 10 g / kg) [2]. When meat or fish is cooked, the Creatine content decreases while the Creatinine content increases [6]. In strict vegetarians, or vegans, dietary Creatine intake is virtually zero and consequently Creatine blood plasma levels are lower [7]. The importance and safety of Creatine for the development of the human body is also reflected by the presence of Creatine in mother's milk [8].

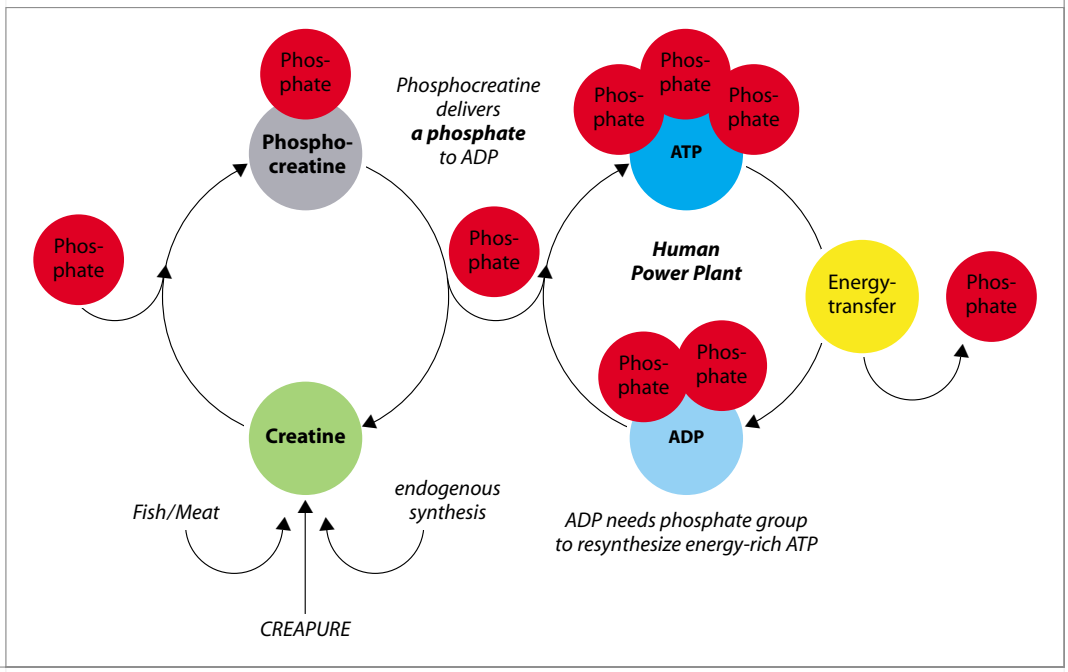
[Fig. 2] **Creatine content in different foods [g / kg]**

FISH			*MEAT*			*OTHERS*		
Herring	→	6.5 – 10	**Pork**	→	5	**Milk (mother's milk)**	→	0.1
Salmon	→	4.5	**Beef**	→	4.5	**Vegetables**	→	traces
Tuna	→	4				**Fruits**	→	traces
Cod	→	3				**Carbohydrates**	→	traces
Plaice	→	2						

All cells use ATP as the immediate energy source, but since ATP stores are limited they must be regenerated by metabolic pathways in order to sustain high power output. Energy is liberated, as the phosphate from ATP is enzymatically removed leaving adenosine diphosphate (ADP) and inorganic phosphate. Creatine and its phosphorylated form, Phosphocreatine, are essential to the regeneration of ATP from ADP. During times of high-energy demand the phosphate from the Phosphocreatine is cleaved off to provide energy for the re-synthesis of ATP. Phosphocreatine serves as a temporary energy buffer in time when ATP consumption exceeds synthesis. Muscles contain 3 – 4 times more Phosphocreatine than ATP, but supply is also limited. Phosphocreatine content and resynthesis are crucial factors during times of high-energy

demand such as sustained high intensity exercise. An increase in Creatine and Phosphocreatine content will increase the capability to resynthesis ATP under conditions of high-energy demand directly translating into more available energy.

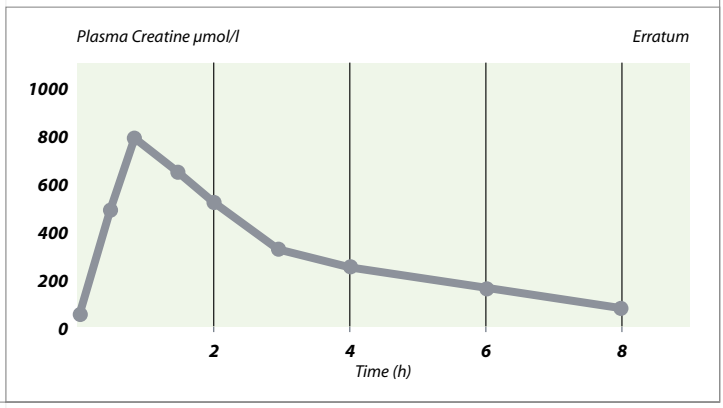
[Fig. 3] **Creatine is involved in the re-synthesis of the body's energy source ATP. Increasing Creatine levels results in increased Phosphocreatine levels and consequently in higher re-synthesis rate of the body's energy source ATP**



Creatine supplementation increases muscle Creatine content

Orally ingested Creatine is absorbed intact from the intestinal lumen and then enters the bloodstream. Low doses of Creatine (1 – 10 g) show a time of maximal plasma concentration of less than 2 hours. At doses above 10 g, time to reach maximum levels shift to more than 3 hours [1].

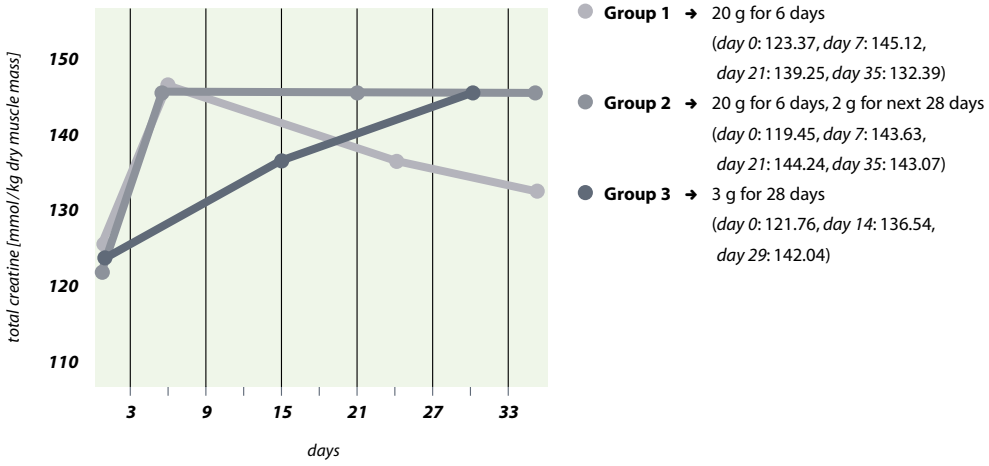
[Fig. 4] **Bioavailability of 5 g Creapure [Creatine Monohydrate]**



One time oral Creatine Monohydrate supplementation (5 g Creapure, Degussa BioActives, dissolved in 450 ml of water) results in a significant increase of Creatine blood plasma levels. Creatinine levels do not increase [9].

Short-term Creatine supplementation of 15 to 25 g for 5 days increases the total muscle Creatine content by 15 – 30 % and Phosphocreatine stores by 10 – 40 % (analyzed by muscle biopsy). After 5 days loading, 2 – 5 g as maintenance dose is sufficient to keep Creatine levels elevated. Without maintenance dose Creatine levels return to baseline after 4 to 5 weeks. Similar increases in Creatine levels can be achieved using low-dose long-term supplementation of 3 g per day for 28 days [10]. However, there seems to be an upper limit of muscular Creatine content (~ 160 mmol per kg of dry muscle mass). Continued use of high amounts of Creatine (15 g or higher) does not increase the Creatine content in the muscle further and is therefore unnecessary.

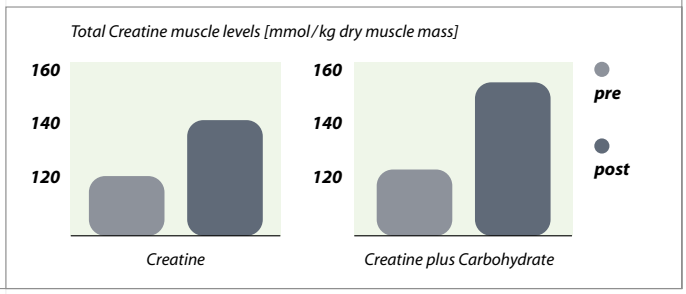
[Fig. 5] **Creatine supplementation increases Creatine muscle content**



Creatine levels significantly increase after Creatine loading (groups 1 and 2). Without maintenance phase Creatine levels drop (group 2). Low-dose long-term supplementation (group 3) results in same increase as short-term high-dose loading.

Creatine retention can be augmented when Creatine is consumed along with simple carbohydrates, such as Glucose, compared to Creatine alone. This increased Creatine absorption has been attributed to insulin mediated stimulation of the Creatine transporter [11]. However, large amounts of carbohydrate (~100 g) or combinations of carbohydrate (47 g) and Protein (50 g) [12] are necessary to significantly improve Creatine uptake. These large doses of carbohydrate are difficult to palate and potentially hazardous to diabetics or those with glucose intolerance. A recent study suggests that adding alpha-Lipoic Acid can reduce the amount of Carbohydrates needed. 25 g Dextrose and alpha-Lipoic Acid (250 mg) resulted in greater muscle Creatine accumulation than Creatine intake alone, or Creatine plus 25 g Dextrose [13].

[Fig. 6] **Insulin stimulation increases Creatine retention**



Addition of large amounts of simple Carbohydrates increases Creatine retention: 4 x 5 g Creatine for 6 days (total Creatine +17 %) vs. 4 x 5 g Creatine plus 90 g of Glucose for 6 days (total Creatine +27 %).

Exercise has also shown to have stimulatory effects on Creatine uptake. Supplementation of 5 g Creatine Monohydrate 4 times daily for 3 – 5 days followed by one-legged cycle ergometry resulted in an increase of total Creatine muscle levels of 37 % in the exercised leg, but only 25 % increase in the control leg [14]. The increase uptake results most likely from an enhanced blood flow or an increase of the translocation of the Creatine transporter to the muscle membrane. Augmented Creatine retention due to carbohydrate ingestion can not be further increased when exercise was performed prior to ingestion [15].

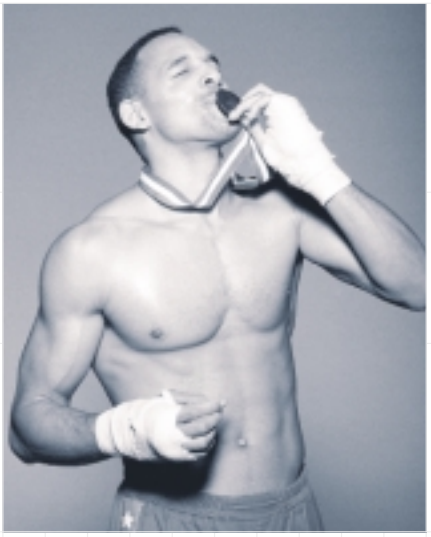
Creatine supplementation has also been reported to increase Creatine content in the brain. A recently published study shows that Creatine supplementation improves mental performance by reducing mental fatigue [44].

Increase in Creatine muscle content increases performance

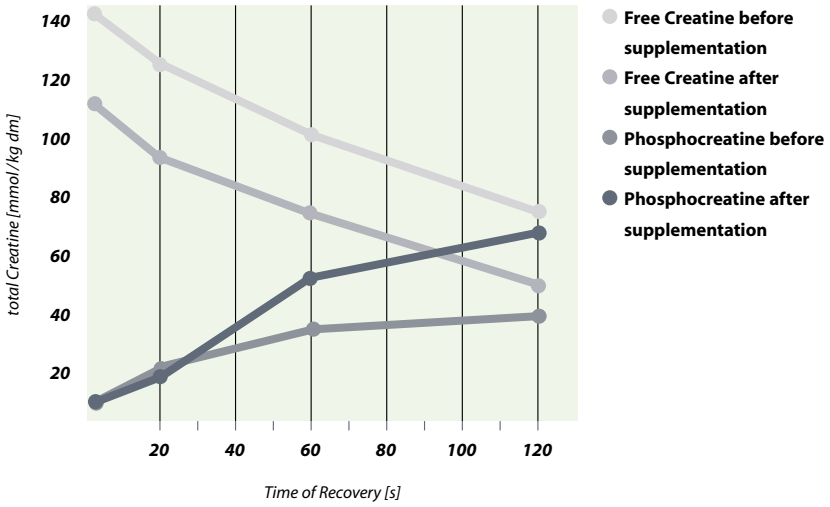
The effects of Creatine supplementation were studied in people with different training background and athletic abilities, from competitive college athletes to relatively untrained beginners. Creatine supplementation significantly increases performance in a variety of sports such as sprinting, repeated jumping, swimming, kayaking / rowing, resistance exercise or cycling. Short-term Creatine supplementation improves, for example, maximal strength / power (5 – 15 %), work performed during sets of maximum efforts (5 – 20 %), power production during short sprints (app. 30 %) and work performed during repetitive sprints (5 – 15 %) [16]. Creatine supplementation is common amongst bodybuilders, power lifters, wrestlers, rowers, cyclists, mountain bikers, tennis players, skiers, and American football, soccer, rugby, basketball, ice hockey, volleyball and handball teams as well as track and field athletes (sprinter, shot put, javelin, discus). Besides Caffeine, Creatine is the only sports nutrition product having scientifically proven ergogenic (performance enhancing) effects [17].

Different mechanisms are involved in the ergogenic effects of Creatine supplementation: (1) higher Phosphocreatine concentrations serving as an immediate buffer to ATP during exercise, (2) increased Phosphocreatine resynthesis rate during and after exercise due to increased levels of free Creatine, (3) smaller decrease in muscle pH during exercise, (4) enhanced training load and (5) increasing muscle mass (absolute power output).

Short-term Creatine supplementation (20 g per day for 5 days) increased the Phosphocreatine resynthesis rate after recovery from intense exercise (mechanism 2) [18]. Phosphocreatine resynthesis during recovery is critical to the restoration of muscle power at the onset of next bout of maximal exercise. An increased resynthesis rate allows increased training units, mainly in explosive sports disciplines.



[Fig. 7] **Creatine supplementation increases the resynthesis rate of Phosphocreatine**



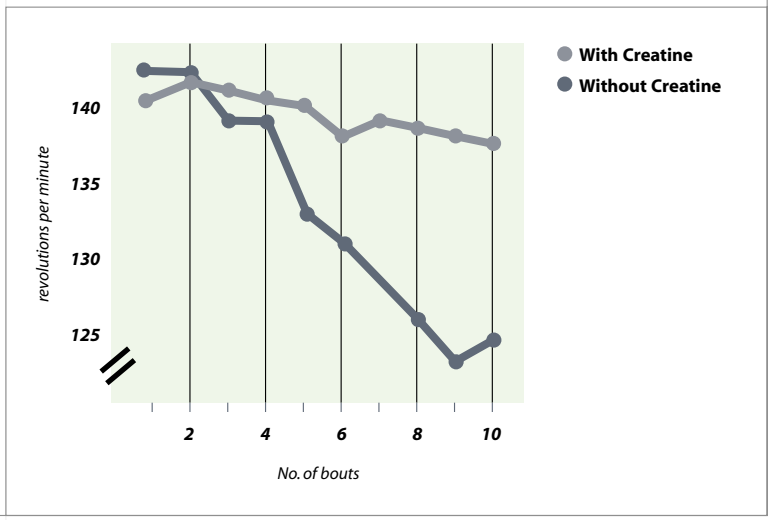
5 days of Creatine supplementation (20 g per day) increases Phosphocreatine resynthesis rate during recovery from intense, electrically evoked isometric contraction. Free Creatine concentrations decrease, while Phosphocreatine concentrations increase, measured in muscle biopsy samples obtained after 0, 20, 60 and 120 seconds of recovery.

During times of high-intensity exercise ATP stores last only 1 – 2 seconds and Phosphocreatine is instantaneously available for ATP regeneration. Phosphocreatine stores, however, last approximately for 10 seconds. Increasing Phosphocreatine levels in the muscle results in a delay of Phosphocreatine depletion and therefore favorably affects muscle performance (mechanism 1). More than 20 clinical trials have shown that Creatine supplementation significantly improves the ability to produce higher muscular force and / or power output during short bouts of maximal exercise.

The greatest improvements in performance can be found during series of repetitive high-power output efforts, separated by a fairly brief period of rest (e.g. 20 – 60 seconds). The rest is sufficient to permit enhanced recovery of Phosphocreatine concentrations (combination of mechanism 1 and 2).

Athletes supplemented with 20 g Creatine Monohydrate per day for 6 days or placebo, had to perform ten 6 second bouts of high intensity cycling exercise, separated by 30 second recovery periods. Athletes were requested to maintain a target speed of 140 revolutions per minute. The resistance on the cycle was determined so that the athletes were unable to maintain the target speed for more than 4 to 6 bouts. No differences were observed between the 2 groups prior to supplementation, however, short-term Creatine supplementation significantly increased performance [19].

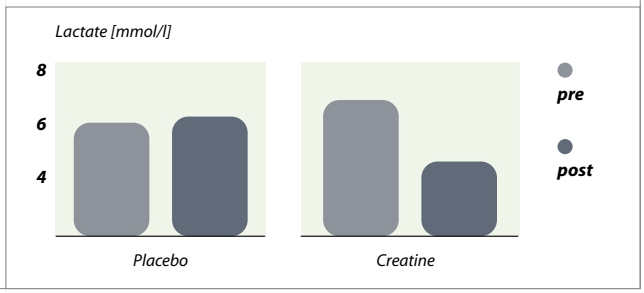
[Fig. 8] **Creatine supplementation significantly improves performance in high-intensity exercise**



Monohydrate for 6 days significantly improved performance during cycling exercise. The resistance was determined so that each athlete was unable to maintain the target speed after 4 to 6 exercise bouts.

Phosphocreatine consumes a hydrogen ion in the process of resynthesizing ATP from ADP, accounting for approximately 30 % of the total muscle buffer capacity. Increasing Phosphocreatine levels through Creatine supplementation increases the buffer capacity and may increase performance by delaying the decrease in muscle pH in short-term high-intensity exercise. In addition, some studies showed that Creatine supplementation results in lower lactate accumulation in the muscle during exercise.

[Fig. 9] **Creatine supplementation results in lower lactate values**



Creatine supplementation results in lower lactate accumulation, although the same amount of work had been performed [19]. In an additional study, muscle lactate accumulation was 70 % lower after Creatine supplementation after 5 standardized 6 second bouts of high intensity exercise [20].

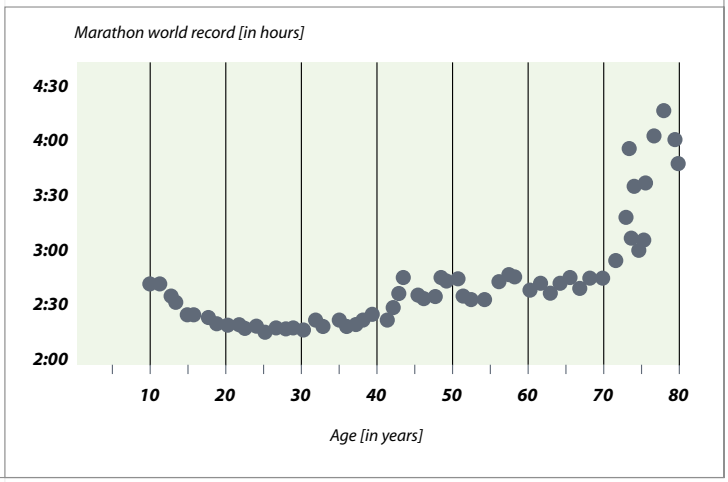
Oxidants such as free radicals can affect muscle fatigue and protein turnover. Creatine acts as a direct antioxidant against radical and reactive species [45].

Creatine significantly improves performance in the mature population

With aging there are decreases in muscle mass, strength and exercise performance. The overall loss of strength during an adult lifespan is typically 30 – 40 % and the reduction of muscle mass between ages 25 to 65 is typically 25 – 35 % [21]. In addition, this loss in strength and power negatively influences daily living such as rising from a chair [22] or climbing stairs. Reduced levels of Phosphocreatine and total Creatine in the skeletal muscle reported in the elderly [23, 24] may be in part responsible for these declines. In addition, re-synthesis rates of Phosphocreatine after exercise decline with age by ~8 % every 10 years after 30 years of age [25].

Short-term (5 to 7 days) [26, 27] and long-term (4 months) [24, 28] placebo-controlled clinical trials proved the efficacy of Creatine supplementation in this age group. Smith et al. (1998) studied the age influence on muscle metabolism during exercise in a placebo-controlled clinical trial [26]. 5 young (31 ± 5.2 years) and 4 elderly (58 ± 4.5 years) men and women took either 0.3 g Creatine Monohydrate per kg body weight (21 g for a 70 kg person) per day for 5 days or placebo. The elderly group had significantly lower Phosphocreatine levels and a lower Phosphocreatine re-synthesis rate during placebo administration. After taking Creatine Monohydrate for only 5 days the Phosphocreatine levels as well as the Phosphocreatine re-synthesis rate increased in the elderly group to a level not different from the young group.

[Fig. 10] **Performance decreases with age**

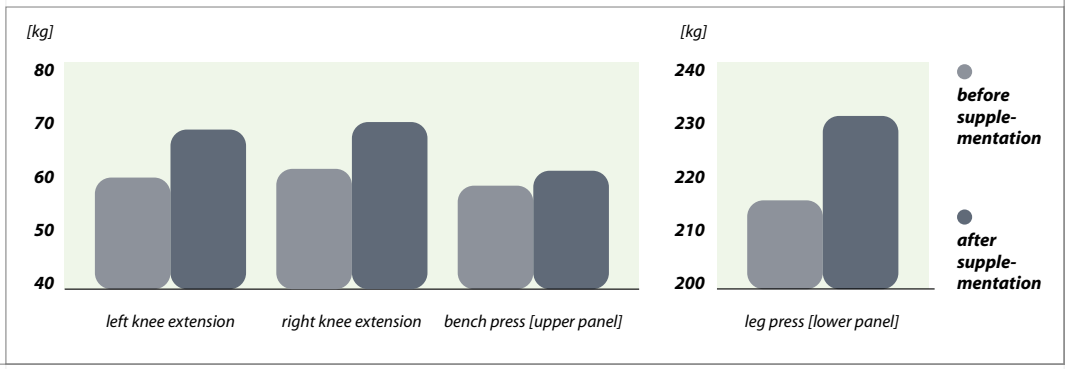


Age-dependent marathon world records (men) shows that performance is closley linked to age. Absolute world records can only be achievedat age 25 – 30 [43].

Performance during a single-leg knee-extension exercise, measured by time to exhaustion, was increased in both groups combined after Creatine supplementation. Gotshalk et al. (2002) supplemented 18 normally active older men (59 – 72 years) either with 0.3 g Creatine Monohydrate per kg body weight (21 g for a 70 kg person) or placebo [27]. Creatine supplementation significantly increased body mass, fat-free body mass, lower-body mean power and lower-extremity functional capacity. Creatine supplementation significant increased (7 – 15 %) maximal dynamic strength of large muscles of the hip/knee extensors (leg press) and chest and shoulders (bench press) as well as maximal isometric strength of smaller muscles of the quadriceps (knee extensions) and hamstrings (knee flexion).

Lower-body motor functional performance significantly increased with Creatine supplementation. Rapid and repetitive transference from a seated to a standing position without the use of upper extremities (sit-stand test) and rapid ambulation along a line by using tandem foot placement (tandem gait test) significantly improved as indicated by 6 – 9 % reduction in time to complete those tests.

[Fig. 11] **Creatine supplementation significantly increases strength in the elderly**



Short-term Creatine supplementation significantly improves maximal dynamic strength (bench (4.1 ± 1.4 kg) and leg press (16.1 ± 4.4 kg)) and maximal isomectric strength (left and right knee extension) in men average age 65.4 ± 1.5 years [27].

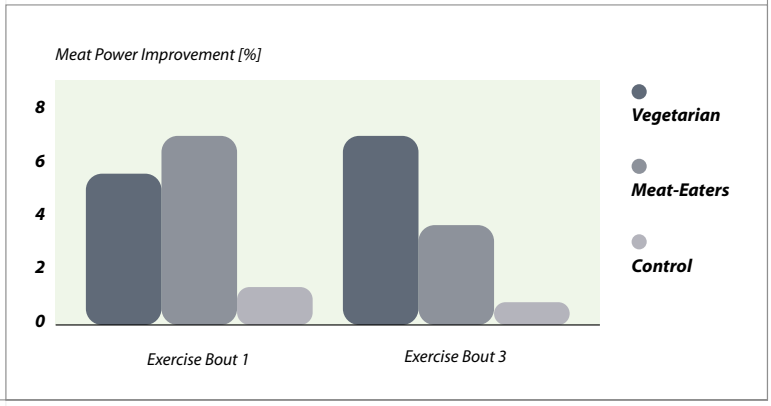
Tarnopolsky et al. (2001) studied in a double-blind, placebo-controlled clinical trial in the elderly the effects of long-term Creatine supplementation on the Phosphocreatine and total Creatine levels, body composition and strength [24]. 14 elderly men (67.8 ± 4.0 years) and 14 women (69.3 ± 6.3 years) took either 5 g of Creatine Monohydrate or placebo during a 4 month supervised strength-training program. Creatine supplementation resulted in a significant increase in muscle Phosphocreatine and total Creatine, fat-free mass and isometric knee extension strength. Similar results could be achieved by the loading phase / maintenance phase supplementation program by Chrusch et al. (2001) in a similar age group (70.4 ± 1.6 years). 4 months of Creatine supplementation resulted in a significant increase in muscular strength (leg press) and endurance (leg press and knee extension) [28].

Creatine supplementation is able to restore Phosphocreatine and total Creatine levels and is able to speed up the re-synthesis rate of Phosphocreatine in the muscle of elderly people to a level not different from young people. Creatine supplementation significantly increased body mass, fat-free body mass, maximal dynamic strength, maximal isometric strength as well as lower-body mean power and lower-extremity functional capacity.

Creatine significantly improves performance in vegetarians

Blood Creatine levels are significant lower (40 – 50 %) in vegetarians since half of our daily Creatine supply is ingested through meat and fish [7, 29]. In a study, investigating the effects of Creatine supplementation, Vegetarians and Meat-Eaters received 21 g Creatine Monohydrate plus 15 g of Glucose for 6 days and exercise performance was tested against a Meat-Eater group only receiving Glucose (15 g daily). Athletes had to perform three 20 second bouts of maximal cycling exercise with 4 min recovery between bouts. Short-term Creatine supplementation results in a significant increase in exercise performance in Vegetarians and Meat-Eaters [29].

[Fig. 12] **Creatine improves performance in vegetarians**



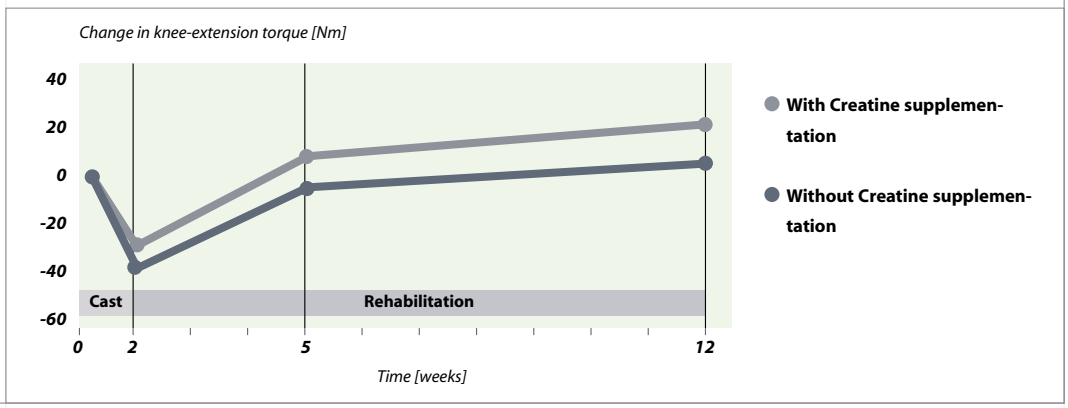
Vegetarians and Meat-Eaters were supplemented with 21 g Creatine Monohydrate and 15 g Glucose. A Meat-Eater group receiving only 15 g of Glucose served as a control.
Vegetarians
bout 1: 5.3 %, bout 3: 6.8 %
Meat-Eaters
bout 1: 6.6 %, bout 3: 3.9 %
Meat-Eaters (Control)
bout 1: 0.8 %, bout 2: 0.5 %

Creatine speeds up recovery after injury

Muscle disuse leads to muscle atrophy (decreased muscle volume), reduced muscle force, reduced muscle energy stores and reduced maximal power. The most prominent condition of muscle disuse is immobilization, for example when a broken leg or an arm is immobilized in a cast for some period of time, or if an athlete has to stop training and competing due to an injury. The muscle atrophy is usually clearly visible by a slimming of the leg or arm. Increased mechanical loading of the muscle either by muscle rehabilitation training or by resumption of a normal level of physical activity reverses the muscle atrophy with time. Creatine's important role in energy metabolism of the muscle raised the question whether Creatine supplementation can reduce the effects on the muscle during immobilization and whether Creatine supplementation can speed up recovery.

Hespel et al. (2001) studied the effects of Creatine supplementation during muscle disuse and subsequent training in a double-blind placebo-controlled trial [30, 31]. The right leg of 22 healthy young volunteers was immobilized using a cast for 2 weeks after which the subjects participated in a 10-week rehabilitation program (heavy resistance training). During immobilization, muscle Phosphocreatine concentration decreased by 15 % in the placebo group and returned to baseline within the first 3 weeks of rehabilitation. In contrast Creatine supplementation negated a decrease of Phosphocreatine and offsets the decline in muscle protein content during immobilization and led to a increase of 12 % Phosphocreatine after the first 3 weeks of rehabilitation. Muscle strength, measured by maximal isometric knee-extension torque, decreased in both groups to the same degree during immobilization, but power output increased at a significant faster rate in the Creatine group during rehabilitation. Power output at the end of rehabilitation compared to baseline was significantly increased in the Creatine group compared to the placebo group.

[Fig. 13] **Creatine supplementation significantly speeds up recovery**



Creatine supplementation significantly speeds up recovery after injury and can shorten the duration of rehabilitation following muscle disuse.

It can be concluded that oral Creatine intake reduces the biochemical and structural deterioration of skeletal muscle during disuse. In addition, Creatine supplementation can shorten the duration of muscle rehabilitation following disuse atrophy. Clinical trials proved that Creatine supplementation in combination with appropriate training decreases recovery time of muscle disuse atrophy due to any cause.

Common dosages and form of administration for athletes

A high-dose loading phase of 20 g (4 times 5 g) Creatine Monohydrate per day for 5 days followed by a maintenance phase of 2 to 5 g (once daily) per day is common for athletes. Based on body weight, 0.3 g Creatine Monohydrate per kg body mass per day as a loading phase and 0.03 g Creatine Monohydrate per kg body mass per day as a maintenance phase are suggested. Alternatively a low-dose long-term usage will result in the same muscle Creatine levels (3 g for 28 days) [32]. Taking larger amounts of Creatine for more than 5 days does not result in significantly higher Creatine levels and is therefore unnecessary.

Dissolve Creatine completely in liquids such as water, fruit juice or tea. 5 g can easily be dissolved in 500 ml of water at room temperature or in a cup (app. 200 ml) of hot tea. Please prepare beverages fresh and consume within the same day (cf. solubility and stability of Creatine in water).

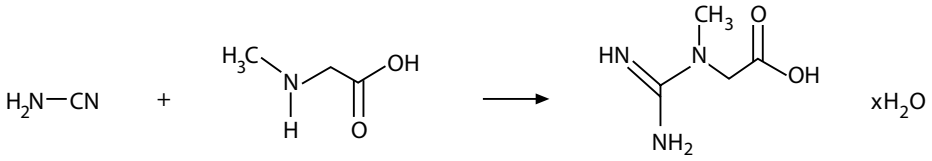
Experts suggest cycling Creatine approximately every 3 months with a 4 weeks rest period. Try to avoid taking high amounts of Caffeine with Creatine. Simultaneous supplementation of large amounts of Caffeine (5 mg per kg body weight per day) eliminates the ergogenic effects of Creatine. Caffeine does not reduce the Creatine uptake by the muscle, however, it interferes with the resynthesis of Phosphocreatine. [33] Lower amounts of Caffeine (e.g. 1 – 2 cups of Coffee) seem not to influence the efficacy of Creatine.

Safety – A question of Quality

Creatine Monohydrate production and potential impurities

Creatine was discovered in the first half of the 19th century as a component of beef tea and for quite some time Liebigs Fleischextrakt (a meat extract) was the only source for Creatine. In the 1990's chemical synthesis became more efficient and two methods starting from different raw materials have been established in the large-scale industrial production. Degussa developed and patented the so-called "cyanamide" route (cf. US patent 5,719,319) guaranteeing highest quality, purity and safety [34].

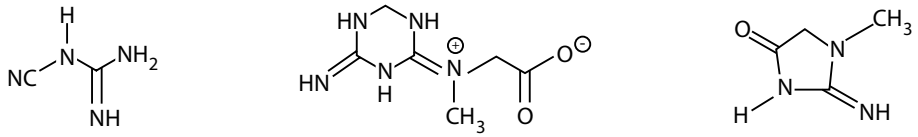
[Fig. 14] **Chemical synthesis of Creapure (Creatine Monohydrate), patent protected by Degussa**



Cyanamide ***Sarcosine*** ***Creatine Monohydrate***

The reaction conditions as well as the treatment of the crude Creatine Monohydrate are crucial for the quality of the product. Cheaper production costs can be achieved using inferior starting materials or reducing the amount of water used to wash the product ("recrystallization"). This results in increasing amounts of impurities such as Dicyandiamide (dimerization product of Cyanamide), Creatinine (Cyclization product of Creatine) and Dihydrotriazines, therefore potentially reducing the safety of the product.

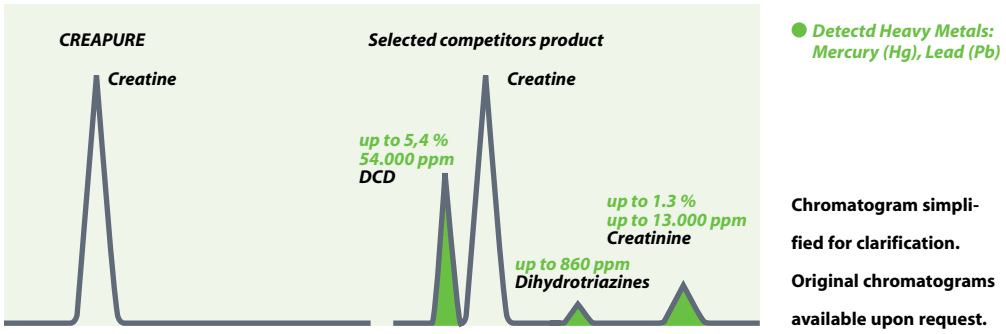
[Fig. 15] **Potential Impurities in inferior Creatine products**



Dicyandiamide ***Dihydrotriazine*** ***Creatinine***

Modern analytical methods can detect impurities in Creatine products in the parts per million (ppm) range. HPLC analytics can quantify impurities at 13 ppm for Dicyandiamide (DCD), 67 ppm for Creatinine and 15 ppm for Dihydrotriazine. Amounts as low as 4 ppm DCD, 20 ppm Creatinine and 4.5 ppm Dihydrotriazine are detectable [35]. Publications on available products on the market showed huge differences in the quality of Creatine products of different manufactures and of end-consumer products [36, 37]. No impurities could be detected in products from the German manufacturer (Degussa, formally SKW, is the only German producer). In certain products, single impurities of more than 5 % could be detected, raising questions on the short and especially long-term safety of such products.

[Fig. 16] **HPLC analytics of different Creatine samples**



Alternative production methods using thiourea as starting material is adding additional health concerns. Worldwide authorities warn about potential health risks resulting from the presence impurities in Dietary Supplements and in Creatine Monohydrate products (e.g. Report of the Scientific Committee on Food (SCF), Opinion on safety aspects of Creatine supplementation (adopted by the SCF on 7 September 2000)) [38].

Degussa's Creapure has the strictest specification on potential impurities in Creatine Monohydrate. Dihydrotriazine, potentially the most harmful impurity, is not detectable in Creapure at all.

All raw materials used in the production of Creapure are subject to in-house specification procedures. Each produced batch is tested for potential impurities such as cyanamide, dicyandiamide and dihydrotriazine. A quality control procedure independent of production and certified under GLP (Good Laboratory Practice) regulations is used to test the lots produced and monitor release of the product for dispatch.

Health risks of potential impurities

Dihydrotriazines

Dihydrotriazine is a compound with unknown pharmaceutical and toxicological properties and may be the most dangerous impurity in Creatine Monohydrate. Dihydrotriazines are unknown to the human body and should not be detectable in Dietary Supplements at all. Structurally related compounds are known to be carcinogenic.

Creapure Fact:
Dihydrotriazines are not detectable in Degussa's Creatine Monohydrate (Creapure).

Dicyandiamide

From human experience it is known, that Dicyandiamide can cause transitory mechanical eye irritation. In addition, a confirmed case of allergic contact dermatitis to Dicyandiamide is reported in literature [39]. Several publications speculate about the formation of highly toxic hydrocyanic acid (HCN) or cyanide out of Dicyandiamide. In the human body DCD might release highly toxic cyanide by pyrolysis. For this case the acceptable daily intake (ADI) for DCD is maximum 76 ppm (based on the ADI for hydrocyanic acid).

Creapure Fact:
Degussa's Creatine Monohydrate (Creapure) contains typically less than 13 ppm of DCD (less than the analytical limit [35]). This amount of DCD is not considered to be a health risk.

Creatinine

The human body converts naturally Creatine into Creatinine. The kidneys excrete Creatinine and Creatinine levels are used as a marker for the correct function of the kidneys. Creatinine ingestion should be as low as possible to avoid potential kidney problems.

Creapure Fact:
Degussa's Creatine Monohydrate (Creapure) typically contains less than 67 ppm Creatinine (less than the analytical limit [35]). This amount of Creatinine is not considered to be a health risk.

Thiourea

Thiourea is harmful if swallowed and has a possible risk of harm to the unborn child. The compound produces thyroid carcinomas in animals. Tumours are also reported occuring in the region of eyes, ears and nose as well as in the liver. In vitro studies with mammalian cells revealed a weak genotoxic effect. Based on these findings, Thiourea is classified as a suspected carcinogen, i. e. as a substance which causes concern for man owing to possible carcinogenic effects. Due to this toxicological profile the use of Thiourea and several derivatives are completely banned in different areas. It is for example mentioned in the list of substances which cosmetics products must not contain.

Creapure Fact:
Degussa's Creatine Monohydrate (Creapure) is synthesized by the patented "cyanamide" route. Therefore, thiourea and other dangerous derivatives such as methyl mercaptan **can not** be present in Creapure.

Toxicity of Creapure and long-term safety

Degussa BioActives sponsored numerous animal and human trials to prove the safety of Creapure supplementation. Besides the classic toxicology, Creapure was tested in more than 50 clinical trials.

People of all ages (from several months up to age 70+) in sports and non-sports applications, over periods of more than 21 months used Creapure without any unwanted side effects. Due to different impurity profiles of different Creatine manufactures, only Creapure has such a proven safety profile.

Classic Toxicology of Creapure

Creapure has intensively been investigated and no toxic effects could be detected in any of those tests (cf. table 2). All studies were based on international accepted guidelines (FDA and EU) under GLP (Good Laboratory Practice) conditions, using Creapure (Creatine Monohydrate, Degussa BioActives, Germany) having the following specification: Creatine Monohydrate: 99.99 % (HPLC), Dihydrotriazine: not detectable (HPLC), Creatinine < 67 ppm (HPLC, limit of quantification), Dicyandiamide < 13 ppm (HPLC, limit of quantification).

[Fig. 17] **Classic toxicity data of Creapure [40]**

1. Acute Toxicity		
1.1. Acute oral toxicity	→	LD50 rat: > 2000 mg/kg
1.2. Acute intraperitoneal toxicity	→	LD50 mouse: > 2000 mg/kg
2. Local Tolerance		
2.1. Skin irritation	→	rabbit: No skin irritation
2.2. Eye irritation	→	rabbit: No eye irritation
2.3. Sensitization	→	guinea pig: not sensitizing
3. Mutagenicity	→	Not mutagenic in the Ames test
4. Subactue Toxicity	→	rat: repeated dose (28 days) toxicity (oral) No treatment-related findings noted, No Observed Adverse Effect Level (NOAEL) > 2000 mg/kg bw/day

Long-term safety of Creapure

Numerous papers have been published over the last years evaluating the long-term clinical safety of oral Creatine Monohydrate supplementation using CREAPURE (Degussa BioActives, Germany). The main focus was to evaluate if long-term CREAPURE supplementation (average of 5 grams / day) might increase incidence of musculoskeletal injury, heat-related disorders (e.g. dehydration and cramping) and renal stress.

Clinical assessments included evaluating a comprehensive panel of serum and whole blood markers (electrolytes, muscle and liver enzymes, substrates, lipid profiles, red and white cells, etc.), renal function tests determined by Creatinine clearance, monitoring of injuries treated by the medical / athletic training staff, as well as collecting medical safety and fatigue inventories.

Results of these safety studies have consistently shown that in comparison to athletes who did not take Creatine, athletes who took Creatine did not experience greater incidence of injuries, heat related disorders (dehydration), cramping, musculoskeletal injuries, or gastrointestinal disturbances. Additionally, subjects taking Creatine did not experience significantly higher muscle and liver enzyme efflux, altered electrolytes, or increased renal stress determined by Creatine clearance [41].

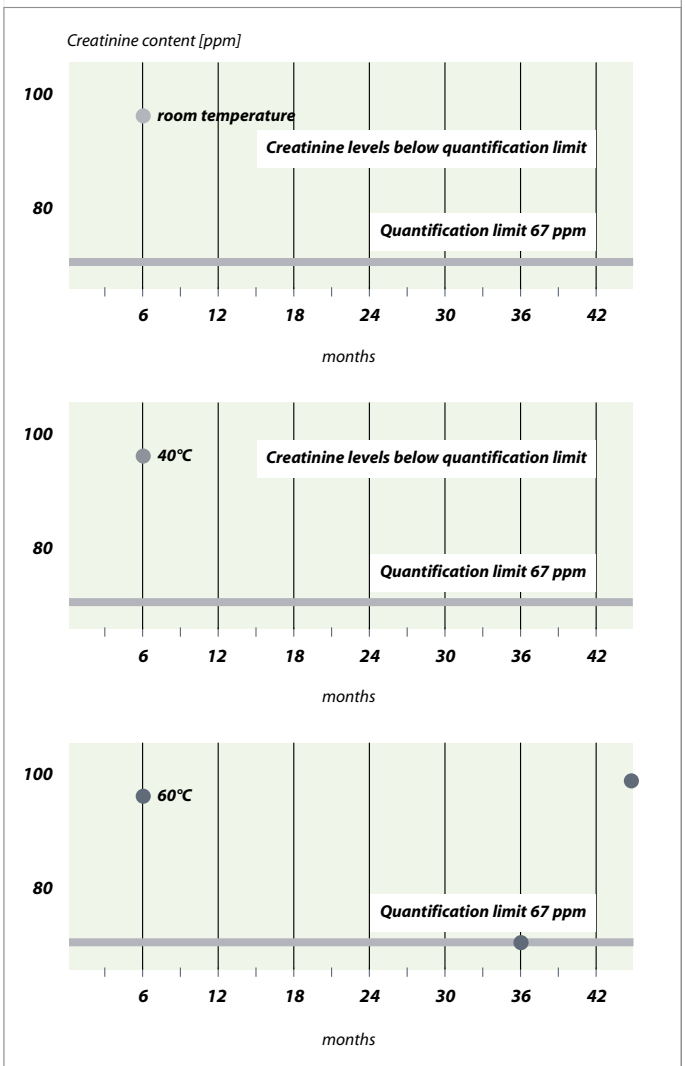
Persons suffering from renal insufficiency should consult their doctor prior to taking Creatine.

Technical data

Shelf-Life

The shelf-life of CREAPURE is 36 months from the date of manufacture, in the original unopened container, under the suggested storage conditions. CREAPURE should be stored at dry and cool to room temperature, in the original unopened container avoiding direct sunlight.

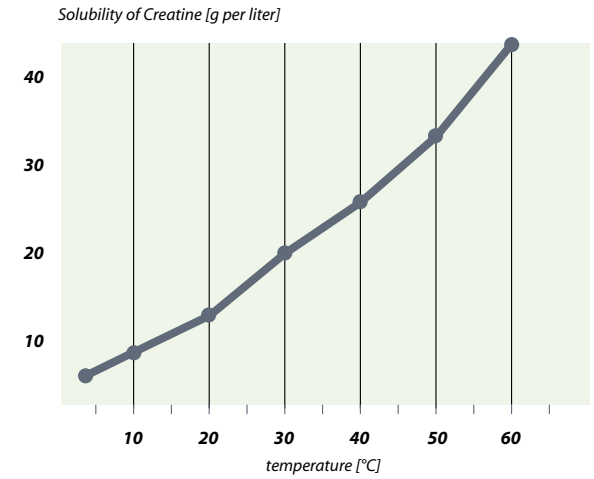
[Fig. 18] **Shelf-life testing of Creapure**



The shelf-life of Creapure was determined by measuring the amount of the degradation product Creatinine with time. Creapure, stored under the suggested storage conditions, is stable for at least 45 months (Creatinine levels stay under the quantification limit of 67 ppm). Storage at increased temperatures of 40°C (104°F) showed no increase in Creatinine levels after 44 months. Even storage at 60°C (140°F) resulted only in minimal amounts of Creatinine (106 ppm) after a period of 44 months.

Solubility in water

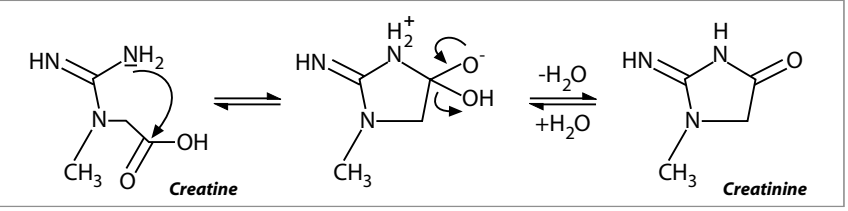
[Fig. 19] **Creatine solubilty in water increases with temperature**



Creatine solubility increases with an increase in temperature (6 g at 4°C, 9 g at 10°C, 14 g at 20°C, 20 g at 30°C, 26 g at 40°C, 34 g at 50°C, 45 g at 60°C)

Stability in aqueous solutions

[Fig. 20] **Creatine is not stable in aqueous solution due to an intramolecular cyclisation to Creatinine:**

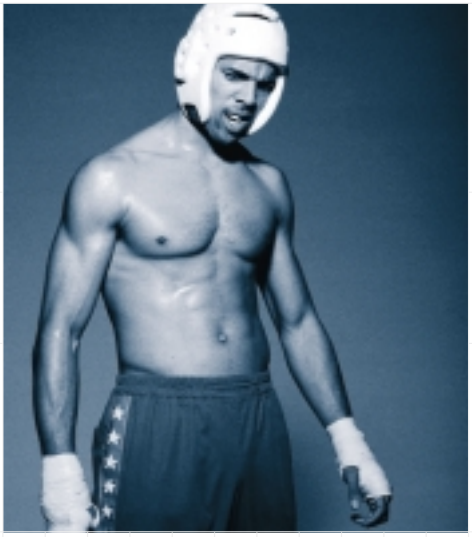


The speed of degradation is:

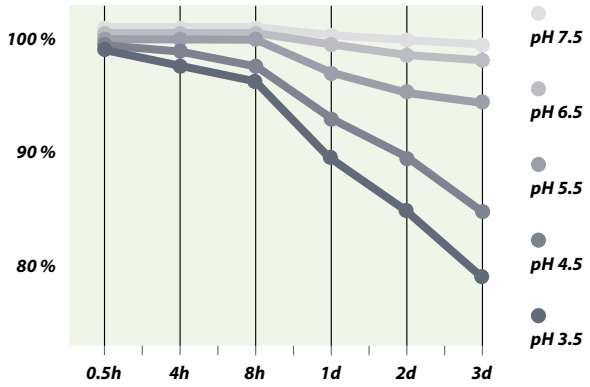
➜ not dependant on the concentration

➜ dependant on the pH (the lower the pH the faster the degradation)

➜ dependant on the temperature (the higher the temperature the faster the degradation)



[Fig. 21]

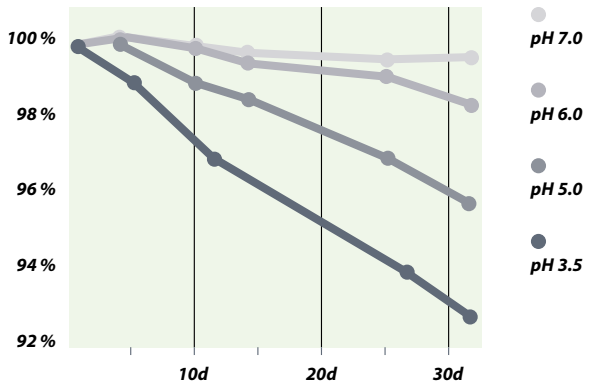


Degradation of Creatine at 25°C, after 0.5h, 4h, 8h, 1d, 2d, 3d at different pH values (7.5, 6.5, 5.5, 4.5, 3.5). The pH of the samples was adjusted to the desired values using 50 % acetic acid or 5N KOH. The pH of the samples was also tested to ensure that pH did not alter during the experiment [42].

Creatine in aqueous solution is reasonably stable for up to 8h at 25°C, pH 7.5 or 6.5. Breakdown after 3 days at pH 5.5, 4.5 and 3.5 was 4 %, 12 % and 21 % respectively.

If Creatine is not used immediately after it is dissolved in water it should be stored at a low temperature to slow down the degradation. The solubility of Creatine Monohydrate is 14 g per liter at 25°C (1.4 %) and 8.5 g at 4°C.

[Fig. 22]



Degradation of Creatine at 4°C, after 2d, 7d, 14d, 28d, 35d at different pH values (7.0, 6.0, 5.0, 3.5). The pH of the samples was also tested to ensure that pH did not alter during the course of experiment [42].

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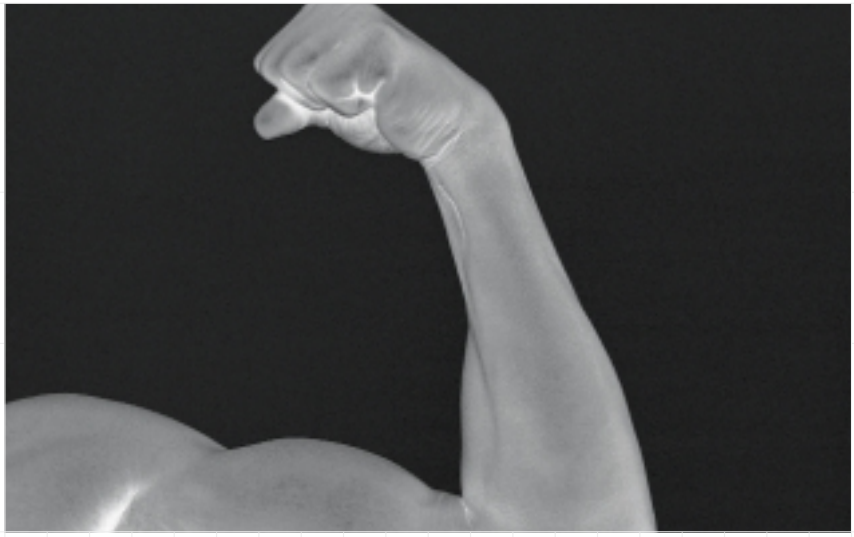

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The statements made in this brochure have not been evaluated by the Food and Drug Administration (FDA) or any other Authority.

This product is not intended to diagnose, treat, cure or prevent any desease.

The difference is inside

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