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Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the risk associated with the consumption of food supplements that contain creatine as an ingredient

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Abstract

Creatine is an endogenous substance synthesised in the pancreas, kidneys and liver, which can also be provided through the diet, mainly through the intake of meat and fish, or in the form of a food supplement ingredient, with creatine monohydrate and creatine hydrochloride being the most common marketed forms of creatine.

Creatine is effective in improving the training and performance of short duration and high intensity physical exercise. In fact, Regulation (EU) No. 432/2012 includes the health claims attributable to foods that provide a daily intake of 3 g of creatine and related to the effect of creatine on physical exercise.

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Based on the information currently available on creatine and considering that creatine monohydrate and hydrochloride are the forms of creatine commonly used in the manufacture of food supplements with creatine, the Scientific Committee considers that the maximum daily amounts of 3.41 g of creatine monohydrate and 3.84 g of creatine hydrochloride provide a maximum daily amount of 3 g/day of creatine and are acceptable from the standpoint of their safety in use as food supplements for a healthy adult population.

Key words

Creatine, food supplement.

Suggested citation

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1. Introduction

As a general consideration, it should be noted that food supplements are intended to supplement the normal diet and provide an additional supply of vitamins, minerals or other substances with a nutritional or physiological effect. On the other hand, the provision of a concentrated amount of nutrients or other substances may pose a risk of excess intake by the population that consumes them. Furthermore, in the case of pregnant or breastfeeding women, children, the elderly and ill people, food supplements should only be used if there are reasons that justify it, since the safety assessment of their use refers to an adult population with a normal physiological situation. Food supplements should in no case replace the use of medicines without suitable medical supervision. They should only be used to supplement the diet and, in general, their use is not necessary if a varied and balanced diet, which they cannot replace, is followed.

Creatine is an endogenous substance synthesised in the pancreas, kidneys and liver from the essential amino acids arginine, glycine and methionine. Approximately 95 % of the total creatine is stored in skeletal muscle, with the remaining amount being found in the brain, testicles and kidneys. Of the creatine present in muscle, approximately 65 % is in the form of phosphocreatine (PCr). This is a source of phosphate necessary for the synthesis of adenosine triphosphate (ATP), on which the muscles depend for energy during short-term and high-intensity physical exercise (Graham and Hatton, 1999) (Fernandez and Hosey, 2009) (Robinson, 2023). Exogenous sources of creatine through food are mainly meat and fish (EFSA, 2011a). Furthermore, creatine is available in the form of a food supplement, with creatine monohydrate being the most commonly sold form of creatine (Robinson, 2023).

The European Food Safety Authority (EFSA) has conducted two assessments on the effect of creatine on physical exercise, specifically on the improvement of physical performance in successive series of short-term high-intensity exercises and on the reinforcement of the effect of resistance training on muscle strength in adults over 55 years of age (EFSA, 2011, 2016).

Currently, Commission Regulation (EU) No. 432/2012 (EU, 2012) includes the health claims attributable to foods that provide a daily intake of 3 g of creatine under certain conditions. Moreover, Royal Decree 1487/2009 (BOE, 2009), relating to food supplements, indicates that the substances with a nutritional or physiological effect included in the Annex to said royal decree, as well as their forms, may be used in the manufacture of food supplements in such an amount that, in accordance with the product dose recommended for daily consumption by the manufacturer, the maximum daily amounts established in that Annex are not exceeded.

However, the stated Annex to Royal Decree 1487/2009 (BOE, 2009) contains creatine monohydrate (with a maximum dose of 3 g) and not creatine, so the health claims for creatine established by Commission Regulation (EU) No. 432/2012 (EU, 2012) and in Commission Implementing Regulation (EU) 2017/672 (EU, 2017) cannot be included.

Therefore, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) was requested to perform a safety assessment of the use of creatine monohydrate as a food supplement ingredient in a maximum daily amount that provides 3 g of creatine, an amount for which two health claims have been approved at European Union level. Likewise, the safety assessment

was requested for the use of compounds other than creatine monohydrate as food supplement ingredients in a maximum daily amount that provide an amount equal to 3 g of creatine per day and are safe for the consumer.

2. Creatine characteristics

Creatine, 2-(carbamimidoyl(methyl)amino) acetic acid (CAS No. 57-00-1), also called (alpha-methylguanido) acetic acid, N-(aminoiminomethyl)-N-methylglycine, N-amidinosarcosine, N-methyl-N-guanylglycine or methyl glycocyamine, is a non-protein amino acid, derived from guanidine, with formula $C_4H_9N_3O_2$ (Figure 1) and molar mass of 131.14 g/mol. It has a limited solubility in water (13 g/l at 18 ºC), which increases with temperature and with the decrease in the pH of the medium. It has very low solubility in alcohol (0.1 g/l) and is insoluble in ether (Jäger et al., 2011) (O'Neil, 2013).

Creatine crystallises as a monohydrate forming monoclinic prisms. The anhydrous form is obtained at 100 °C. It has a melting point of 255 ºC and decomposes at 303 ºC. It is a weak base, with pKb of 11.02 at 25 ºC, and can form salts with strong acids, through the protonation of guanidine; in addition, it can act as a complexing agent (Jäger et al., 2011) (O'Neil, 2013) (Yalkowsky et al., 2016).

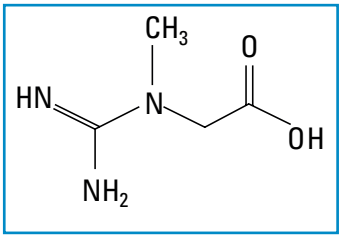

Figure 1. Chemical structure of creatine.

This molecule was first isolated from skeletal muscle by M.E. Chevreul in 1835, giving it its name derived from the Greek *kreas* (meat) (Demant and Rhodes, 1999).

Creatine is largely included in food supplements in the form of creatine monohydrate (Jäger et al., 2011), sold since the 1990s. This form has a solubility in water of 12.3 g/l at 20 °C, yielding a pH of 7. Subsequently, other possible sources of creatine have been developed, with different solubility, stability, bioavailability and efficacy properties, such as, among others, the following:

- Creatine hydrochloride, which can be dissociated into creatine and hydrochloric acid, demonstrating better solubility than creatine monohydrate, due to the lowering of the pH that occurs (Escalante et al., 2022) (Kreider et al., 2022).
- Creatine salts with different acids: malate, citrate, pyruvate, taurinate, pyroglutamate, decanoate, gluconate or creatine ascorbate, which can be dissociated into free creatine and the corresponding acid. When dissolved, they also cause a decrease in pH, due to the acid part of the molecule, which can reach values of up to 2.6 in the case of creatine pyruvate, increasing its solubility. Thus, if normalised by the amount of creatine they contain and compared to creatine monohydrate, creatine citrate is 1.55 times more soluble and creatine pyruvate is 2.63 times more soluble than the monohydrate (Jäger et al., 2011).

- Complexes of several creatine molecules with polyprotic acids, such as, for example, citric, such that the first carboxylic group is sufficiently strong to form a salt with creatine (pKa= 3.09), while the following molecules (pKa2= 4.75; pKa3= 5.41) form complexes with the salt. The compound formed would be, in this case, tricreatine citrate (1 citrate:3 creatine), which when dissolved produces an acidic pH of 3.2 (Jäger et al., 2011). The use of tricreatine orotate has also been proposed; however, it should be noted that the salts of orotic acid have a narrow safety margin (EFSA, 2009) (Andrés et al., 2017) (Escalante et al., 2022).
- Creatine chelate in anionic form with divalent magnesium, in a 2:1 ratio (Kreider et al., 2022).
- Creatine methyl or ethyl esters, which could be hydrolysed to release creatine. Nevertheless, it has been observed that, contrary to what one might imagine, creatine ethyl ester is rapidly degraded to creatinine, in the gastric acid medium, which would imply a very low efficacy for this source of creatine (Giese and Lecher, 2009).
- Creatine alcohols, such as creatinol-O-phosphate and dicreatinol sulfate (Jäger et al., 2011) (Kreider et al., 2022).

The different forms of creatine are stable in the solid state; for example, creatine monohydrate remains without signs of degradation for at least 3 years at 40 ºC (Jäger, 2003). However, its stability is much lower in solution, resulting in intramolecular cyclisations, which produce creatinine. Generally, degradation is intensified by lowering the pH and increasing the temperature, although extreme pHs (less than 2.5 or greater than 12.1), prevent the degradation of creatine (Kreider et al., 2022). In general, creatine salts and esters are less stable than creatine monohydrate, being degraded by intramolecular hydrolysis mechanism. It has also been observed that the presence of carbohydrates can increase the stability of some creatine salts in solution, due to the decrease in pH they generate (Howard and Harris, 1999) (Jäger et al., 2011).

However, compared with creatine monohydrate, so far there is no clear evidence that these new forms of creatine are significantly more bioavailable, effective and safe than monohydrate (Jäger et al., 2011).

3. Creatine sources

Creatine is naturally produced in the human body from amino acids and is transported through the blood to be used by the muscles. Approximately 95 % of the total creatine in the human body is found in skeletal muscle (Stec and Rawson, 2010). In humans and animals, about half of the stored creatine comes from food, mainly meat. Creatine is synthesised by the kidneys, pancreas and liver (approximately, 1 g/day), in addition to creatine ingested through food (approximately, 1-5 g/day) (Alves et al., 2013). Creatine kinase (CK) catalyses the reversible transfer of the N-phosphoryl group from PCr o adenosine diphosphate (ADP) to regenerate ATP (Wyss and Kaddurah-Daouk, 2000). It is important to highlight that genetic deficiencies exist in the creatine biosynthesis pathway that result in several serious neurological alterations.

There are two main sources of creatine supply (Mercimek-Andrews and Salomons, 2009):

- Endogenous sources: it is estimated that the daily needs of creatine for a 70 kg man are approx-

imately 2 g. If a person follows a standard Mediterranean diet, they ingest between 0.25 and 1 g of creatine daily. Therefore, the rest of the creatine needed to cover daily requirements must be synthesised by the body itself. This amount of endogenously synthesised creatine varies between 1 and 1.7 g, depending on the amount of creatine-rich foods consumed in the diet.

- Exogenous sources: the foods that contain the highest amount of creatine are fish and red meat, which have 3 to 5 g per kg of raw food, and somewhat less when they have been cooked.

Some of the main dietary sources of creatine are:

- Fish: especially fish such as herring (6.5-10 g/kg), cod (3 g/kg), salmon (4.5 g/kg), tuna (4 g/kg) or flounder (2 g/kg) are rich in creatine.
- Beef: red meat, such as steak (4.5 g/kg) and other parts of beef, is one of the richest sources of creatine.
- Pork: pork also contains significant amounts of creatine (5 g/kg).
- Chicken and rabbit meat: although it has less creatine than beef, chicken is still a good source of creatine (3.4 g/kg), as is rabbit (3.4 g/kg).
- Seafood: some seafood also contains creatine, although to a lesser extent than fish and meat.

A person who follows a standard Mediterranean diet usually eats between 0.25 and 1 g of creatine daily. However, fully vegetarian people consume practically no creatine, since plant-based foods contain very little or no amount of creatine (traces).

4. Creatine content equivalence

The commercial creatine formulas seek, on the one hand, to achieve a more acidic environment that favours its dissolution in the form of more dissociable salts of this compound and, on the other, as much creatine contained as possible in these forms of creatine salt. In this regard, creatine hydrochloride is one of the most soluble forms of this compound, providing 78.2 % of creatine. However, creatine monohydrate, being a less soluble form than the previous one, provides a higher content of creatine available in blood and tissues (87.9 %). Other soluble forms of creatine provide a lower systemic creatine content, such as creatine pyruvate, creatine dicitrate and creatine citrate, which provide 59.8 %, 57.7 % and 40.6 % of creatine, respectively (Kreider et al., 2022).

5. Nutrition and metabolism

The ingested creatine is absorbed in the intestine and passes into the bloodstream. Once in the blood, creatine is transported to several tissues such as the heart, smooth muscle, brain and skeletal muscle. However, the vast majority of creatine stores are found in skeletal muscle.

The reasons why the highest amount of creatine is located in skeletal muscle are as follows:

- Creatine is transported from blood to muscle by an active, saturable, sodium- and chlorine-dependent transport process, i.e., it is transported against a concentration gradient, mediated by insulin.
- Once inside the muscle cell, creatine is trapped (trapping) by two mechanisms:

– by converting (phosphorylating) 60 to 70 % of the total muscle creatine into PCr, which cannot exit the muscle because it does not cross the cell membrane, and
– free creatine in the muscle probably remains bound to an internal component that is not yet known. Furthermore, creatine is an osmotically active substance, meaning that its presence in muscle induces an increase in intracellular fluid due to the passage of water from the extracellular space into the cell.

Endogenous creatine is synthesised from three amino acids: glycine, arginine, and methionine. The main organ that produces creatine is the liver, although the pancreas and kidneys also synthesise small amounts. Muscle does not have the ability to synthesise creatine. The endogenous synthesis of creatine is regulated by the amount of creatine and its precursors present in the diet. For example, if the amount of creatine ingested is low, endogenous synthesis is stimulated. However, if the amount of creatine ingested is high, endogenous synthesis is inhibited or even suppressed. Moreover, high consumption of creatine precursors (such as glycine and arginine) can stimulate their endogenous synthesis (Bonilla et al., 2021).

Creatine is endogenously synthesised from arginine and glycine by arginine-glycine amidinotransferase (AGAT) to guanidinoacetate (GAA). GAA is then methylated by the enzyme guanidinoacetate N-methyltransferase (GAMT) with S-adenosyl methionine (SAM) to form creatine (Bonilla et al., 2021). The kidney, pancreas, liver and some regions of the brain contain AGAT, and most of GAA is formed in the kidney and is converted by GAMT into creatine in the liver (da Silva et al., 2009). Endogenous creatine synthesis provides approximately half of the daily creatine needs; the remaining amount of creatine needed to maintain normal tissue levels is obtained from the diet, mainly from red meat and fish, or from food supplements (Brosnan and Brosnan, 2016). The ingested creatine is absorbed in the intestine and passes into the circulation. Once in the blood, creatine is transported to various tissues, largely being stored in skeletal muscle and in the brain.

Approximately 95 % of the body's creatine reserve is in skeletal muscle. High creatine levels are also found in other cells with high energy demands such as cardiomyocytes, hepatocytes, renal cells, inner ear cells, enterocytes, sperm and photoreceptor cells. After synthesis or absorption, creatine reaches target tissues through the bloodstream and intracellular transport mediated by a solute transporter protein called sodium- and chloride-dependent creatine transporter (CRT, also known as SLC6A8). This transporter belongs to a family of neurotransmitter transporters known as solute carrier 6 family. Creatine is one of the main osmolytes of the central nervous system, which can play important roles in pathophysiological conditions of the brain. Although some of the aforementioned tissues could synthesise creatine, CRT is necessary to transport endogenous and exogenous creatine to cells with high and fluctuating energy demands for adequate physiological function (Kreider and Stout, 2021).

Once creatine enters the cytosol through the CRT transporter (Tarnopolsky et al., 2001) (Santacruz and Jacobs, 2016), thanks to the associated cytosolic and glycolytic CK enzyme isoforms, the glycolytic ATP levels, the cytosolic ATP/ADP ratio and the consumption of cytosolic ATP are maintained (Wallimann et al., 2011). In addition, creatine diffuses into mitochondria and couples

with ATP produced from oxidative phosphorylation and the Adenine Nucleotide Translocator (ANT) via mitochondrial CK. Then, PCr diffuses back into the cytosol and helps meet energy needs. This coupling reduces the formation of Reactive Oxygen Species (ROS) and, therefore, creatine acts as a direct and/or indirect antioxidant (Rahimi, 2011) (Saraiva et al., 2012). The PCr shuttle is important in the translocation of ATP produced from oxidative phosphorylation in the mitochondria to the cytosol and to areas of the cell that need ATP for energy metabolism (Wallimann et al., 2011). Therefore, the PCr transporter is an important regulator of cellular metabolism.

In muscle tissue, 50-80 % of creatine is in the phosphorylated form, PCr, which is in balance with ATP. The reaction rate is highly influenced by the enzyme CK. PCr serves as an energy reservoir and has a higher phosphoryl group transfer potential than ATP. Hence, when muscle is stimulated for a prolonged period in the absence of glycolysis or respiration, the supply of PCr will be depleted within a few hours by maintaining the ATP concentration. This is especially the case of *post mortem* muscle, when ATP supply has significantly decreased due to oxidative respiration (Bonilla et al., 2021).

The role of creatine in energy metabolism and the impact that creatine has in maintaining energy availability in diseases that depend on the CK/PCr system provides the metabolic basis for how creatine can affect health and disease or provide therapeutic benefits. Creatine plays a vital role in the supply of energy through the CK/PCr system (Wallimann et al., 2011). In this regard, the free energy produced by the enzymatic degradation of ATP into ADP and inorganic phosphate (Pi) by CK serves as the primary fuel to replenish ATP for cellular metabolism. The decomposition of PCr into Pi and creatine with the enzyme CK produces around 10.3 kcal of free energy that can be used to resynthesise ADP + Pi into ATP (Schlattner et al., 2016) (Ydfors et al., 2016) (Bonilla et al., 2021). The ability to replenish depleted ATP levels during states of high energy demand such as intense exercise or in conditions where energy production is impaired (e.g., ischaemia or hypoxia) or insufficient, due to increased demand (e.g., in some disease states), is important for maintaining ATP availability. In fact, alterations in creatine concentrations due to CRT, AGAT or GAMT deficiencies can produce functional changes in these tissues, leading to a wide range of diseases that are grouped in the creatine deficiency syndrome. For example, CRT malfunction results in low levels of intracellular creatine which, although not lethal, induces an alteration of brain energy metabolism to the same extent as deficiencies in creatine biosynthetic enzymes. A dysregulation in creatine metabolism has also been implicated in several pathological conditions, including muscle dysfunction, cardiomyopathy and cancer, among others (Kreider and Stout, 2021).

Approximately 2/3 of the creatine that is stored in muscle is bound to Pi and is stored as PCr, while the remainder is stored as free creatine. The total creatine reserve (creatine + PCr) is about 120 mmol/kg dry muscle mass for a 70 kg individual who has a diet that includes red meat and fish. Vegetarians have been reported to have 20-30 % lower muscle reserves of creatine and PCr than non-vegetarians (Green et al., 1996) (Hultman et al., 1996). The body degrades approximately 1-2 % of muscle creatine per day into creatinine, which is excreted in the urine. Degradation of creatine to creatinine is greater in individuals with larger muscle mass and in individuals with higher physical activity levels. Therefore, a normal-sized individual may need to consume 2-3 g/day of creatine to

maintain normal creatine stores based on diet, muscle mass, and physical activity levels (Brosnan and Brosnan, 2016). In fact, Wallimann et al. (2011) noted that since creatine stores are not fully saturated in normal vegan or omnivorous diets, which generally provide 0 or 0.75-1.5 g/day of creatine, daily dietary needs for creatine may be in the order of 2-4 g/day to promote overall health.

6. Safety

In relation to the safety of the use of creatine in food supplements, there are now more than 500 publications on this supplementation (Antonio et al., 2021). However, despite the high number of studies related to the effect of creatine as a food supplement, there is still a relative uncertainty regarding the safety related to the use of this substance in certain population groups.

The normal intake of creatine through food corresponds, approximately, to 1 g creatine/day, which is equivalent to the amount produced endogenously (Balsom et al., 1994). In populations with special diets, for example, vegan or vegetarian, this amount due to food is even lower (Harris et al., 1992) (Rogerson, 2017) (Kaviani et al., 2020). According to Venderley and Campbell (2006), when using creatine supplementation regimens based on an intake of 20-25 g/day of creatine for 3-7 days, very high doses of creatine are being ingested, which is impossible to ingest only through the diet.

It seems to be widely accepted that the most common side effect of creatine supplementation relates to water retention (Rosene et al., 2015) (Buck et al., 2023). For example, in a study with loading doses of 0.3 g/kg body weight (b.w.)/day between 5 to 7 days and maintenance doses of 0.03 g/kg b.w./day in periods of 4 to 6 weeks, this water retention was verified (Hall and Trojian, 2013). However, there is at present controversy regarding this statement, since a large number of studies that include wide ranges of creatine supplementation (between 5 and 10 weeks) seem to contradict this fact, since they do not show this described retention (Powers et al., 2003) (Spillane et al., 2009) (Rawson et al., 2011) (Jagim et al., 2012) (Andre et al., 2016). In short, there is currently more evidence to support that creatine supplementation does not cause this stated fluid retention.

Therefore, the main concern regarding the possible toxic effect of creatine use is mainly based on the possible effects on renal function. It should be noted that, after non-enzymatic degradation to creatinine, both of creatine and PCr, it is transported via the blood and excreted via the urine (Ropero-Miller et al., 2000) (Wyss and Kaddurah-Daouk, 2000). Under normal conditions, creatine is not present in the urine but, during its supplementation, creatine can reach very high levels in the urine irrespective of the age of the subjects (Rawson et al., 2002). Although oral creatine supplementation is consumed in amounts of 20 g/day for 5 days, the significant increase in creatine in the urine and plasma would not correspond to significant changes in creatinine in the urine, creatinine clearance or albumin excretion rate (Mihic et al., 2000). There are many studies that investigate the effects of creatine supplementation on renal function in different population groups of healthy (Derave et al., 2004) (Carvalho et al., 2011) (Lugaresi et al., 2013) (Blancquaert et al., 2018) and sick individuals (Bender et al., 2008) (Gualano et al., 2011) (Hayashi et al., 2014) (Domingues et al., 2020) and, in general, they indicate that there is no evidence that this supplementation may be harmful to the general population (Lonmgobardi et al., 2023).

Taking these data into account, creatine monohydrate supplementation up to 30 g/day for 5 years could be safe in the adult population, since there is currently no scientific evidence of harmful effects, both among healthy individuals and among populations with clinical pathologies, who could benefit from this supplementation (Kreider et al., 2017). It should be noted that creatine supplementation should not be used by individuals with pre-existing kidney disease. However, although a slight increase in mutagenic agents (methylamine and formaldehyde, mainly) in the urine has been determined after a high loading dose of creatine (20 g/day), its excretion remains within a normal range (Francaux and Poortmans, 2006). Another piece of data to take into consideration is that the consumption of creatine could simulate the indicators of kidney disease, since there are data of misdiagnosis of nephropathies and suspicion of drug nephrotoxicity (Pritchard and Kalra, 1998) (Willis et al., 2010) (Williamson and New, 2014).

The possible adverse effect of its concomitant consumption with ethanol has also been disclosed since, despite the fact that the supplementation of male mice with creatine monohydrate seems to protect the liver from a possible production of fatty liver in diets with high lipid content, it has been confirmed that it aggravates alcoholic liver disease induced by ethanol (Marinello et al., 2022). Although this effect has not been confirmed in humans, nor in moderate ethanol consumption, it is an issue that should be taken into account.

A population group of special interest is the adolescent population, as there has been an increase in the use of creatine in this group for sports (Jagim et al., 2018). Therefore, efforts aimed at determining the safety of the consumption of this food supplement in this population group should be increased. In this sense, Simpson et al. (2019) conducted a study with elite athletes between 16 and 21 years, with a supplementation of 0.3 g/kg b.w./day for 7 days and 5 g/day for 7 subsequent weeks, finding indications that it cannot be excluded that creatine supplementation has an adverse effect on the airways of these athletes, with special attention to individuals with allergic sensitisation. Today there is a lack of studies on the safety and efficacy of creatine supplementation in adolescents (Metzger et al., 2023), so the number of studies relating to this population group should be enhanced.

Conclusions of the Scientific Committee

Based on the information available to date on creatine as an ingredient in food supplements and taking into account the general considerations reflected in this report, the Scientific Committee concludes that the maximum daily amounts of 3.41 g of creatine monohydrate and 3.84 g of creatine hydrochloride, according to the equivalence reflected in this report, provide a maximum daily amount of 3 g/day of creatine, and are acceptable from the safety point of view for their use as food supplements for a healthy adult population.

The safety assessment studies of the use of creatine monohydrate and creatine hydrochloride in food supplements for the non-adult population are insufficient.

The above conclusions may need to be revised in the future in light of new scientific evidence.

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