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Intradialytic creatine supplementation: A scientific rationale for improving the health and quality of life of dialysis patients

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ABSTRACT

The CK/PCr-system, with creatine (Cr) as an energy precursor, plays a crucial role in cellular physiology. In the kidney, as in other organs and cells with high and fluctuating energy requirements, energy-charged phospho-creatine (PCr) acts as an immediate high-energy source and energy buffer, and as an intracellular energy transport vehicle. A maximally filled total Cr (Cr plus PCr) pool is a prerequisite for optimal functioning of the body and its organs, and health. Skeletal- and cardiac muscles of dialysis patients with chronic kidney disease (CKD) are depleted of Cr in parallel with the duration of dialysis. The accompanying accumulation of cellular damage seen in CKD patients lead to a deterioration of musculo-skeletal and neurological functioning and poor quality of life (QOL). Therefore, to counteract Cr depletion, it is proposed to supplement CKD patients with Cr. The anticipated benefits include previously documented improvements in the musculo-skeletal system, brain and peripheral nervous system, as well as improvements in the common comorbidities of CKD patients (see below). Thus, with a relatively simple, safe and inexpensive Cr supplementation marked improvements in quality of life (QOL) and life span are likely reached. To avoid Cr and fluid overload by oral Cr administration, we propose intradialytic Cr supplementation, whereby a relatively small amount of Cr is added to the large volume of dialysis solution to a final concentration of 1–10 mM. From there, Cr enters the patient's circulation by back diffusion during dialysis. Because of the high affinity of the Cr transporter (CRT) for Cr affinity for Cr (Vmax of CRT for Cr = 20–40 μM Cr), Cr is actively transported from the blood stream into the target cells and organs, including skeletal and cardiac muscle, brain, proximal tubules of kidney epithelial cells, neurons, and leukocytes and erythrocytes, which all express CRT and depend on the CK/PCr system. By this intradialytic strategy, only as much Cr is taken up by the body as is needed to fill the tissue Cr pools and no excess Cr has to be excreted, as is the case with oral Cr. Because aqueous solutions of Cr are not very stable, Cr must be added immediately before dialysis either as solid Cr powder or from a frozen Cr stock solution to the dialysate,

Abbreviations: AGAT, arginine-glycine amidinotransferase (mostly in the kidney); AMPK, AMP-activated protein kinase; ANT, mitochondrial adenine nucleotide or ATP/ADP carrier of the inner mitochondrial membrane; ATP, adenosine-trisphosphate, the universal energy currency of living systems; CK, creatine kinase: MM-CK: cytosolic muscle-type MM-CK dimer, cytosolic non-muscle or brain-type BB-CK dimer: mtCK octameric mitochondrial CK; CKD patients, chronic kidney dialysis patients; CKD, chronic kidney disease patients; Cr, creatine, Crn: creatinine, total Cr: Cr plus PCr; CrT, creatine transporter or $2Na^+$:$1Cl^-$:1Cr-cotransporter belonging to the solute carrier family SLC6A8; ECs, erythrocytes; EPO, erythropoietin; GAMT, guanidino acetate methyl-transferase (mostly in the liver); GAT2, gamma-aminobutyric acid transporter-2; GFR, glomerular filtration rate; HCys, homocysteine; HIF, hypoxia-induced factor; mPTP, mitochondrial permeability transition pore; mtCK, mitochondrial octameric CK isoform, sandwiched between inner and outer mitochondrial membranes; NAFL, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PCr, phosphoryl-creatine or phospho-creatine; QOL, quality of life; SAM, S-adenosyl-methionine; TBARS, thiobarbituric-acid-reactive substances; tHCys, total plasma homocysteine concentration; VDAC, voltage-dependent anion carrier of the outer mitochondrial membrane.

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Protection by creatine of erythrocytes and immune cells
Protection from oxidative damage and mechanical stress by creatine
Sparing of erythropoietin (EPO)
Diabetes mellitus type-2
Insulin sensitivity
Metabolic syndrome
Dyslipidemia
Fatty liver disease
NASH
NAFL
X-ray contrast media induced kidney failure

or alternatively, Cr could become an additional component of a novel dry dialysate mixture in a cartridge device.

Introduction

The creatine kinase/phosphocreatine (CK/PCr) system and the actions of creatine

Creatine (Cr) is a prominent guanidino component of skeletal, cardiac, and smooth muscle; brain and peripheral nervous tissues; and the kidney and other organs [1,2]. Cr can be charged to the high-energy compound, phosphoryl-creatine (PCr) by creatine kinase (CK) and ATP. PCr acts as an energy source and buffer and energy transporter, shuttling energy from subcellular sites (mitochondria) of energy production by glycolysis to sites of energy consumption where cellular ATPases (facilitating hydrolysis) and ATP-dependent ion pumps are located [1,3]. The main function of the CK-PCr circuit or shuttle (see Fig. 1) to optimize the free energy change of ATP hydrolysis ($\Delta G_{ATPhydrolysis} = \Delta G_{obs.} - RT \times \ln([ATP]/[ADP] \times [P_i])$, which is obviously strongly dependent on the local ATP/ADP ratio. That is, the CK/PCr system maintains high local levels of ATP in the vicinity of ATPases in resting and working cells and thus guarantees the highest work-output per ATP hydrolysed [1,4]. The prerequisite for this to happen is that cytosolic CK isoforms, either muscle-type MM-CK or brain-type BB-CK are forming functionally coupled microcompartments with various ATPases, such as the sarcoplasmatic reticulum Ca^{2+}-ATPase and the sarcolemmal Na^+/K^+-ATPase [4] (Fig. 1). At the mitochondrial site of ATP production, mitochondrial mtCK maintains a high ADP/ATP ratio and forms a functional microcompartment with the mitochondrial ATP/ADP-carrier (ANT), which is located in the inner membrane, and the voltage-dependent anion-selective channel (VDAC) in the outer membrane [5]. There, mtCK, sandwiched between ANT and VDAC, enables efficient high-energy export from the mitochondria to the cytosol in the form of PCr [1,3–5] (Fig. 1). Because PCr and Cr are present in cells at much higher concentrations relative to ATP and ADP (up to 10- and 100-fold higher, respectively) and have bigger diffusion coefficients compared to the bulky and charged adenosine nucleotides, ATP and ADP, PC and Cr are ideal molecules for shuttling energy equivalents from the sites of energy production either via mitochondrial oxidative phosphorylation or glycolysis to the sites of energy (ATP) consumption facilitated by ATPases. The action of the CK/PCr shuttle has been directly demonstrated in spermatozoa, where the distance from the sperm mitochondria in the midpiece to the distal

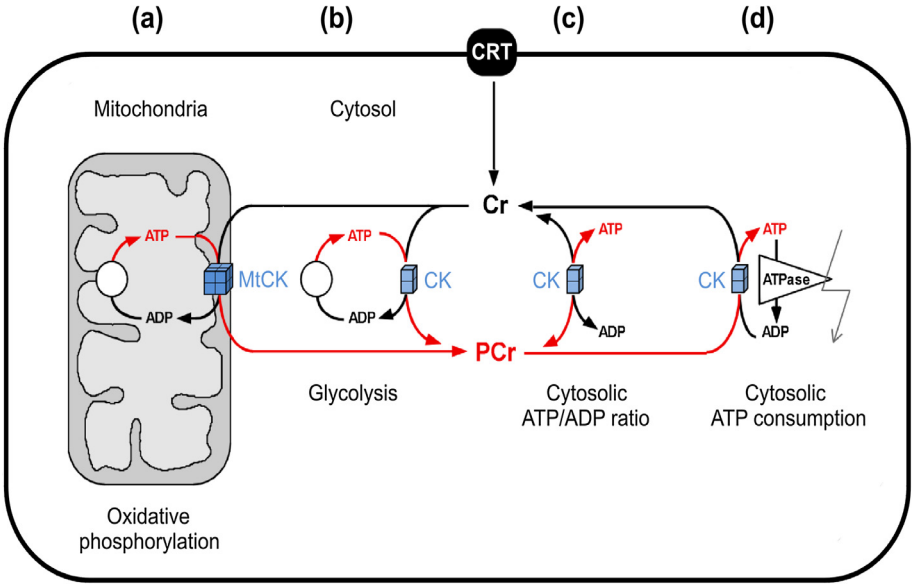

Fig. 1. The creatine kinase/phosphocreatine (CK/PCr)system. The CK/PCr shuttle or circuit [4] also functions in kidney epithelial cells. The model shows the compartment-specific localization of the isoenzymes of creatine kinase that are found in mitochondria (Mt) (**a**) (octameric MtCK, left) and in the cytosol (**b** and **c**) (dimeric MM-CK, BB-CK, MB-CK, right). In the kidney, the main CK players are the ubiquitous (u) uMtCK and BB-CK isoenzymes, which are highly expressed in this tissue. CK isoenzymes are either associated with ATP-delivery processes (mitochondrial oxidative phosphorylation [**a**] or glycolysis [**b**]) or ATP-consuming processes (ATPases [**d**] rightmost) to maintain local ATP/ADP ratios; or occur in soluble form (to maintain global cytosolic ATP/ADP ratios [**c**]). A large cytosolic PCr pool of up to 30 mM results from the action of CK on creatine plus ATP derived from oxidative phosphorylation (e.g., in aerobic skeletal or heart muscle) or glycolysis (e.g., in fast-twitch [anaerobic] skeletal muscle). The large PCr pool is then used as a temporal energy buffer to maintain constant global and local ATP/ADP ratios over a wide range of workloads. The higher diffusibility of PCr, as compared with ATP, together with localized CK isoenzymes, is used for spatial energy buffering, i.e., for an energy shuttle between ATP-providing and -consuming processes. This energy shuttle seems to be most important for cells that are polarized, e.g., spermatozoa, and/or have very high or localized ATP consumption, e.g., skeletal and cardiac muscle and neuronal cells (slightly modified figure and legend taken from Schlattner et al., 2006 [5]).

flagella is very long [4,6]. However, the CK/PCr shuttle, which is schematically depicted in Fig. 1, is also active in kidney epithelial cells, where Na^+/K^+-ATPase ion pumps [7] and a variety of solute or metabolite transporters such as the Cr-transporter (CrT) [8] are also active (Fig. 1). In addition the CK/PCr system is also thought to be involved in the energetics and regulation of the cell cycle [9].

Numerous investigations have corroborated the role of the CK/PCr system, as we have described. For example, the striking phenotype, the double CK-knockout mouse, lacks both the muscle-type MM-CK and sarcomeric mtCK isoforms. The double CK-knockout mouse manifests significant changes in muscle contraction and generation of muscle force, and most interestingly, manifests difficulties in relaxation of the fast muscle fibers [10,11]. The ablation of the brain-type BB-CK isoform in transgenic animals, on the other hand, leads to neurological disorders such as hearing and equilibrium deficits and difficulties in thermoregulation [12] also see [4]. The human creatine-deficiency syndromes due to genetic defects that lead to deficiencies of either of the two enzymes (AGAT or GAMT) involved in the synthesis of endogenous creatine or the deficiency of the creatine transporter (CrT), which is a $2Na^+$:$1Cl^-$:1Cr-cotransporter, belonging to the solute carrier family SLC6A8a [8], are manifested as hypotonia and muscle weakness; and more prominently as developmental delays (speech delay), autism, brain atrophy, and severe brain pathology [13]. The data from transgenic mice with CK deletions highlight the physiological importance of CK and the PCr/Cr system for a normally functioning nervous system [14]. These data are completely validated by the recently available transgenic CrT-knockout mouse. Its phenotype shows inefficient energy utilization of muscle tissues, early onset of cognitive impairment [15], and neurohistopathological findings associated with brain ageing [16]. Cr-deficient patients and transgenic animals can accumulate a certain amount of Cr by endogenous synthesis of Cr and/or from food, but Cr cannot be directly taken up by cells such as neurons that depend on importation of Cr from the circulation. As shown by the three Cr deficiency syndromes (AGAT, GAMT, and CrT deficiencies) in animal models and humans and by the double CK-knockout animal models, life without Cr and/or the CK/PCr system must be miserable, with a number of more or less severe disorders [17].

Creatine metabolism and the kidney

The adult human body contains from 120 to 140 g of total Cr (2/3 as PCr and 1/3 as Cr) in the muscles (80%-90% of total Cr) and in the brain and other organs. Approximately 2% of total Cr is nonenzymatically daily converted into the cyclic degradation product, creatinine (Crn), which can leave the cells via the Crn-permeable cell membrane, enter the circulatory system, and be excreted by the kidneys into the urine [2]. The serum Crn level is easy to measure, and was chosen as an indicator of kidney function, despite some uncertainties and problems with its use for that purpose. A serum Crn concentration that exceeds a certain threshold value, which depends on race, gender, and the amount of total muscle mass, can suggest kidney failure, which must be verified by more direct clinical testing. This diagnostic paradigm, the association of high serum Crn concentrations with kidney malfunction, and the fact that the terms *Crn* and *Cr* are often confused with each other, even by nephrologists, have led to the general misconception that Crn *per se* could be toxic to the kidney and that ingestion of Cr should be also avoided, to spare the kidneys. However, Crn is in actuality not toxic and Cr, as part of the PCr/Cr energy shuttle, is required for adequate kidney function [18]. In addition, because Cr manifests pleiotropic effects on a variety of cells and organs [4], it might indirectly provide benefits to kidney cells, aside from improving cellular energetics (see below).

For optimal physiological functioning, that is, for ion homeostasis and clearance of metabolic waste products, the kidney depends on a variety of ion pumps and transporters of other solutes or metabolites for the secretion into or reabsorption of a plethora of substances from primary urine [7]. Many pumps are energetically demanding and depend on a localized high ATP/ADP ratio, which is guaranteed by the presence of CK and PCr, as explained previously. Thus, the CK/PCr system which is also prominent in kidney epithelial cells, where both BB-CK and mtCK isoforms have been identified and localized by immunohistochemical staining [14], is of paramount importance for normal kidney function [7] (Fig. 1). Both cytosolic BB-CK and the ubiquitous mtCK isoforms have been found in kidney epithelial cells, and BB-CK has been found to be coupled to and support the energetics of Na^+/K^+-ATPase pump functioning these cells [7]. Incidentally, in kidneys damaged by hypertension, cytosolic BB-CK is one of the most prominently upregulated proteins [19], corroborating its physiological importance in situations of cellular energy crisis.

Moreover, the first step in the endogenous biosynthesis of Cr takes place primarily in the kidney, and to a much smaller extent also in the pancreas [20]. AGAT, which is highly expressed in the kidney, facilitates the synthesis of guanidino acetic acid (GAA) from arginine and glycine [21,22]. Primarily synthesized in the kidney, GAA, enters the blood stream from where it is transported into the liver by the GAT2 transporter [23]. There, in the second step of Cr synthesis, GAMT catalyses GAA to form methyl-GAA, or Cr [20,24]. By a mechanism that still remains unknown, Cr is then exported from the liver into the blood stream and from there is taken up by target organs (muscle, brain and kidney [25,26] via the Cr-transporter (CrT) [8]. The simultaneous importation of 2 sodium ions and 1 chloride ion for each Cr into the cell adds to its osmotic load, which is demonstrated by its retention of water. And the excess of imported sodium must be removed from the cell by the ATP-dependent Na^+/K^+-pump [2].

Since in humans, only a certain fraction of the daily requirement of Cr is endogenously synthesized in the kidney and liver, carnivores and omnivores, and to a greater extent vegetarians and vegans, seem to depend on dietary Cr (fresh meat, fish, and also milk) to supply the total demand of Cr by the body [27]. An increased dietary intake of Cr is required for individuals with peak physical and/or mental demands [28,29]. A re-evaluation of human Cr metabolism revealed that dietary Cr must account for as much as 50% of daily Cr requirements in nonvegetarians [30]. In order to take up dietary Cr, carnivores and omnivores, but not herbivores (such as horses, cows, and sheep) express high levels of CrT in their small intestines, where alimentary Cr is taken up and transported into the blood stream [31]. Vegetarians and even more so vegans, who ingest little or no dietary Cr, show decreased total Cr levels in serum and erythrocytes [32,33], as well as decreased tissue levels of total Cr and PCr, paralleled with a decreased PCr/ATP ratio in skeletal and cardiac muscles [34]. More recent data on total Cr content and Cr transporter gene expression in vegetarians prior to and following Cr supplementation clearly show that skeletal muscles of vegetarians contain a lower total Cr content than non-vegetarians, but seem to have an increased capacity toad Cr into their skeletal muscles following Cr supplementation [35]. This indicates that vegetarians are in need of oral Cr supplementation that will work quite effectively to restore normal tissue levels of Cr that is paralleled by an increase in muscle performance [36], cognitive functions [37] and most likely also by other Cr-related positive effects [4].

There is plenty of evidence that prehistoric humans have evolved from carnivores as hunter-gatherer, eating large quantities of raw or later of cooked meat [38–41], to modern omnivores, ingesting a mixed diet. As a consequence, modern man depends to a variable extent on Cr that is, however, no longer provided by

fish and meat. In order to obtain the necessary 3–6 g of Cr per day one would need to ingest 0.5–1 kg of fresh beef per day. However nowadays, only a minority of people regularly eat such quantities of meat. The change in dietary eating habits together with the state of awareness for Cr as a physiologically important energy precursor, resulted in the compensatory use of oral Cr supplements, such that millions of professional and recreational athletes world-wide are regularly using either the recommended 3–6 g/day or higher dosages of chemically pure Cr. Cr has been convincingly demonstrated to improve muscle mass and muscle performance [29,42,43]. Most importantly, since Cr supplementation also leads to increased glycogen storage in muscle; Cr not only improves short-term, high-intensity exercise performance, but also endurance exercise capacity [44]. The most recent data suggest that Cr supplementation is specifically effective for endurance performance at high ambient temperatures under heat-stress conditions [29].

In conclusion: *first*, Cr is a guanidino compound that is essential for optimal organ function, including the kidney, and is neither damaging nor toxic to the kidney [18,45] or other organs [46]. Large multicenter international studies of more than 1500 patients with neurodegenerative conditions, who ingested a daily dose of 9.5 g of Cr for a total of 5480 patient-years of Cr consumption, did not reveal any significant adverse effects on the kidney and liver or on blood pressure [47]. Indeed, kidney function, especially the function of the epithelial cells of the proximal tubules, relies on the CK/PCr shuttle [7]. Mitochondrial ATP is transphosphorylated by mtCK to yield energy-rich PCr, which is then shuttled to those subcellular sites that require ATP, such as the plasma membrane, the location of energy-demanding ion pumps and carriers of solutes and metabolites and the BB-CK isoform, which converts the shuttled PCr in situ to ATP, the energy source. The CK/PCr shuttle guarantees efficient function of the kidney, which also depends on a high cellular PCr/ATP ratio and thus on an optimally large pool of total Cr in the kidney [18]. This function is supported by the identification of BB-CK and mtCK isoforms and the CrT at specific subcellular sites in the kidney [7,22,48,49]. In the kidney, these CK isoenzymes, together with Cr and PCr, work exactly the same way [7], as described previously for muscle [1] (Fig. 1). As a matter of fact, Cr supplementation did indeed increase urinary output and renal excretory function to some extent in mice [26].

Second, as previously mentioned, the first step of endogenous CR synthesis occurs in the kidney. The kidney provides the liver with GAA for Cr synthesis. To maintain its own pool of Cr, the kidney, which is a target organ for Cr, takes up the Cr that was synthesized in the liver from the blood stream. The Cr uptake is facilitated by kidney CrT.

Third, because Cr can be reabsorbed from native urine by a specific CrT located on the apical membrane of kidney proximal tubules [49] the kidney can prevent the loss of metabolically valuable Cr into the urine [50,51]. Preventing the loss of Cr by excretion also prevents using even more of the metabolically precious S-adenosyl-methionine (SAM) that is needed for Cr synthesis. This is important, for already 50%–70% of the methyl groups from SAM are used for methylation of GAA for the synthesis of Cr [30,52].

Hypotheses

Rationale for creatine supplementation for patients with CKD and recipients of kidney transplants

1) Cr is a safe and effective nutritional supplement with many beneficial pleiotropic effects, as demonstrated in studies of healthy participants [29,53,54], and of patients (primarily those with muscle and neuromuscular diseases [55,56]. Cr supplementation has not yet been studied in CKD patients. Cr supplementation has been shown to increase the total Cr content (Cr + PCr) of target organs such as skeletal and cardiac muscles, brain, and the kidneys [25,26]. Since Cr supplementation has been shown to significantly improve several parameters of health in young and elderly study participants, it is likely to also improve the QOL and survival of long-term dialysis patients with CKD. These patients present with significantly decreased total Cr levels, aswell as with a decreased PCr/ATP ratio in their skeletal and cardiac muscles [57,58], and most likely also in other organs. The expected benefits of Cr encompass not only reinforcement of the musculoskeletal and cardiovascular system, but also improved function of the nervous system. Parameters reflecting physical and mental fatigue and depression are also improved (see below). In addition, Cr supplementation may benefit many of the comorbidities of chronic dialysis patients, including cardiovascular disease, anemia, type 2 diabetes mellitus and metabolic syndrome, inflammatory condition, dyslipidemia, and non-alcoholic fatty liver disease (NAFL) or non-alcoholic steatohepatitis (NASH), which are also often associated with diabetes, metabolic syndrome and ageing [59,60] (see below). The early onset of cognitive impairment and neurohistopathological findings associated with brain ageing in transgenic mice with CrT deficiency [16] seem to explain the premature ageing in individuals with chronic depletion of creatine, who include patients with CKD.

Many CKD patients tend to appear catabolic. The administration of Cr to these patients will allow them to spare the precious methyl donor (SAM), since the endogenous synthesis of Cr consumes about 50% of the body's methylation potential [52]. SAM provides methyl groups for more than 50 critical transmethylation reactions, including protein synthesis. Cr supplementation is especially important if a patient is nutritionally deficient in methionine [61]. Therefore, Cr can be classified as an anticatabolic nutritional supplement, and is very likely to have specific beneficial effects for CKD patients.

2) Erythrocytes (ECs) are subjected to mechanical and oxidative stress during hemodialysis treatment and undergo hemolysis, which eventually leads to anemia. Cr was found to protect ECs against such external stressors [62–64]. If dialysis is performed with intradialytic PCr and/or Cr, the reduced loss of ECs to hemolysis could reduce the erythropoietin (EPO) requirements of the supplemented CKD patient. Furthermore, the assumption that PCr/Cr will also support the energy-demanding process of erythropoiesis is reasonable. Thus, Cr supplementation of CKD patients should further reduce the need for EPO if they are optimally loaded with Cr. The reduced administration of EPO should markedly reduce costs to the healthcare system and at the same time circumvent possible EPO-supported progression of tumors [65,66], which is a major risk for CKD patients.

3) Oral Cr (5–10 g of crystalline powder per day) is not a feasible option for dialysis patients, because of problems with compliance and the requirement of relatively large volumes of water to dissolve the Cr powder. Dry crystalline Cr powder would have to be ingested, which is problematical, and the 1 to 1.5 L of water needed to dissolve 5 g of Cr would not be possible for a CKD patient who needs to restrict fluid intake. In addition, the ingestion of such a dosage of oral Cr has not been investigated for CKD patients so far and the possibility exists that the higher serum Cr and Crn levels may be a burden for CKD patients [67]. For that reason, we propose intradialytic Cr supplementation. Cr should be either be directly dissolved in the dialysis fluid just before use, or be added as a solid constituent to a dry dialysate mixture, which could be packed into a cartridge where it will remain in stable form. The contents of the cartridge would be dissolved immediately before use at the bedside of the patient. The intradialytic administration

of Cr would also be advantageous over oral ingestion of 5 to 10 g of Cr, because the amount of Cr absorbed from the dialysate would only be the amount required at the time of dialysis, avoiding the need to clear surplus Cr from the system, which would be necessary with the oral administration of Cr.

4) For kidney transplantation, explanted kidneys should be perfused with Cr and/or PCr to better preserve the organ before implantation and protect it from ischemic-reperfusion damage after transplantation [68]. In addition, Cr and/or PCr may also confer some protection to the kidney against toxicity related to immunosuppressive therapy.

5) Similarly, prophylactic Cr loading could protect the kidneys of patients who undergo contrast-enhanced X-ray-based imaging from contrast-induced kidney damage, which is considered to be a rather common and serious side effect [69].

State-of-the-art Cr supplementation of dialysis patients

Unfortunately, to date only a very few studies of Cr supplementation of very small numbers of dialysis patients have been performed. Generally, Cr supplementation is known to lead to Cr loading in target organs, including muscle, brain, and the kidney [25,26]. Therefore, the chronic ingestion of large amounts of Cr is not rational, since Cr that exceeds the storage capacity of an organ must be eliminated in the feces and/or excreted by the kidney into the urine. However, although Cr has very rarely been reported to be associated with kidney damage, the role of Cr in kidney damage has not been definitively established [45,70]. Chemically pure Cr taken at the recommended dosage (3–10 g daily with sufficient fluid) is definitely considered safe for healthy individuals such as athletes, and also for the patients with neuromuscular and neurodegenerative diseases, who have been studied [47]. Cr supplementation is also assumed to be safe for patients with CKD [18].

Gualano et al. investigated Cr supplementation in a young man with a single kidney and mildly decreased globerular filtration rate (GFR). The patient received high-dose supplementation with Cr (20 g/day) for 5 days followed by Cr (5 g/day) for 30 days without negatively affecting his kidney function [71]. A retrospective study of long-term high-dose Cr supplementation (13 g Cr/day for up to 4 years) that assessed 65 variables, including independent clinical markers for kidney function, did not observe untoward health effects, except for occasional gastrointestinal upset [72]. Notably, a double-blinded, placebo-controlled clinical study of 18 healthy participants who received 10 g of Cr per day for 3 months found a slight but significant increase in the glomerular filtration rate for the participants receiving Cr versus the controls, as measured by the cystatin clearance method [73].

Although not significant because of low numbers of patients, the few studies of Cr supplementation for patients with CKD undergoing dialysis did not report any negative effects. Furthermore, a single study of 10 CKD patients undergoing hemodialysis, who received 12 g of Cr/day for 4 weeks found significant improvement in muscle cramps [74]. A decrease in the number of episodes of symptomatic muscle cramps by 60% among the patients receiving Cr compared to those receiving placebo is an important result that is good news for dialysis patients. Muscle spasms can be very painful and greatly reduce a patient's QOL. The reduction in muscle cramps due to Cr supplementation indicates [57,75] that Cr improves the energetics of the skeletal muscle cells of CKD patients whose skeletal muscles are depleted of Cr. Ca^{2+}-sequestration, which is needed for efficient muscle relaxation, is one of the most energy-demanding processes executed by the ATPase for the SR Ca $^{2+}$-pump. The enzyme depends on a high $\Delta G_{ATPhydrolysis}$, and thus on a high cellular concentration of total Cr and high PCr/ATP ratio, which are provided by CK, normally located near the SR Ca^{2+}-pump, using a locally high PCr/ATP ratio [76]. The MM-CK knockout mouse, with a phenotype that manifests major problems with muscle relaxation, provides validation for these ideas [10].

The study by Chang et al. showing reduced muscle cramps in CKD patients receiving a relatively high dose of Cr (12 g/day) also did not observe adverse effects during the treatment and washout periods [74]. A study that analyzed plasma guanidino compounds in 20 dialysis patients taking 2 g/day of Cr for 4 weeks found that serum GAA concentrations were reduced, and while the serum concentrations of some other guanidino compounds such as keto-guanidino-valeric acid and argininic acid increased, the levels of guanidino-succinate did not change. Cr was obviously well tolerated, and there was no information on in the study report [67]. In light of the fact that the safety of higher dosages of oral Cr and/or a higher burden of accompanying Crn have not been established in detail for patients with CKD [67], larger clinical studies on Cr supplementation for CKD patients are needed. These studies must include more clinical endpoints than the smaller published studies and a detailed questionnaire about subjective changes noticed during the treatment with Cr. To support our hypotheses on the various health benefits of Cr supplementation, we will now discuss the wealth of data from investigations on Cr supplementation for healthy athletes and study participants, as well as for patients with conditions other than CKD.

Scientific basis and empirical data supporting the hypotheses

Dialysis patients are energy- and Cr-depleted, which is likely to be also true of their failing kidneys

That a general deficit in total body Cr and decreased PCr/ATP ratio indeed exist in CKD patients is based on the findings of significantly decreased total Cr levels in the skeletal and heart muscles of animal models of experimental uremia, as well as in those of CKD patients, concomitant with significantly decreased PCr/ATP energy-charge ratios in their hearts [58,77,75] and skeletal muscles [57,75]. Decreased total Cr levels and decreased PCr/ATP energy-charge ratios probably are also true for failing kidneys. Although these levels have not been directly assessed in kidney tissues, the decreased levels are probable; CKD patients have significantly lower serum Cr concentrations compared to healthy controls, which is indicative of the global depletion of total body Cr [34]. In addition, the first step in the endogenous Cr synthesis occurs in the kidney via GAA, and diseased kidneys with marked renal cell damage very likely show impaired synthesis of GAA, or no synthesis at all in CKD patients, which would contribute to the overall deficit of total Cr. The Cr deficit in skeletal and cardiac muscles and other organs (see above) due to impaired endogenous Cr synthesis, is additionally exacerbated by the washout and loss of Cr during each dialysis treatment. Additionally, the alimentary uptake of Cr is likely to be markedly lower in CDK patients because dialysis patients often present with anorexia and are also not allowed to eat large amounts of meat and fish, which are the best sources of alimentary Cr. The meat and fish intake of patients with CKD must be rigorously controlled and reduced, because meat and fish contain a large amount of inorganic phosphate, which must be avoided. In addition, the energy-demanding CrT resorption of Cr from primary urine in the distal tubules of the kidney [49] is also probably impaired in CKD patients. Impaired resorption is accounted for by the fact that the CrT reuptake of Cr is regulated by AMP-activated protein kinase (AMPK), which downregulates CrT expression when the energy state of the kidney is reduced (low PCr/ATP and low ATP/ADP ratio) in order to reduce the energy spent on processes not essential for cell viability [49]. As a matter of fact, it has recently been shown by a metabolomics analysis of urine metabolites of experimental animals undergoing ischemia/

reperfusion injury that urine Cr levels are significantly elevated in the experimental animals versus controls, indicating that a damaged kidney is less capable to reabsorb Cr from the distal tubuli urine [78]. Thus, because of decreases in the overall functions of the kidney, which include Cr synthesis and resorption, the metabolism of whole-body creatine is changed and impaired such that overall total Cr is markedly decreased, which is reflected by generally decreased serum Cr concentrations in patients with CKD [34]. The overall decrease in energy charge in the organs and cells of CKD patients, which is reflected by decreased PCr/ATP and ATP/ADP ratios, is bound to interfere with optimal physiological functioning of various organs and cells, including the kidneys of these CKD patients.

Since chronic dialysis leads to the washout of not only metabolic waste products, but also essential substances such as amino acids, fatty acids, cofactors, hormones, mineral salts, trace elements, and Cr; we propose that Cr together essential substances should be directly added to the dialysis fluid. These valuable intradialytic supplements can be reabsorbed from the circulating blood during dialysis. Cr could be reabsorbed via CrT, which is expressed on the surfaces of target organs and cells [8].

Creatine supplementation improves muscle mass, function, and coordination

Patients undergoing chronic dialysis have generalized energy depletion, which is indicated by the generally decreased cellular PCr/ATP ratio in skeletal and heart muscles [58,75,77]. This low energy state partially accounts for the loss of muscle mass in CKD patients, which is concomitant with muscle weakness and muscle fatigue. Since many dialysis patients are elderly, these signs and symptoms of sarcopenia, which are also frequently associated with senescence, are even more exacerbated in CKD patients. In fact, a low GFR is a risk factor for muscle weakness associated with sarcopenia [79]. Studies of athletes have yielded a surfeit of evidence, with more than 1000 publications showing that Cr supplementation leads to increased muscle mass and muscle strength in healthy young-to middle-aged study participants, especially with simultaneous physical training or sports [29,44]. There is also evidence that Cr provides similar benefits for elderly study participants undergoing moderate exercise [80]. Additionally, Cr improves the QOL of the elderly even without associated resistance training, by delaying muscle atrophy, improving endurance and strength, and helping to decrease the loss of ability to perform the activities of daily living [81,82]. Thus, the scientifically valid effects of Cr supplementation on muscle, such as maintaining and increasing muscle mass and function and improving coordination, represent important and decisive benefits for the QOL and survival of patients with CKD undergoing dialysis. The loss of mobility, either because of falls or at the time of initiation of dialysis, has been associated with significantly increased mortality in elderly CKD patients [83]. The reduction or prevention of falls by the simple addition of Cr to the dialysis solution would greatly benefit patients with CKD.

Creatine positively affects bone mineralization and bone density

Bone cells ubiquitously express the cytosolic BB-CK isoform, in addition to the mtCK isoform. The PCr/Cr system is also active in bone [14]. The BB-CK isoform also plays a crucial role in osteoclast-mediated bone resorption [84]. Cr was shown to stimulate the in vitro differentiation and mineralization of bone cells in a dose-dependent fashion [85]. Based on study design, some human studies have found that Cr supplementation increased bone density, especially in conjunction with exercise [82]. The preservation of a well functioning skeletomuscular system of patients with CKD for as long as possible is of paramount importance, especially for elderly CKD patients [83]. The patient will be able to maintain an independent life style, and falls and related comorbidities can be avoided.

Creatine is effective against mental fatigue and depression

Physical and mental fatigue are common problems of CKD patients who may also have depression and insomnia. Recent reports show that Cr is highly effective in this context. First, small double-blinded cross-over studies of humans have shown that Cr supplementation is beneficial for memory, learning, and mental and cognitive performance [29,86,87]. The effect of Cr on cognitive performance was especially pronounced if the study participants were sleep deprived before taking cognitive tests; at the same time, Cr supplementation reduced mental fatigue [88]. Notably, a human study showed that Cr supplementation (20 g/day) for 7 days led to increased corticomotor excitability with reduced cognitive decline when measured under mildly hypoxic conditions [89]. Most remarkably, a double-blind placebo-controlled trial of oral Cr supplementation showed an augmentation for enhanced response to a selective serotonin reuptake inhibitor in women with major depressive disorder [90]. Recently, in a dose-dependent Cr supplementation study involving antidepressant-resistant major depressive disorder, in which in vivo ^{31}P NMR spectroscopy was used to assess the brain PCr content, an obvious inverse correlation between brain PCr content and depression score was found [91]. Most recently using a similar patient cohort, it was shown that N-acetylaspartate levels and rich club connections increased after Cr augmentation of selective serotonin reuptake inhibitor treatment [92]. It was discussed that the antidepressive effects of creatine administration on brain energy metabolism and network organization may partly underlie its efficacy in treating women mit major depressive disorder [92]. These results suggest that Cr was indeed effective against depression. These data are certainly relevant for CKD patients, who often have depression. In a very recent publication, animal experiments were described showing that Cr supplementation counteracts in a similar fashion as a conventional anti-depressant drug, fluoxetine, the behavioral changes of mice accompanied by astrogliosis in the dentate gyrus of the hippocampus elicited by high-dose corticosterone, a pharmacological model of depression that mimics exposure to stress [93]. In a most recent publication it was shown that even a single treatment of creatine has partial effects as an antidepressant in mice with chronic mild stress-induced depression and that creatine treatment combined with exercise has synergistic effects and is a more effective prescription than a single treatment [94].

Creatine is an energy booster and protects cells against a variety of stressors

As mentioned previously, supplementation by Cr, which is a precursor of "energy-rich" PCr, leads to increased total Cr (Cr plus PCr) in target organs and cells and increased cellular PCr/ATP and ATP/ADP ratios, thus improving cellular energetic states to allow optimal physiological performance and protection against energy stressors. However, Cr is more than a mere energy precursor, and has pleiotropic effects [4]. Some of these effects are listed below:

Creatine stimulates mitochondrial functioning and protects mitochondria and tissues against oxidative damage

Preservation of mitochondrial function results in decreasing the rate of accumulation of damage to cells and organelles, thus retarding the ageing process and improving survival [95]. Cr provides both direct and indirect antioxidant effects and protects various

cells from oxidative damage [64,96]. The indirect antioxidant activity of Cr is due to the reduced production of free radical oxygen species (ROS) by mitochondria [97] in conditions where Cr-stimulated mitochondrial respiration via the mtCK isoform is functioning optimally. This mechanism was discovered in mitochondria isolated from skeletal and cardiac muscle and in permeabilized muscle fibers with intact in situ mitochondria [98]. Since kidneys also contain a sizable amount of mitochondria and rely on mitochondrial respiration, Cr probably also stimulates the functioning of kidney mitochondria, leading to reduced production of mitochondrial ROS and ameliorating oxidative damage and inflammation in the kidney [99].

Creatine acts as an antiapoptotic agent by suppressing the first steps of programmed cell death

The opening of the mitochondria permeability transition pore (mPTP), which is a central event in apoptosis, can be initiated by the release of Ca^{2+}, cytochrome-c, and ROS from mitochondria. This is accompanied by mitochondrial swelling and subsequent activation of caspases. At the same time, the expression of the Bcl2 protein is increased and the Bax protein is decreased, which is followed by eventual formation of apoptosomes [100]. The mtCK isoform is sandwiched between the ANT of the inner and the VDAC of the outer mitochondrial membrane, at the so-called mitochondrial contact sites. There, the inner and outer mitochondrial membranes are in close proximity, and the octameric mtCK stabilizes the contact sites between mitochondrial membranes [101]. This location is also where the mtCK isoform plays an important role in maintaining closed mPTPs [102]. Cr and its analogues can prevent the Ca^{2+}-induced opening of mPTPs and thus protect the mitochondria from swelling and initiating cellular apoptosis [103]. The stabilization of mitochondrial membranes by Cr or its analogues [103], as well as the binding of mitochondrial hexokinase to the outer and the mtCK isoform to the inner mitochondrial membrane, also require Mg^{2+} [104]. Cr and Cr plus D-ribose were shown to protect nonirreversibly injured ischemic cardiomyocytes from apoptosis, as measured by reduction in the activation of caspase-3 and reduced poly (ADP-ribose) polymerase (PARP) cleavage [105,106]. In addition, PCr was shown to protect human umbilical vein endothelial cells from apoptosis induced either by oxidized low-density lipoprotein [107] or by lipopolysaccharides [100]. A battery of tests was used to quantify cellular apoptosis. These data clearly demonstrate that Cr and PCr are bona fide antiapoptotic agents. Thus, because of its protective antiapoptotic effects on mitochondria and its potency as a direct or indirect antioxidant, Cr can be envisaged as an authentic *anti*-ageing agent. The findings that the brains of CrT-deficient mice manifested neurohistopathological findings consistent with early brain ageing support this claim [16]. The *anti*-ageing activity of Cr is also relevant for CKD patients, who manifest varying degrees of Cr deficiency, and who often seem to age prematurely.

Creatine protects organs from ischemic insults

Cr and PCr protect the heart and brain from ischemic insults and ROS-meditated reperfusion damage, both in vitro and in vivo [68,108]; and these protective effects of Cr and PCr probably extend to the kidney. Renal oxygen tension is low, especially in the inner medulla, which continuously functions under almost hypoxic conditions [109]. Oxygen tension cannot be increased in the medulla by an increased velocity of blood flow, which would increase the GFR and increase energy consumption. Since the efficiency of oxygen utilization by the kidneys is associated with paracellular sodium resorption by the tubular epithelial cells, which is facilitated by Na^+/K^+-ATPase [109], the overall energy charge reflected by the PCr/ATP ratio is critical. With chronic hypertension, which leads to an energy deficit, regions of the kidney rapidly become ischemic, a condition that leads to kidney failure or permanent damage. Under hypoxic conditions, mitochondrial function may be impaired, leading to vascular hypertrophy, accumulation of extracellular matrix, and the eventual development of glomerular sclerosis, tubular atrophy, and interstitial fibrosis [19]. These authors used a proteomic approach to find that under conditions of hypertension-induced renal damage, the cytosolic BB-CK isoform is one of the most highly upregulated proteins in the kidney, suggesting that the upregulation of the BB-CK isoform is a compensatory response to the increased energy demand in the damaged organ. Thus, a kidney under oxidative stress due to hypoxia/reperfusion or chronic hypertension, is an energy-starved organ, similar to the failing heart [110] or brain [111]. Animal studies have shown that after transient occlusion of a coronary artery, oral administration of a Cr analogue or direct intravenous infusion of PCr provided cardioprotection by preventing ventricular dysfunction. The maintenance of myocardial ATP levels correlated with improved hemodynamic parameters at multiple time points [68,112]. These findings are relevant for patients with CKD, because ischemia/reperfusion damage is the primary cause of acute kidney injury, which is characterized by a rapid decrease in the glomerular filtration rate and/or decreased urine output. Approximately 30%–40% of all cases of acute kidney injury occurring during hospitalization are observed in operative settings, particularly after cardiovascular surgery, where the reduced or interrupted renal perfusion with subsequent reflow induces inflammation and significant perturbation of kidney cell metabolism [78]. Thus, as for hypoxic cardiac muscle, Cr or PCr supplementation should be useful for preserving the residual function of the kidneys in CDK patients by alleviating damaging episodes of ischemia/reperfusion. These data are also important for CKD patients because of the many cardiovascular risk factors associated with deteriorating renal function and the adaptive changes that the heart undergoes during chronic dialysis, which include left ventricular hypertrophy and dilation, with concomitant systolic and diastolic dysfunction. Subsequent myocardial fibrosis is due to impaired angio-adaptation, reduced capillary angiogenesis, myocyte-capillary mismatch, and myocardial micro-arteriopathy, as stated by Amann et al. (2006)[59] and Wanner et al. (2016)[113]. Cr and/or PCr supplementation might be a pragmatic protective intervention for both the heart and kidney during renal replacement therapy. The benefits of Cr discussed previously above are supported by human studies showing that Cr supplementation attenuates endothelial cell dysfunction, as well as key inflammatory markers in patients with heart failure patients [114]. The presence of cytosolic CK and mtCK isoforms and high PCr has already been documented in vascular endothelial cells, which suggests that the high-energy PCr-shuttle is also used by these endothelial cells, and that these cells will benefit from Cr supplementation [115]. The preservation of and prevention of damage to vascular endothelial cells is also relevant for dialysis patients, who often develop calcified atherosclerotic plaques [113]. In a model of brain ischemia, Cr supplementation led to significant neuroprotection in rat pups after arterial occlusion [108] by improving endothelial cell functions, which led to increased cerebral blood flow [111]. Most relevant in this context is the fact that recently, it was convincingly shown that in the spiny mouse, a model for a precocious mammal, Cr added to the food of pregnant mice led to the astonishing protection of the new-born mouse kidneys after an oxygen-deprived birth [116]. The kidneys of nonpregnant and pregnant mice who received Cr supplementation took up significant amounts of Cr and retained the functional parameters of normal renal excretion [26]. Based on the additional findings in pregnant mice who received Cr supplementation that Cr protected the brain [117], skeletal muscles [118], diaphragm [119], and the

kidneys [26,116] of newborn mice from hypoxic/ischemic tissue damage; Cr supplementation was proposed as a general dietary intervention for perinatal protection of multiple organs [117]. These findings from investigations using the spiny mouse model suggest that Cr supplementation might be used to reduce fetal and neonatal morbidity and mortality in high-risk human pregnancy [120]. Finally, an open-label study of young human adults found that dietary Cr supplementation improved the reactivity of systemic endothelial-dependent microvasculature and increased capillary density in the skin [121]. All of these promising results described above are certainly highly relevant for dialysis patients as well. That is, Cr and/or PCr supplementation may not only prevent further damage to failing kidneys, but may at the same time positively affect other major organs and cells (cardiac muscle, brain, and skin), which are all energy-deprived in CKD patients. For example, cardiovascular events, which affect and involve cardiac myocytes and endothelial cells, are the primary comorbidities and risk factors associated with mortality in CKD patients [59]. Thus, as described above, the protective effects of Cr and/or PCr are likely to be relevant for maintaining cardiac health in patients with CKD. Likewise, Cr and/or PCr should be useful for improving residual function of kidneys in CKD patients, and protecting and maintaining the physiological functioning of kidney grafts.

Creatine acts as antioxidant and protects erythrocytes from oxidative and mechanical stress

Optimally sized energy reserves, that is, high cellular PCr/ATP ratios, are necessary for efficient body functions, because the work output per hydrolyzed ATP molecule is optimized; and the body has extra energy needed for endogenous cell repair and for defense against external cell stressors such as oxidative and mechanical stressors, which affect all types of cells in the body, including ECs and leukocytes. The protection of blood cells is important for CDK patients, because those cells are subjected to mechanical and oxidative stress during dialysis.

Erythroblasts as well as mature ECs and lymphocytes contain CK and Cr [122]. The concentration of Cr in ECs decreases with the age of the cells [123]; the ECs of uremic patients [124] and CKD patients undergoing chronic dialysis [125] have generally higher Cr concentrations than seen in the ECs of healthy controls.

Oral Cr supplementation of humans leads to a marked increase of Cr, not only in skeletal muscle and other organs including the kidneys [25,26], but also in ECs [33]. Thus, human ECs also express CrT. As observed with muscle [126], the PCr concentration also increases in ECs upon supplementation with Cr, because these cells contain CK. CK and high-energy PCr in ECs also play a role in swelling-induced ATP-dependent K^+/Cl^--cotransport [127] and Na^+/Mg^{2+}-antiport [128].

In addition, Tokarska et al. found that PCr and Cr directly protect cell membranes and intact ECs from mechanical and oxidative stress, respectively [63]. ECs can take up Cr, and roughly two-thirds of Cr is converted to intracellular PCr by CK. PCr interacts specifically with the phospholipids of EC plasma membranes, thus stabilizing the membranes against mechanical stress. The same protection to the membrane can be exerted by extracellular PCr, which cannot cross the cell membrane because of its negative charge [63]. In addition, creatine acting directly and/or indirectly as an antioxidant [129] significantly protects ECs against oxidative damage [63], which is a problem during dialysis. The antioxident properties of Cr on ECs have been fully corroborated and extended by a demonstration that Cr, and to a certain degree Crn, can act as antioxidants that protect ECs against oxidative stress and maintain the viability of lymphocytes after oxidative stress [122]. These findings were supported by an investigation that used scanning electron microscopy to assess the morphological integrity of ECs and lymphocytes [122]. The protective effect of Cr resulted in significantly reduced hemolysis, decreased release of methemoglobin, and reduced lipid peroxidation and intracellular protein carbonyl content [122]. Similar data were obtained from muscle cells and neurons that were exposed to oxidative stressors. Cr, acting as a direct and indirect antioxidant [64,96,129] was shown to exert significant cell protection, as well as trophic, prosurvival, and prodifferentiation effects in vitro and in vivo [64]. These data are corroborated by the findings of decreased levels of plasma markers of lipid peroxidation in laboratory animals receiving Cr supplementation [130]. Most importantly, when ECs of diabetic patients with uremia were exposed in vitro to 3–5 mM Cr, the rheological characteristics of the EC suspension were favorably altered so that they could be filtered through a 5 μm filter, indicating improved flexibility of the cell membrane [62]. In addition, hydrogen peroxide-induced formation of malondialdehyde was significantly inhibited in a dose-dependent fashion by addition of Cr. At the same time, Cr inhibition of the lipid peroxidation of EC membranes improved EC filterability and therefore improved maintenance of normal EC deformability. These effects were especially pronounced for ECs of diabetic uremic patients [62]. Since erythroid cells seem to be especially susceptible to oxidative stress [131], the antioxidant properties of Cr should also protect erythroblasts as well, and thus positively affect erythropoiesis in chronically anemic CKD patients [64,96,129].

The findings on the antioxidant effects of Cr are important, since they are relevant to the need to preserve as many intact ECs as possible during the hemofiltration process of hemodialysis. The reduced survival of ECs due to hemolysis associated with mechanical and oxidative stress mainly accounts for the chronic dialysis-induced anemia of CKD patients undergoing long-term dialysis. The concentration of Cr in ECs decreases with the age of the cells [123]; the ECs of both uremic patients [124] and CKD patients undergoing chronic dialysis [125] have generally higher Cr concentrations than seen in the ECs of healthy controls.

Thus, these data on the effects of Cr should have a beneficial translational impact on the management of dialysis patients, since fewer ECs and leukocytes would be damaged because of mechanical and oxidative stress during hemodialysis. At the same time, fewer erythroblasts would be lost to oxidative damage within the bone marrow of CKD patients, resulting in the maintenance of erythropoiesis at a higher level. And finally, less EPO would be needed for stimulating erythropoiesis in chronically anemic CKD patients [124]. In addition to the economic benefits of less EPO needed for CKD patients, patients receiving less EPO might have reduced risk of tumor progression, since EPO was recently shown to stimulate tumor growth [65,66]. These authors identified the EPO receptor EphB4 as a critical mediator of EPO-induced tumor progression in patients with cancer of the ovary and breast, which expands the clinically significant dimensions of EPO biology. Thus, the possible reduction of EPO usage in the management of CKD patients could lead to multiple important benefits.

Creatine reduces inflammatory markers and levels of total plasma homocysteine

Two human studies show that Cr supplementation reduces oxidative stress, as well as inflammatory markers in humans after repeated exercise [132], and reduces inflammatory and endothelia dysfunction markers in heart failure patients [114].

Total plasma homocysteine (tHCy) is an independent risk factor for cardiovascular disease; recent data have demonstrated a relationship between tHCy and free radical formation. In normal rats, Cr supplementation was found to reduce plasma tHcy levels, as well as lipid peroxidation biomarkers (TBARS) [133]. In addition, Cr supplementation markedly decreased tHCy in acutely exercised

rats [132], in Walker-256 tumor-bearing rats [134], and in animal models of chronic uremic renal failure [135]. However, tHCy was not reduced in CKD patients receiving high doses of folic acid and vitamin B6 and B12, who were also supplemented with low-dose Cr (2 g/day) [136]. As discussed by the authors, the high vitamin dosages might have masked the tHcy-lowering effect of Cr, and at the same time, the Cr dosage might have been too low, as corroborated by [137]. Most important within this context, the arginine-creatine pathway was found to be disturbed in adolescent recipients of renal grafts who were on immunosuppressive therapy. In these study patients, Cr excretion was negatively correlated with plasma concentrations of tHCy, which were significantly higher in the transplant recipients than in the controls, leading to hyper-homocysteinemia; although the transplanted kidneys worked well and showed no evidence of renal failure [138]. Defective methylation and impaired production of nitroxide contributed to the endothelial dysfunction observed in renal transplant patients [138]. Therefore, a fresh look at tHcy in CKD patients before and after supplementation with graded dosages of Cr is certainly warranted.

Creatine is an antidiabetic agent

Since type-2 diabetes mellitus in CKD patients is a serious comorbidity, the finding that Cr supplementation has antidiabetic activity is also of paramount importance for these patients. An early study revealed that Cr supplementation led to a reduction in accumulated collagen type IV in the diabetic kidney of db/db mice [139]; collagen accumulation is also observed in diabetic CKD patients. An in vitro study of pancreatic β-cells found that Cr in the presence of glucose potentiated insulin release, compared to glucose alone [140]. An in vitro study found that Cr is scavenges methylglyoxal, a prominent dicarbonyl compound that can be found in relatively large amounts in diabetic patients, which leads to the formation of advanced glycation products in vivo [141]. Thus, by eliminating methylglyoxal, Cr is likely to reduce the "carbonyl stress" of pathophysiological concentrations of glyoxal in diabetic CKD patients. Interestingly, short-term Cr supplementation (3 g Cr twice daily) of recently diagnosed patients with type-2 diabetes led to a reduction in blood glucose levels comparable to that of metformin (500 mg twice daily) [142]. The same was true for Cr (3 g once daily) compared to glibenclamide (3.5 mg once daily) [143]. Thus, short-term Cr supplementation appears to have a potent antihyperglycemic effect in human diabetics similar to established antidiabetic agents, although the long-term Cr supplementation of diabetics for controlling hyperglycemia has not been evaluated [144].

A study of healthy sedentary male participants found that Cr supplementation combined with aerobic training significantly improved glucose tolerance, but did not affect insulin sensitivity [145]. A randomized, double-blind, placebo-controlled clinical trial found that Cr plus an exercise program improved glycemic control in patients with type 2 diabetes [146]. The increased glucose uptake and translocation of glucose transporter type 4 observed in the skeletal muscle of the type-2 diabetics following Cr supplementation was associated with an increase in the muscle content of AMP-activated protein kinase (AMPK)-α, suggesting that signaling by the cellular energy state sensor AMPK may be involved in the effects of Cr supplementation on glucose uptake in type 2 diabetes [147]. The findings that Cr, provided as a safe and efficacious supplement, has antidiabetic activity suggests that Cr may be useful as a prophylactic or adjuvant therapeutic intervention, which might delay or prevent the onset of type 2 diabetes mellitus or treat type 2 diabetes in CKD patients. Importantly, Cr supplementation does not impair kidney function either in healthy athletes [73] or in type-2 diabetic patients [148], and Cr did not reduce the GFR of the kidneys of postmenopausal women [149].

Creatine is a lipid-lowering agent

Dyslipidemia and the fatty liver diseases, NASH and NAFL, are prevalent co-morbidities in chronic dialysis patients. Older findings in humans and recent findings from studies of animal models on the effects of Cr supplementation on lipid profiles and fatty liver disease, are noteworthy [150]. In a 1996 study, supplementation with Cr (5 g/day) for 56 days resulted in the significant reduction of blood lipids in men and women [151]. More recently, Cr was shown to prevent the accumulation of fat in the livers of rats fed a high-fat [152] or choline-deficient diet [153]. In parallel, Cr was shown to reduce the accumulation of hepatic triglycerides by stimulating fatty acid oxidation [154]. Thus, Cr supplementation may be envisaged as a potentially new approach for fatty liver diseases [150] also for CKD patients. Since hypercholesterolemia and inflammation in atherogenesis are two sides of the same coin [155], and since, as shown above, Cr supplementation favorably affects both of these pathologies, Cr supplementation should be an attractive therapeutic strategy for reducing atherogenesis in high-risk individuals, including patients with CKD [156].

Creatine modulates the immune system

The small number of studies that have examined the impact of creatine on the immune system have shown changes in the production of soluble immune mediators and in the expression of molecules involved in recognizing infections, specifically, toll-like receptors. Thus, there is increasing evidence that Cr supplementation may have a regulatory impact on the immune system [157].

Ingested creatine contributes to intestinal health

Mucosal surfaces of the lower gastrointestinal tract are subject to frequent, pronounced fluctuations in oxygen tension, particularly during inflammation. CK was found to be expressed in a hypoxia-inducible transcription factor (HIF)-dependent manner in an animal colitis model. Supplementation with dietary Cr markedly ameliorated both disease severity and the inflammatory response [158]. These findings confirm that HIF-regulated CK plays a role in the energy homeostasis of epithelial cells, which is needed for an intact mucosal barrier, functioning intestinal mucosa, and resolution of disease [158]. Since intestinal inflammatory bowel disease can be a comorbidity in CKD patients and kidney transplant recipients, the results of Glover et al. [158], if confirmed in humans, should be relevant to these patients.

Consequences of the hypotheses and discussion

Many signs, symptoms, and comorbidities of chronic dialysis patients are likely to be ameliorated by Cr and/or PCr. Thus Cr and/or PCr, by positively influencing mitochondrial physiology and function and by acting as antiapoptotic agents that prevent opening of the mPTP, which is the initial triggering event for apoptosis [100,107], may help to decrease the rates of cell damage and the accumulation of damaged intracellular organelle and thus retard the ageing process, improve the QOL, and prolong the survival of CKD patients.

To prevent the potential burden on residual kidney function by overloading the system of CKD patients with high-dose Cr, the intradialytic administration of Cr, proposed herein, would provide many advantages. In contrast to oral or intravenous administration of 5-10 g of Cr before the hemodialysis session, intradialytic

administration of Cr would allow the body to absorb only as much Cr from the dialysis fluid as needed by the body, with no need to clear the system of excess Cr. This is especially important in light of the fact that the safety of higher dosages of oral Cr or a potential burden of Crn have not been established for patients with CKD [135,136]. Since CrT has high affinity for Cr, with a Vmax for Cr of 20–40 μM Cr [8], relatively low concentrations of Cr, ranging from 1 to 5 mM, would be needed in the large volume of dialysis fluid. For example, if a CKD patient were exposed to 100 L of dialysis fluid containing 1 mM or 5 mM Cr, the patient would be exposed to a total of 13 to 65 g of Cr during a dialysis session. It can be assumed that diffusion of dialytic Cr will enter equilibrium with the blood, eventually at a constant Cr concentration of 1–5 mM in the serum as well as dialysis fluid. Therefore, it is also assumed that because of the high affinity of CrT for Cr, sufficient amounts of Cr could be taken during each 3–4-h dialysis session. However, to date, investigations on intradialytic Cr supplementation have not been performed, and the equilibration of Cr concentration gradients and Cr uptake kinetics would have to be determined under this novel scheme. Based on the finding that a Cr concentration of 3 mM significantly improved the rheology, ie, filterability of ECs [62], a 3-mM Cr concentration in the dialysate seems reasonable. If a higher concentration of Cr is required, a Cr concentration of 10 mM would still be entirely feasible, based on the maximum solubility of Cr in aqueous solutions (100 mmoles/l, strongly temperature dependent). Thus the optimal Cr concentration in the dialysate in relation to maximal Cr uptake by CKD patients has to be established and then the effects of Cr under the established conditions have to be studied by measuring a variety of parameters, and administering a comprehensive assessment questionnaire.

Future directions

Cr is consumed worldwide in huge quantities. It was officially approved by the European Food Safety Agency (EFSA) as a safe and effective nutritional supplement with a few valid health claims accepted by EFSA. Provided that the Cr supplement is chemically pure and is consumed at the recommended dosage, Cr has an excellent validated safety record and is without any serious side effects, as testified by millions of athletes worldwide and thousands of patients with neuromuscular or neuronal diseases (Parkinson's Huntington's etc), who were participants in several large multicenter clinical studies [47]. Cr is a metabolic amino acid that is essential for normal physiological function; and life without Cr is miserable, as opined by leading experts in the field [17]. Contrary to what is still believed worldwide by a majority of physicians, Cr is not toxic to the kidney, and is needed for normal kidney function [4,18,71,73,148]. Thus, we propose that Cr supplementation should be investigated in patients with renal insufficiency, before failure, to assess its efficacy at preserving residual function of the kidney and delaying the progression to irreversible kidney failure [26,116]. As proposed and scientifically justified in our review, intradialytic Cr supplementation should be clinically tested in CKD patients. For the same reasons as we presented for CKD patients, the usage of Cr and/or PCr is also recommended for recipients of kidney transplants. We propose that kidney donors should be loaded with Cr before kidney removal and that kidneys removed from deceased donors should be perfused with Cr and/or PCr, to delay the onset of irreversible tissue damage in donor kidneys during cold-storage and transport of the organ without an energy and oxygen supply. As soon as the donor organ is connected to the blood-stream of the recipient, the kidney transplant recipient should be flushed intravenously with a Cr/PCr-containing "renoplegic" solution to protect renal function as much as possible and to prevent ischemia/reperfusion damage to the kidney. PCr-containing cardioplegic solutions are similarly used for heart revascularization [159] and after myocardial infarction [68,160]. Furthermore, the arginine-creatine pathway was found to be disturbed in adolescent recipients of renal transplants on immunosuppressive therapy. In these study patients, excretion of Cr was negatively correlated with plasma concentrations of tHCy, which was significantly higher (hyperhomocysteinemia) in the transplant recipients compared with the controls, although the transplanted kidneys functioned well with no signs of renal failure [138]. Defective methylation and impaired production of NO contribute to the endothelial dysfunction observed in renal transplanted patients [138]. The possible benefits of appropriate therapeutic measures, including Cr supplementation, warrant further investigation. Finally, imaging media-induced nephropathy, seen after contrast-enhanced computed tomography or angiography, accounts for 10% of hospital acquired iatrogenic renal failure and is the third most common cause of acquired nephropathy [69]. Since Cr has been demonstrated to be effective against a number of different cellular and extracellular stressors and/or noxious substances, e.g., against damage induced by free oxygen radicals (ROS) etc. (see above), as well as against toxins such as the mitochondrial poison, rotenone, or the contact herbicide, paraquat [161]; a clinical study using preventive Cr loading before and during exposure to contrast media should be initiated.

Conclusions

In their review: "In sickness and in health: the widespread application of creatine supplementation", Bruno Gualano and coworkers [162] wrote: "There is an extensive and still growing body of the literature supporting the efficacy of Cr supplementation. In sports, creatine has been recognized as the most effective nutritional supplement in enhancing exercise tolerance, muscle strength and lean body mass. From a clinical perspective, the application of Cr supplementation is indeed exciting. Evidences of benefits from this supplement have been reported in a broad range of diseases, including myopathies, neurodegenerative disorders, cancer, rheumatic diseases, and type-2 diabetes. In addition, after hundreds of published studies and millions of exposures creatine supplementation maintains an excellent safety profile. Thus, we contend that the widespread application of this supplement may benefit athletes, elderly people and various patient populations" [162]. Many of these health claims are very likely to be relevant for CKD patients as well, and may contribute significantly to the QOL and health status of dialysis patients [163], as pointed out previously [4,164]. As shown mostly by studies on healthy individuals, Cr supplementation of dialysis patients are likely to positively affect not only the common health risks and problems of dialysis patients, such as muscle wasting, cardiovascular events, anemia, fatigue, and depression; but will also prevent and/or improve co-morbidities such as type 2 diabetes mellitus, metabolic syndrome, dyslipidemia, fatty liver disease, and inflammatory conditions [163]. These are good reasons to go back to the drawing board to design large controlled studies of different modalities of Cr and/or PCr supplementation for CKD patients, preferentially including relatively low concentrations of Cr and/or PCr added directly to dialysis fluid. Clinical studies are clearly needed to verify our hypotheses.

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Conflict of interests

Theo Wallimann, PhD and Prof. Emeritus of the Biology Dept. ETH Zurich, Switzerland, is a member of the scientific board; Michael Möddel, MD and Nephrologist at Klinik Im Park, Zürich, Switzerland, is a member of the medical board; and Uwe Riek, PhD, formerly at ETH Zurich, Switzerland, is a member of the directorial board of Crearene Ltd Bahamas, a start-up company that is dedicated to the development and promotion of Cr/PCr supplementation for dialysis patients. Crearene holds two related patents on this subject: US20120135943A1 and US 8791078 B2.

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Interested readers are recommended to consult for review of the current creatine literature the two Special Issues: (1) "Creatine in Health, Medicine and Sport", Amino Acids 2011; 40(5):1265–1418 (Roger Harris, Editor) and (2) "Creatine for life: in Health, Sport and Medicine", Amino Acids 2016; 48(8): 1739–2065 (Theo Wallimann and Roger Harrs, Guest Editors), where world-renown experts present up-to-date reviews and original papers on Cr, CK, CRT and related topics, where the progress in this field over the last 20 years can be felt. The emphasis of Cr research has been moved over these years from sport in the direction of health, prevention and diseases.

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