5 Creatine Consumption in Health

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1. INTRODUCTION

Concerns about the deleterious consequences of oral creatine (Cr) supplementation were initiated in Spring 1998. Two British nephrologists published a paper in "The Lancet" suggesting that there is "strong circumstantial evidence that Cr was responsible for the deterioration in renal function" *(1)* (details given in Section 3.3.2). Three days after this publication a French sport newspaper "L'Equipe" (28th April 1998) stressed that Cr is dangerous for the kidneys, in any condition. This news was handed over to several European newspapers. Cr became the champion's viagra with eventual death! Indeed, Pritchard and Kalra commented on the case of three American college wrestlers who died *(1)*. This later turned out to be false and the Food and Drug Administration (FDA) ruled out Cr supplementation as a primary cause of the deaths of these young athletes *(2)*.

A website of the "Food and Drug Administration" (http://vm.cfsan.fda.gov/~dms/aems.html) gives regularly reported complaints from voluntary consumers or healthcare professionals. The search of 20th October 1998 revealed 32 matches for Cr. They specify: dyspnea, fatigue, grand mal seizure, intracerebral haemorrhage, vomiting, diarrhoea, nervousness and anxiety, polymyositis, myopathy, rabdomyolysis, severe stomach cramps, deep vein thromboses, atrial fibrillation, cardiac arrhythmia, chest pain, and death! Potential adverse effects of oral Cr supplementation was also critically reviewed by Juhn and Tarnopolsky who concluded in 1998 that future studies should include large randomized controlled trials evaluating the short- and long-term effects on the renal

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and hepatic systems, as well as the many other organ systems in which Cr plays a metabolic role *(3)*. Furthermore, the FDA asks the reader to keep in mind "there is no certainty that a reported adverse event can be attributed to a particular product or ingredient. The available information may not be complete enough to make this determination." In other words there is no scientific evidence to correlate oral Cr monohydrate supplements with any of these reported adverse effects. Nevertheless, the FDA, the "Association of Professional Team Physicians," and the "American College of Sports Medicine" *(4)* concluded that, although short-term studies look positive for oral Cr supplementation, much more long-term research needs to be done before one can issue a verdict on its health status. In its consensus statement, the "American College of Sports Medicine" said that "the fact that Cr is a naturally occurring compound does not make supplementation safe, as numerous compounds are good, even essential in moderation, but detrimental in excess. The lack of adverse effects does not equal safety, since unending research must be performed to eliminate the possibility of all potential complications" *(4)*. In doubt anyone should be left to its free choice and interpretation. In 2004, a Scientific Panel of the "European Food Safety Agency" concluded that "the safety and bioavailability of Cr, Cr monohydrate, in food for particular nutritional uses, is not a matter of concern provided there is adequate control of purity of this source of Cr" *(5)*. However, that same year, a report of the "Agence Française de Sécurité Sanitaire et Alimentaire (AFSSA) claimed that "one should not encourage publicity of Cr in order to protect sport participants to any potential pathological consequences" *(6)*. Eventually, a world expert on Cr metabolism, M. Wyss, came to the conclusion that there are still many open questions related to Cr metabolism, which are worth being analyzed in detail *(7)*.

As will be observed later in this chapter, there are still several reports or reviews pointing out the uncertainties related to health hazard of Cr utilization in sport events or training. Moreover, commercials are adding ahead many positive allegations without any definite or formal evidences.

2. MODIFICATIONS INDUCED BY CR SUPPLEMENTATION WITHIN HEALTHY CONDITIONS

2.1. Total Body Mass

One of the purported effects of oral Cr supplementation regularly mentioned by the consumers is the increase in total body mass (BM) with special attention to muscle mass. On scientific grounds, the situation is less

clear. Indeed the average increase in BM reported in the literature amounts to 1–2 kg or 1–2.3% of total BM (Table 1). However, one has to explore the effects of short-term (<10 d), and medium-term (>10 d) on BM. In fact, the short-term supplementations are based on a mean 20 g/d whereas most of the medium-term supplements are consumed as 2–10 g/d. Table 1 emphasizes that the difference between short-term and medium long-term is not so important. The reported literature indicates 75% and 71%, respectively. Thus, despite the reduced daily charge of Cr supplements, most of the reports stressed the increase in BM. Nevertheless about 30% of published papers do not report any change in BM either after short-term or medium medium-term oral Cr supplementation.

Tentative explanations may be put forward to explain this observation on BM: the characteristics of the subjects and the supplementation protocol. Sedentary people, physically active individuals, and recreational athletes seem to respond equally to oral Cr supplementation. Thus, individuals need not be physically active to increase BM to oral Cr supplementation despite different daily energy intake. It cannot be concluded from specific studies on female vs male subjects. Moreover, none of these few studies controlled the menstrual cycle of the female subjects. Presently, it is difficult to postulate a sex dependence as there is no scientific evidence to support those differences. Eventually, not all studies have been made using a control group (no training session) in addition to a placebo and Cr supplementation groups.

Also it will be investigated if the increase in total BM could be attributed to the effect of training itself (*see* Table 1). Apparently, no statistical differences were found in BM in inactive control subjects followed during 9 wk as compared with active people involved in resistance training for the same period of time *(8)*. Thus, even during endurance training, the increase in BM was clearly related to Cr supplementation.

2.2. Free-Fat Mass

Of course, the increase in total BM should be in favor of a more specific characteristic for the athlete; namely free-fat mass (FFM). A dozen studies did investigate the modifications of FFM after Cr supplementation (Table 2). As expected, 11 out of 12 studies reported a higher increase of FFM, as compared with total BM. The increase may even reach 6% after sustained Cr load.

The increase in FFM could be attributed to carbohydrate supplements added to Cr ingestion in several studies. Indeed, water binding to muscle and liver glycogen occurs after glucose loading. Green et al. *(9)* and Robinson *(10)* observed that whole body- and muscle-Cr retention was increased when large amounts of simple carbohydrates were ingested in

Table 1
Cr Supplements and BM Changes

Gender	*Population*	*Dose (g/d)*	*Duration (d)*	*Effect on BM(% change)*	*References*
Short-term (<10 d)					
M	Active	24	6 d	+1.5	*140*
M	Trained	20	6 d	+1.2	*141*
M	Active	20	5 d	+1	*142*
M	Active	20	5 d	+1.3	*143*
M	Active	20	6 d	+1.4	*144*
M	Active	20	5 d	+1	*145*
M	Sedentary	20	5 d	+1.1	*9*
M, F	Swimmers	20	5 d	+1.1	*146*
F	Active	0.5/kg body wt	6 d	Stable	*147*
M	Weight lifters	20	5 d	+2.3	*148*
M, F	Cyclists	20	5 d	Stable	*149*
M, F	Swimmers	21	9 d	Stable	*150*
F	Active	25	7 d	Stable	*151*
M, F	Active	18.8	5 d	Stable	*152*
M	Football	21	5 d	Stable	*153*
F	Runners	20	5 d	Stable	*154*
F	Active	20	4 d	Stable	*51*
M	Runners	0.35/kg body wt	3 d	+2	*14*
M, F	Active	20	5 d	Stable	*90*
M	Active	11.4	5 d	+2.3	*91*
M	Karatekas	20	5 d	+1.3	*155*
M	Active	30	5 d	+1.3	*156*
M	Active	20	5 d	+1.4	*10*
M	Trained	25	7 d	+2.1	*157*
M	Westlers	20	5 d	+1.3	*158*
M	Active	20	5 d	+1	*159*
M	Active	20	5 d	+1.8	*58*
M	Active	5	5 d	+2.3	*160*
M	Weight lifters	20	5 d	+0.9	*160*
M	Active	20	5 d	+1.9	*161*

(Continued)

Table 1 *(Continued)*

Gender	*Population*	*Dose (g/d)*	*Duration (d)*	*Effect on BM(% change)*	*References*
F	–	–	–	+0.6	–
M	Soccer	20	6 d	+0.8	*83*
M	Senior	20	5 d	+0.5	*17*
M	Active	20	5 d	Stable	*162*
M	Active	21	7 d	+1.4	*163*
M	Rowers	20	6 d	+1.9	*164*
M	Football	20	5 d	+3.4	*15*
M	Active	20	6 d	Stable	*165*
M	Triathletes	20	5 d	+1.1	*166*
M	Active	20	4 d	+1.5	*87*
M	Active	20	5 d	+0.8	*167*
M	Active	20	7 d	+0.2	*168*
M, F	Runners	0.35/kg body wt	3 d	+1.1	*169*
M	Active	20	5 d	+1.4	*170*
M	Weight lifters	20	4 d	+2.2	*81*
M	Handball	20	5 d	+3.4	*82*
M	Active	20	5 d	+1.2	*94*
M	Active	20	5 d	+1.8	*171*
M	Active	0.25/kg body wt	7 d	+2.7	*16*
M	Swimmers	20	8 d	+2	*172*
M	Active	20	5 d	+1.6	*127*
M, F	Active	20	7 d	+2.4	*173*
M	Active	21	5 d	Stable	*174*
M	Swimmers	20	5 d	Stable	*175*
Medium and long-term (>10 d)					
M	Weight lifters	20	14 d	+1.9	*176*
F	Swimmers	2	42 d	Stable	*177*
M	Football	3	14 d	+0.8	*178*
M, F	Active	0.3/kg body wt	42 d	+2	*179*
F	Active	10.5	51 d	Stable	*153*
F	Active	5	60 d	Stable	*51*
M	Active	3	47 d	+1.8	*180*

(Continued)

Table 1 *(Continued)*

Gender	*Population*	*Dose (g/d)*	*Duration (d)*	*Effect on BM(% change)*	*References*
M, F	Active	3	47 d	Stable	*90*
M	Football	16	28 d	+1	*50*
M	Active	3	63 d	+2.9	*8*
M, F	Swimmers	10	14 d	Stable	*181*
M	Football	8	35 d	+1.4	*182*
M	Active	7	77 d	+6.3	*157*
M	Senior	20	30 d	+0.6	*183*
M	Active	21	14 d	Stable	*184*
M	Active	0.1/kg body wt	21 d	+2.4	*185*
M	Active	0.03/kg body wt	365 d	+10.6	*64*
M	Active	0.1/kg body wt	42 d	+0.9	*92*
M	Senior	0.3/kg body wt	84 d	+3.4	*186*
M	Weight lifters	10	56 d	+5.4	*72*
M	Active	6	84 d	+5.8	*43*
M, F	Active	5	14 d	–	*187*
M	Senior	5	180 d	Stable	*188*
M	Senior	5	98 d	+1.2	*187*
F	–	–	–	+1.7	–
M	Active	5	30 d	+1.3	*189*
M	Active	0.2/kg body wt	42 d	+4	*190*
F	–	–	–	Stable	–
M	Active	0.3/kg body wt	28 d	+2.9	*191*

conjunction with Cr. However, the conclusions of the two studies are not similar. Green reported that Cr loading (20 g daily during 5 d) increased total BM by 0.6 kg but when 370 g of glucose was added over the course of the day the subjects gained an additional 1.5 kg *(9)*. On the contrary, Robinson did not observe significant total BM change after 5 d of 277 g glucose supplements but did report a 1.4% increase (1 kg) postsupplementation with Cr + carbohydrate *(10)*.

Table 2
Cr Supplements and FFM in Humans

Gender (Nb)	*Population*	*Dose (g/d)*	*Duration (d)*	*Effect on FFM (% increase)*	*References*
F (20)	Active	5	70	+5.7	*51*
M (10)	Active	5	5	+3.9	*160*
M (10)	Active	20	5	+2.2	*160*
M (24)	Active	0.1/kg body wt	21	+2.8	*185*
M (7)	Active	2	42	Stable	*161*
F (8)	–	20	5	Stable	–
M (9)	Active	20	5	+3.7	*15*
M (16)	Senior	0.3/kg body wt	84	+6.1	*186*
M (6)	Active	6	84	+4.4	*43*
M (11)	Active	10	56	+6.4	*72*
M (10)	Active	20	4	+3	*81*
M (23)	Active	5	180	+1.5	*188*
		5	365	+1.9	
M (5)	Active	5	98	+2.5	*192*
F (5)	–	–	–	+5.9	–
M (9)	Active	0.3/kg body wt	28	+3.4	*191*

It has been demonstrated that supraphysiological circulating concentrations of insulin, as induced by glucose ingestion, are required to augment muscle-Cr accumulation in humans *(11)*. Consistent with this observation, Willott et al. *(12)* reported that the highly insulin-sensitive rat soleus muscle had higher rates of Cr uptake than the relatively less insulin-sensitive extensor digitorum longus muscle. However the same authors pointed out that insulin had no direct effect on ^{14}C-labeled Cr uptake rates at concentrations in which effects are seen on glucose uptake, glycogen synthesis, and glycolysis. Moreover, using a competitive inhibitor (β-guanidoproprionic acid) and low-extracellular Na^+ concentrations to study the rate of ^{14}C-labeled Cr uptake in isolated rat muscle, Willott et al. suggested that the rate of Cr uptake is strongly dependent on the extracellular Na^+ concentration, insulin playing only a minor role in the regulation in Cr transfer *(12)*.

2.3. Skeletal Muscle Mass

The increase in FFM could be attributed more specifically to muscle mass volume changes. There are a few recent reports that investigate muscle volume changes using either anthropometric dual energy X-ray absorptiometer (DEXA), electrical bioimpedance, or magnetic resonance imaging techniques after Cr supplementation (Table 3). Local muscle groups were measured after resistance training under Cr load. In most cases, the direct effect on muscle volume was observed with a mean increase of 12% when supplementation and training were maintained for several weeks. Thus, one may conclude that oral Cr supplementation has a direct effect on muscle mass volume. However, this increase may be because of water retention in the muscle and/or to real accretion of muscle protein.

2.3.1. Water Distribution in Muscle Tissue

The increase in muscle mass by Cr may be because of water retention in the intracellular compartment or to an increase in dry mass. Hultman et al. *(13)* suggested that the increase in BM during acute Cr feeding is likely to be attributable to body water retention as they observed a 0.6-L decline in urinary volume after ingestion of 20 g of Cr for 6 d.

Multifrequency bioimpedance technique can distinguish between, and assess changes in the body fluid compartments of human subjects (Table 4). Ziegenfuss *(14)* reported 6.6% in tight skeletal muscle volume and a 2–3% increase in total body and intracellular water volume in aerobic and cross-trained men after 3 d oral Cr feeding. In 1999, the incorporation of intra- and extracellular water (by multifrequence bioimpedance) in subjects under Cr supplementation over a period of 9 wk was measured *(8)*. The observed increase in BM (2 kg) could be attributed partially (55%) to an increase in the body water content and more specifically to an increase in the volume of the intracellular compartment (+4.9%). A few more studies repeated these short-term Cr loadings *(15–18)*. All studies but one reported a stable proportion of extracellular water and a 3–8.9% increase of intracellular water. The mechanisms by which Cr supplementation increases intracellular water remains unclear. It is known that Cr transfer into muscle sarcoplasm is governed by a Na^+-dependent, saturable transporter included in the plasma membrane *(19)*. It remains also possible that an osmotic draw of fluid into the intracellular compartment would explain the increase in muscle mass volume. But it may also be suggested that the gain in muscle BM should not be attributed only to water retention, but probably to dry matter growth accompanied with a normal water volume.

Table 3
Cr Supplements and Muscle Volume Changes

Gender (Nb)	*Population*	*Dose (g/d)*	*Duration (d)*	*Exercise (type)*	*Muscle*	*Technique used*	*Effect on muscle (% increase)*	*References*
M (20)	Active	20	5	Resistance training	Thigh	Anthropometry	+6	*185*
M (6)	Untrained	6	84	Resistance training	Thigh	Anthropometry	+18.1	*43*
M (12)	Active	0.25/kg body	7	Power training	Total	DEXA	+1.3	*16*
M (6)	Active	0.2/kg body wt	42	Resistance training	Elbow	DEXA	+12.8	*190*
F (5), M (12)	Active	20	5	Sprint cycling	Thigh	MRI	Stable	*127*
M (12)	Swimmers	20	8	Sprint training	Total	BIA	Stable	*172*
F (6), M (9)	Active	0.3/kg body wt	28	Power training	Total	DEXA	+5.1	*191*

DEXA, dual energy X-ray absorptiometer; MRI, magnetic resonance imaging; BIA, electrical bioimpedance.

Table 4
Cr Supplements and Body Water Distribution Modification

Dose (g/d)	*Duration (d)*	*Total body water (% change)*	*EC water (% change)*	*IC water (% change)*	*References*
0.35/kg body wt	3	Stable	Stable	+3	*18*
3	63	Stable	Stable	+4.9	*8*
20	5	Stable	Stable	Stable	*17*
0.3/kg body wt	7	+0.8	–	–	*88*
20	5	+5.3	Stable	+8.9	*15*
0.25/kg body wt	7	+4.5	+4	+4.9	*16*

EC, extracellular; IC, intracellular.

2.3.2. Protein Muscle Mass

Most, if not all, commercial allegations argued on the basic consequences of sustained creatine supplementation: the increase in skeletal muscle protein mass. Several scientific publications also emphasize the higher content of muscle proteins by indirect implications of the BM or FFM increases. Do we have enough experimental arguments to support this important allegation?

One has to avoid inaccurate mismatch of publications mixing animal studies with human experiments, embryonic and growing models with stable adult situations. Thus, experimental information shall be separated and conclusions refrained to specific models.

2.3.2.1. Animal Studies. The hypothesis of a dry mass increase under Cr supplementation has been already introduced in 1972 by Ingwall et al. *(20)* from in vitro experiments on mononucleated cells and from breast muscle from 12-d chick embryos. Their experiment demonstrated that skeletal muscle cells synthesize myosin heavy-chain faster when supplied with Cr in vitro. The response was apparent within 4 h after addition of Cr to the culture medium and was concentration-dependent over the range of 10–100 μmol (1.3–13 mg/L). Two years later Ingwall et al. showed that Cr stimulated selectively in cultures of differentiating skeletal muscle the rate of synthesis, and not the rate of degradation, of the two contractile proteins, actin and myosin heavy chain *(21)*. The same group extended their previous research on isolated hearts from 17 to 21-d fetal mice maintained in organ culture *(22)*.

Using C2C12 cells, Louis et al. *(23)* were able to confirm that when 5 mmol Cr monohydrate were added to the differentiation medium this supplementation promoted myotube growth whereas guanidopropionic acid depressed it (Francaux, unpublished). This observation postulates that myotube growth could be controlled by the energy status of the cell. Ovine *(24)* and rat *(25)* myogenic satellite cells also appeared to have an effect on growing myofibrils. Both papers suggested that Cr monohydrate supplementation induces differentiation of myogenic satellite cells.

Flisinska-Bojanowska conducted an experiment on rats supplemented with Cr (10 mg/100 g body wt/d) *(26)*. She electrostimulated (50 Hz, 10 min daily for 14 d) the gastrocnemius muscle, and fragments of the white and red portions of the muscle were analyzed for soluble and myofibrillar proteins. The Cr supplemented rats had a 16% increase in myofibrillar proteins, specifically in the white portion of the gastrocnemius when compared with the control group (no Cr, no stimulation). However, it appears that when rats were electrostimulated without Cr supplementation the increase in myofibrillar proteins amounts 50% in the white portion and 37% in the red portion of the muscle. Cr supplementation alone did not change the content of the white muscle but increased the myofibrillar proteins by 18%. So far, these results do not establish a clear-cut on the beneficial effect of a Cr supplements on muscle proteins.

Two further studies do support the conclusion of Ingwall and Flisinska-Bojanowska. Brannon et al. *(27)* investigated the combined effects of Cr supplementation (3.3 mg Cr/g of chow diet) and high intensity run training on the performance capacity and biochemical properties of rodent skeletal muscle. There were no significant changes in either phosphorylcreatine kinase (PCK) activity or myosin heavy-chain isoform distribution following training or supplementation. However, these authors did not give any data on the synthesis of myosin. Fry et al. *(28)* re-examined the effects of Cr supplementation on muscle protein synthesis in tissue culture. They could not support the observation of Ingwall *(20–22)* and Flisinska-Bojanwoska *(26)*. On the contrary, it seems that when adult rats are depleted for Cr through administration of the analog β-guanidopropionic acid, there was a reduction of muscle myofibrillar proteins and atrophy of fiber II *(29,30)*. Adam et al. *(31,32)* investigated the running performance and myosin isomers after β-guanidopropionic acid treatment. This specific drug did not induce any change in running performance but the myosin isomers appear to be reoriented toward the type I phenotype. The interpretation of results obtained in rodents regarding Cr supplementation must be taken with caution. Indeed, although it is generally well accepted that

Cr supplementation increases total muscle-Cr content by about 20% in humans *(13)*, such changes are systematically observed in rodents *(33)*. Moreover, protocols of Cr depletion by β-guanidopropionic acid treatment may not be interpreted as inducing opposite effects of Cr supplementation. Indeed β-guanidopropionic acid can impair ATP level whereas Cr supplementation did not modify muscle-ATP concentration.

Moreover, Murphy et al. *(34)* analyzed the effect of Cr on contractile force and calcium sensitivity in mechanically skinned single fibers from 24- to 28-wk old rat skeletal muscles. They added Cr to the contracting solution in combination with an appropriate volume of water to maintain osmolarity constant and they observed that this solution had beneficial effects on performance of contractile apparatus. This finding suggests that the initial improvement in performance observed with Cr supplementation could be because of a decrease in ionic strength induced by water retention rather than to an energetic effect provoked by higher muscle phophorylcreatine content.

To conclude, there is indirect evidence that Cr supplementation induces muscle protein in vitro and in growing cells and animals.

2.3.2.2. Human Experiments. Already in 1990, Bessman et al. *(35)* suggested that Cr could induce muscle hypertrophy in adult subjects. They founded their hypothesis on the increased uptake of amino acids by the muscle and thereafter an enhanced biosynthesis of myofibrillar proteins. However, they stated that the concentration of Cr *per se* might not be responsible for the stimulation of protein synthesis seen in physiological active muscle. It might well be the increased transport of phosphorylcreatine in the intervening space during contraction that makes more energy available for the ribosomes. Along the same line, it has been shown that cell swelling act as an anabolic signal stimulating protein synthesis and net protein deposition *(36,37)*.

The effect of acute Cr monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis has been studied more directly in adult human subjects by Parise et al. *(38)*. Young healthy men and women were allocated to Cr (20 g/d for 5 d followed by 5 g/d for 4 d) and tested before and after the supplementation period under rigorous dietary and exercise controls. Intravenous infusion of L-[1-^{13}C]-leucine and mass spectrometry were used to measure mixed-muscle protein fractional synthetic rate and indexes of whole body leucine metabolism. These authors conclude that there was no increase of whole body or mixed-muscle protein synthesis under Cr supplementation. Healthy men were tested before and after oral Cr supplementation (21 g/d during 5 d), myofibrillar protein synthesis in the vastus lateralis and muscle protein breakdown using intravenous infusion of L-[1-^{13}C]-leucine and

L-[$^2H_5$]-phenylalanine, without and with maltodextrin and protein feeding *(39)*. Feeding led to a doubling of myofibrillar protein synthesis and a 40% depression of muscle protein breakdown, but no effect of Cr monohydrate was found on these parameters either in the fed or fast states. Furthermore, the possible stimulatory effect of Cr loading (21 g/d for 5 d) was examined in conjunction with acute resistance exercise on an isokinetic dynamometer (20 × 10 repetitions of leg extension-flexion at 75% of one repetition maximum one leg, before and after Cr intake) *(40)*. Muscle biopsies and arterio-venous differences, under L-[1-^{13}C]-leucine and L-[$^2H_5$]-phenylalanine venous infusion, were used to measure synthetic rates of myofibrillar and sarcoplasmic proteins or muscle protein breakdown. Exercise increased the synthetic rates of myofibrillar and sarcoplasmic proteins by two- to threefold and leg phenylalanine balance became more positive, but Cr loading was without any anabolic effect. Clearly, both exercise in itself and food are much more stronger stimuli for protein synthesis than Cr intake in healthy adult individuals *(41,42)*.

However, a few studies have recently utilized different technical tool of molecular biology to investigate the membrane Cr transporter, muscle-specific gene expression, and some regulatory signals of protein synthesis. Willoughby and Rosene investigated the effect of oral Cr on myosin heavy chain expression in adult male subjects after resistance training *(43)*. Their results on 12 wk Cr supplementation suggested that the expression of myosin heavy chain mRNA are reflected in the observed increase in myofibrillar protein content. Additionally, Deldicque et al. *(44)* investigated the effect of Cr supplementation on insulin-like growth factor (IGF-I and -II) mRNA expression, including the PI3K-Akt/PKB-mTOR signaling pathway, in adult human skeletal muscle. IGF-I and -II mRNA were slightly, but significantly, increased after Cr supplementation (5 d, 21 g/d). IGFs stimulate the PI3K-Akt/PKB-mTOR signaling pathway, which is involved in the regulation of skeletal muscle fiber size and in the stimulation of translation initiation by activating mTOR and two of its downstream effectors, namely $p70^{s6k}$ and 4E-BP1 (eukaryotic initiation factor-4E binding protein-1). The subjects were submitted to a resistance exercise session consisting in a one-leg knee extension and muscle biopsies were taken before and after the exercise test. Although resistance exercise was shown to increase both IGF-I and -II mRNA, Cr did not potentiate this effect. Three hours after stopping the exercise, Cr supplementation did not induce any change in $p70^{s6k}$ or 4E-BP1 expression, as compared with placebo experiment. However, the phosphorylation of the 4E-BP1 factor displayed a slight increase at the 24 h postexercise under the Cr supplementation condition. These conclusions

on Cr transporter mRNA in young and elderly healthy humans are also reported by Tarnopolsky et al. *(45)*. However, Willoughby and Rosene reported a positive effect of oral Cr and resistance training on some myogenic regulatory factor (MRF) expression *(46)*. Their study suggested that 12 wk of Cr supplementation, in conjunction with heavy resistance training, increase the mRNA expression of muscle-Cr kinase by way of pretranslational mechanism, likely owing to the concomitant increases in the expression of myogenin and MRF4.

A recent publication by Olsen investigated the influence of Cr monohydrate (6–24 g/d) or protein (20 g/d) supplementation on satellite cell frequency and myonuclei number in healthy adult men during 16 wk of heavy resistance training *(47)*. The results of this study showed that, after 16-wk training, muscle mean fiber area increased by 17% or 8% under Cr or protein supplementation respectively, as compared with 4% in a placebo group. The author concluded that Cr supplementation in combination with strength training amplifies training-induced increase in satellite cell number and myonuclei concentration in adult human skeletal fibers, thereby allowing an enhanced muscle fiber growth in response to strength training.

Eventually, under induced immobilization in adults, Cr supplementation may lead to the expression of muscle myogenic factors as shown by a few papers *(48,49)*. Immobilization of the leg by cast during 2 wk decreased the cross-sectional area by about 10% and maximal knee-extension power by nearly 25%. Oral Cr supplementation stimulated muscle hypertrophy during rehabilitation strength training. This effect appears to be mediated by MRF4 and myogenin expression.

2.4. Muscle Cramp Incidences

Anecdotal reports from athletes have claimed that Cr supplementation may induce muscle cramps (*see* the FAO website). In a recent unpublished study, 12 young healthy males were fed with Cr 3 g/d over a period of 28 d. The subjects were physically active. Five subjects present at least one cramp during sport activities over the supplementation period. Nevertheless, there is no proof to certify that these cramps are directly related to the Cr supplementation. The prevalent hypothesis to explain this potential side was an imbalance in muscle electrolytes. However, Kreider investigated athletes involved in heavy resistance training (5 H/d) for 28 d *(50)*. They were supplemented daily with 15.75 g of Cr monohydrate. There was no evidence of muscular cramping during resistance training sessions or during performance trials. Along the same line, Vandenberghe et al. *(51)* pursued a study on sedentary female subjects who were involved in a 10-wk resistance training with Cr supplementation

(20 g/d for 4 d, then 5 g/d up to 10 wk). No spontaneous side effects were reported during the entire duration of the study. Nevertheless, Juhn et al. *(52)* reported muscle cramping in 25% of 52 baseball and football players who were supplemented with 6–8 g/d during 5 and 3 mo, respectively. A few later studies on 96 young healthy subjects trained during 3 yr *(53)* or on 10 older men *(54)* involved in resistance training did not report cramping incidences owing to Cr supplementation.

The anecdotal reports of muscle cramping might be owing to the intensity of exercise rather than Cr supplementation. Staying well-hydrated could reduce this risk. Moreover, psychological stimulation could foster an individual to exercise over his/her optimal intensity. Meanwhile, further epidemiological studies should be performed to evaluate this potential side effect.

2.5. Gastrointestinal Complaints

Even if rumors sometimes evoke gastrointestinal distress (stomach upset, vomiting, and diarrhoea) among consumers of oral Cr, these assertions are not supported by real evidences. The scientific literature lacks precise information on this matter. Nevertheless, one report from Vandenberghe et al. *(51)* stated that one-third of their subjects (3/9) had minor gastrointestinal distress during 3 d of Cr (40 g/d) and caffeine (400 mg/d) supplementation. As well, Juhn et al. *(52)* reported diarrhoea in 31% of their baseball and football players who were supplemented with 6–8 g of Cr monohydrate during 5 (baseball) and 3 (football) mo, respectively. They suggested that this side effect may be the result of the unusually high osmotic load imposed on the digestive tract of some subjects. On the contrary, Kreider et al. *(50)* did not observe any disturbances among their subjects. Greenhaff supported this observation with his population ingesting 20 g Cr/d *(55)*. However, he mentioned that some discomfort can occur if Cr is incompletely dissolved before ingestion.

In conclusion, there is no reason to believe that oral Cr supplementation had any detrimental effect on the gastrointestinal tract.

3. EVALUATION OF POTENTIAL DETRIMENTAL EFFECTS OF CR SUPPLEMENTATION

3.1. Liver Dysfunctions

Despite the allegations published in sports newspapers and periodicals there are seldom information on liver metabolism changes induced by oral Cr supplementation. Some publications have reported data on liver function while consuming Cr supplements *(50,56–64)*. These results are summarized in Table 5.

Table 5
Cr Supplements and Liver Enzymes in Human Plasma (Mean ± SD)

Enzymes (IU/L)	*Base line*	*Cr*	*Dose (g/d)*	*Duration (wk)*	*References*
ASP	27.5 ± 2.1	32 ± 2.1	20	5	*58*
	42.8 ± 22.6	35.7 ± 10.7	3	4	*62*
	21.3 ± 1.4	22 ± 1.2	10	12	*56*
	19.6 ± 3.7	19.1 ± 1.6	3	9	*62*
	23.1 ± 8.6	26.9 ± 13	15.75	8	*50*
	25 ± 17	32 ± 13	13.7	216	*64*
	26.4 ± 12.9	29.9 ± 8.6	13.9	150	*60*
	34 ± 24	32 ± 22	5–10	76	*50*
	27.2 ± 7.2	29.7 ± 9.2	5	300	*193*
ALT	12.2 ± 1.2	12.7 ± 1.1	20	1	*58*
	27 ± 8	30 ± 7	20	9	*63*
	29.3 ± 15.4	27 ± 8.8	3	4	*62*
	65.8 ± 3.9	70.7 ± 3.7	10	12	*56*
	21.6 ± 2.7	18.7 ± 2.9	3	9	*62*
	24.1 ± 4.7	28.1 ± 9.8	15.75	8	*50*
	24 ± 13	29 ± 15	13.7	216	*64*
	14.5 ± 5.2	14 ± 6.3	13.9	150	*60*
	27 ± 11	27 ± 14	5–10	76	*50*
	21.9 ± 6.1	21 ± 8.9	5	300	*193*
GGT	17.4 ± 2.4	16.4 ± 2.4	20	5	*58*
	27 ± 7	27 ± 9	20	9	*63*
	19.5 ± 5	19.8 ± 7	3	4	*62*
	24.7 ± 2.8	20.1 ± 1.8	10	12	*56*
	24.7 ± 13.2	25.2 ± 15.8	15.75	8	*50*
	27 ± 14	21 ± 9	13.9	150	*60*
	31.4 ± 8.9	28.4 ± 6.7	5	14	*192*
	16.5 ± 6.2	15.3 ± 5.3	5	300	*193*
ALP	72 ± 9	72 ± 9	20	9	*63*
	81.3 ± 18.3	79 ± 16.7	3	4	*62*
	74 ± 13	81 ± 19	13.7	216	*64*
	65.2 ± 15.3	65 ± 17	13.9	150	*60*
	91 ± 29	93 ± 17	5–10	76	*50*
	237 ± 57	209 ± 37	5	300	*193*

ASP, aspartate-oxaloacetate aminotransferase; ALT, alanine-pyruvate aminotransferase; GGT, γ-glutamyl aminotransferase; ALP, alkaline phosphatase.

Indeed, the same group investigated serum enzymes levels, which are of interest for liver origin *(56)*. No changes in enzyme levels were observed during the 8 wk supplementation. Additional information was obtained after oral Cr supplementation in trained subjects (Table 5). No statistical differences were observed throughout the study as far as alkaline phosphatase, aspartate transaminase, alanine transaminase, and γ-glutamyl transpeptidase are concerned. Thus, there is no reason to believe that oral Cr supplementation would induce changes in liver function in human healthy subjects.

However, a report by Duarte et al. *(65)* reported that mice supplemented with oral Cr (0.3 g/body wt) for 6 d had a liver protein content increased by 23%. Among the measured liver enzymes, the authors mentioned that the aspartate transaminase decreased and that the alanine transaminase tended to increase. Moreover, Keys et al. *(66)* reported that mice assigned to 0.05 g Cr monohydrate/kg body wt supplementation for 8 wk underwent chronic hepatitis. Thus, at least in mice, there might be some concern regarding the potential for Cr toxicity. Therefore, Tarnopolsky et al. *(67)* initiated a study to characterize pathological changes of intermediate- and long-term Cr monohydrate supplementation in mice and in rats. They supplemented the animals with 2% (wt/wt) Cr during 1 yr. Histological assessment (20 organs/tissues) was performed on healthy and transgenic (SOD1) mice and in normal rats before and after Cr supplementation. The administration of Cr monohydrate to mice resulted in histological evidence of hepatitis with no evidence of pathology in a variety of other tissues and organs *(67)*. Cr administration to rats did not result in any pathology of all organs/tissues examined. These results clearly show a species- and tissue-specific responses to Cr administration. The authors also insisted that the Cr supplementation were made for one-third to one-half of the life-span of the animals at doses that are those habitually consumed by humans *(67)*.

In addition, several studies on humans *(57,68–72)* did not show any significant increase in plasma urea throughout the duration of Cr supplementation (20 g daily for 5 d or up to 10 g daily for 5 yr) (Table 6). Meanwhile, Earnest pointed out that Cr supplementation did increase by 17% serum urea in females *(57)*. However, the urea levels remain within the range of a normal population. Earnest et al. *(57)* suggested that chronic high dose of Cr supplementation elicited minimal changes in the markers of hepatic function that were evaluated. Looking at their data and knowing that serum urea is not an accurate representative of liver function the authors of this chapter do not share their conclusion.

Table 6
Cr Supplements and Plasma Measurements (Mean ± SEM)

Subjects	Doses (g.d)	Duration (d, wk, mo, yr)	Urea (mg/L)		Crn (mg/L)		References
			Pre-Cr	Post-Cr	Pre-Cr	Post-Cr	
Active	10	5 d	158 ± 7	138 ± 10	11.2 ± 0.5	11.3 ± 0.4	*57*
Active	21	5 d	–	–	9.4 ± 1.6	10.2 ± 1.5	*86*
Active	3	63 d	152 ± 1	151 ± 8	9.6 ± 0.5	9.1 ± 0.8	*71*
Active	<10	5 yr	150 ± 6	147 ± 9	8.8 ± 0.6	8 ± 1.5	*70*
Active	9.7	4 yr	120 ± 18	154 ± 34	9 ± 0.3	13 ± 3[a]	*64*
Active	20	7 d	139	164	12.2	12.2	*68*
Active	10	56 d	192 ± 18	219 ± 75	11.1 ± 3	13.4 ± 3.3[a]	*72*
Active	0.3/kg body wt	7 d	–	–	12.3 ± 0.2	14.03 ± 0.4[a]	*88*
Senior	0.3/kg body wt	7 d	–	–	9.8 ± 1.7	10.8 ± 1.9[a]	*54*
Football	13.9	5.6 yr	–	–	–	12 – 19	*60*
Active	5	19 mo	151 ± 37	155 ± 36	12.3 ± 1	11.6 ± 2	*69*
Active	5	1 yr	–	–	11.3 ± 0.2	12.4 ± 0.4[a]	*188*
Active	5	14 wk	–	–	12.6 ± 0.7	4.2 ± 1[a]	*187*
Active	0.3/kg body wt	4 wk	–	–	16.5 ± 0.9	17.9 ± 1.3[a]	*191*
Active	20	8 d	–	–	10.1 ± 1.2	11.8 ± 2.1	*172*
Active	21	14 d	–	–	11 ± 1.4	8.52 ± 1.2	*95*

[a] $p < 0.05$ (post- vs pre-Cr).

Table 7
Effect of Oral Cr Supplementation on Plasma PCK Activity (Mean ± SD)

		Activity (IU/L)		
Dosage (g/d)	*Duration (d)*	*Baseline*	*Cr*	*References*
10	84	89.3 ± 10.2	97.8 ± 7.8	*56*
10	56	118.8 ± 18.6	181.3 ± 23.6[a]	*56*
20	5	–	No change	*61*
20	5	–	No change	*73*
15.75	28	239 ± 106	609 ± 366[a]	*50*
20	5	–	No change	*74*
5	98	53.3 ± 23.6	107.4 ± 76.4[a]	*187*
20	5	48.3 ± 27.3	32.2 ± 16.4	*75*
5	300	398 ± 253	405 ± 255	*193*

[a] $p < 0.05$ (Cr vs baseline).

3.2. Muscle Markers

PCK is commonly used in clinical pathology as a marker of muscle enzyme efflux and thus muscle dysfunction. Contradictory results were recorded under Cr supplementation (Table 7). Five publications did not report changes in total plasma PCK after 5–84 d of 5–20 g Cr/d supplementation *(56,61,73–75)*. Some studies even followed the increase of plasma PCK under either maxilla isometric contractions or endurance 30-km race with control groups without Cr loading. Apparently, even with exercise-induced muscle damage and muscle soreness, there was no modification of indirect muscle markers under Cr supplementation. Moreover, one study investigated also on two other plasma markers of inflammatory muscle markers (prostaglandin E2 and tumor necrosis factor-α). The results indicated that Cr supplementation reduced cell damage and inflammation after the exhaustive 30-km running.

On the contrary, Kreider et al. *(50)* reported a mild elevation in PCK after 28 d of 15.75 g/d. Nevertheless, it is difficult to get a clear situation about these mild changes in PCK levels because the athletes were practicing heavy training, which might induce muscle enzymes efflux *per se*. Moreover, the plasma PCK exists in different isoforms, (M for muscle, B for brain) namely MM for skeletal muscle, MB for heart, and BB for brain. Today there is no specific report available on the enzyme isoforms that are released from the tissues. Most probably, the slight increase observed in a few reports could be attributed to the skeletal muscle isoform but precise information is needed to confirm this hypothesis.

3.3. Kidney Impairments

Already in 1926, Chanutin investigated the fate of Cr when administered to two subjects during 29 and 44 d with a daily intake of 10–20 g *(76)*. The absorption of Cr appeared to be complete. He found an increased creatinine (Crn) excretion as well as significant positive nitrogen balance. Unfortunately, the excretion was measured for only 1 or 2 d after stopping the administration of Cr. Thus, the carry-over effects on Crn excretion were not determined. Two years later, Rose et al. *(77)* reported that after 49 d of feeding 1 g/d, one man and one woman had a 22–25% increase in the Crn excretion. Hyde *(78)* extended the study of Rose et al. looking at 14 subjects of varying age who were fed 1 g Cr daily for 4–10 wk. Eight of their subjects had increased Crn excretion whereas six individuals did not increase Crn excretion when fed with Cr. Subsequently, Crim et al. *(79)* fed healthy young men with Cr 0.23 g daily for 9 d and 10 g daily for 10 d, consecutively. The subjects were trained on a treadmill (5 d/wk) during the 9-d low-Cr feeding. Crn excretion increased by 10–30% during Cr feeding. There was no significant increase in fecal nitrogen during the Cr-feeding period. Moreover, using oral [^{15}N] Cr feeding in humans, Hoberman et al. *(80)* observed that urine is the only major excretatory route of Cr and Crn. Additionally, sweat loss of Cr collected after exercise was insignificant *(79)*.

More recently, there has been several publications on plasma levels of urea and Crn under Cr supplementation (Table 6). None of the seven reports on plasma urea did observe any modification of urea handling by the kidney. On the contrary, 44% of the publications on plasma Crn level did show a mean 15% increase after Cr supplementation. However, there does not seem to be any relationship between the daily load, the duration of supplementation, and the observed slight plasma increase, which mostly remains with the normal range of a healthy population.

Several publications investigated the modifications of the excretion of urea and Crn after Cr supplementation. Some showed an increase in Crn excretion when individuals were fed with Cr *(13,51,81–85)*. However, several authors did not observe any statistical changes in Crn excretion after short-term *(68,86–89)*, medium-term *(71,72)*, or long-term *(69,70)* oral Cr supplementation in trained individuals (Table 8). The urine output was also measured after Cr supplements in some publications and the results are contradictory, either an increase *(90)*, a stable output *(71,91)*, or a decline in urinary volume *(13)*. Hultman et al. *(13)* suggested that the increase in BM during acute Cr loading is likely to be attributable to body water retention. This explanation does not follow from the other studies.

Urea output was also taken into consideration in a few studies. No modification in 24 h urea was observed after 4–7 d *(51,68,81,86)*,

Table 8
Cr Supplements and Urine Measurements in Humans (Mean ± SEM)

Dose (g/d)	Duration (d, mo, yr)	Subjects	Urea (g/24 h)		Crn (g/24 h)		References
			Pre-Cr	Post-Cr	Pre-Cr	Post-Cr	
20	6 d	Active	–	–	0.99	1.36[a]	*13*
0.25/kg body wt	5 d	Active	–	–	1.41 ± 0.53	2.09 ± 0.66[a]	*85*
21	5 d	Active	18.8 ± 2.3	22.2 ± 1.1	1.19 ± 0.08	153 ± 0.005	*86*
20	4	Active	27.5 ± 1.1	26.8 ± 1.8	1.86 ± 0.08	2.25 ± 0.14[a]	*51*
3	58 d	Active	12.6 ± 2.8	10.3 ± 2.1	1.73 ± 0.14	1.90 ± 0.10	*71*
9	5 d	Active	–	–	1.63 ± 0.35	2.21 ± 0.26[a]	*84*
3–10	8 mo–5 yr	Active	11.3 ± 0.9	12.3 ± 1.3	1.80 ± 0.07	1.53 ± 0.12	*70*
20	6 d	Active	–	–	2.15 ± 0.69	2.62 ± 0.60[a]	*83*
10	56 d	Active	41.4 ± 27.8	43.3 ± 23.1	1.38 ± 0.67	1.85 ± 0.77	*72*
0.3/kg body wt	7 d	Active	–	–	2.34 ± 0.36	2.72 ± 0.73	*88*
20	4 d	Active	–	–	1.71 ± 0.09	1.85 ± 0.20	*87*
20	7 d	Active	47.2	38.2	1.87	1.81	*68*
20	6 d	Active	–	–	1.41 ± 0.53	2.09 ± 0.66[a]	*82*
20	4 d	Active	41.6 ± 5.6	45.5 ± 9	1.30 ± 0.20	1.80 ± 0.20[a]	*81*
5	19 mo	Active	–	–	2.82 ± 1.6	2.67 ± 1.1	*69*
0.06/kg body wt	7 wk	Active	–	–		No change	*16*
20	8 d	Active	–	–	1.56 ± 0.39	2.71 ± 1.04	*172*
21	14 d	Active	–	–	1.86 ± 0.14	2.22 ± 0.14	*95*
5	20 wk	Active	–	–	1.57 ± 0.10	1.64 ± 0.07	*134*

[a] $p < 0.05$ (post- vs pre-Cr).

Table 9
Cr Supplement Excreted vs Oral Doses

Dose (g/d)	*Duration (d)*	*Excreted (%)*	*References*
10	10	73	*76*
20	10	67	*93*
20	5	67	*13*
0.25/kg body wt	5	57	*85*
21	5	60	*86*
10	5	44	*91*
9	5	33	*84*
25	5	72	*98*
20	1	67	*97*
0.1/kg	7	46	*92*
20	5	55	*94*
20	5	47	*96*
21	14	77	*95*

9–10 wk *(71,72)*, or 10 mo–5 yr *(69,70)* of oral Cr supplementation (Table 8). Therefore, it seems reasonable to say that the liver does not seem to be overproducing urea production when healthy subjects ingest Cr in excess.

As mentioned earlier, Cr load (from 2 to 20 g/d) seems to be totally absorbed by the intestinal tract. However, skeletal muscle cannot take up all this excess Cr and Cr must be excreted in the urine *(13,71,76,84–86,91–98)*. The excreted Cr represents 40–72% of the original load (Table 9).

An early report from Earnest et al. *(57)* stated that Cr supplementation had minimal changes in the markers of renal function. Unfortunately, they used plasma urea, which does not represent a valuable marker when taken without any urine determination to assess renal function. More troublesome, their data did not show any differences in plasma urea for male subjects and a modest increase (17%) for female consumers.

The first investigations on renal functions in healthy individuals who consumed oral Cr supplementation was published 7–9 yr ago *(70,71,86)*. Renal clearances of Crn, urea, and albumin were compared in three different groups of active subjects who consumed Cr during 5 d, 9 wk, and up to 5 yr as compared with control groups. Statistical differences were not observed between the control groups and the Cr consumers (Table 10). Lately, several other investigations supported the authors' primary results on glomerular filtration rate

Table 10
Cr Supplements and GFR in Humans (Mean ± SEM)

Gender (M, F, Nb)	*Age (yr)*	*Population*	*Dose (g/d)*	*Duration (d, mo, yr)*	*Effect on GFR (mL/min)*		*References*
					Pre-Cr	*Post-Cr*	
M (5)	25.1	Active	21	5 d	130 ± 16	138 ± 25 (2)	*86*
M (20)	21	Active	3	58 d	130 ± 16	143 ± 11 (2)	*71*
M (8)	24	Active	3–10	8 mo, 5 yr	145 ± 8	143 ± 11 (2)	*70*
F (1), M (10)	28.4	Active	20	5 d	124 ± 9	125 ± 6 (2)	*58*
M (7)	22.4	Active	20	5 d	137 ± 18	129 ± 23 (2)	*161*
F (8), M (10)	20.5	Football	13.5	35 mo	124	126 (1)	*60*
M (12)	19.2	Football	15.75	5 d	162 ± 15	120 ± 18 (3)	*69*
M (25)	19.2	Football	5	9.1 mo	162 ± 15	168 ± 33 (3)	
M (17)	19.2	Football	5	1.6 yr	162 ± 15	177 ± 44 (3)	
M (17)	21	Active	5	23 d	109 ± 2.2	110 ± 2.7 (4)	*197*
M (20)	24.1	Active	21	14 d	127 ± 6	137 ± 10 (2)	*95*

Determination of GFR by:
(1) Crn, colorimetric method (urine, plasma).
(2) Crn, enzymatic assay (urine, plasma).
(3) Crn, HPLC (plasma).
(4) Iohexol plasma clearance.

(GFR). From these experimental protocols it can be stated that GFR and the tubular reabsorption process were not affected by oral Cr supplementation using the usual daily amount (20 g/d for 5 d, less than 10 g/d thereafter). However, the use of Crn clearance to assess GFR in healthy athletes who consume oral Cr monohydrate has been criticized by Kuehl et al. *(99)*. In a recent communication at the annual meeting American College of Sports Medicine these authors investigated athletes consuming oral Cr supplements (10 g/d during 56 d) looking at their GFR using the Crn and the iohexol clearances *(100)*. There was a 0.99 correlation between the two methods and all values tracked the same pattern. The authors are thus confident to use the Crn clearance, which is less invasive and more practical to assess impairment of the filtration process at the renal side. A large survey (100 subjects) made by Richard B. Kreider on regular users of Cr who consumed the supplement during 1 yr reached the same conclusion *(101,102)* (and Kreider 2000, personal communication).

Furthermore, the specific immunochemical techniques did not observe any modification induced by Cr loading on urine albumin excretion rate, which remained within the physiological range for healthy subjects *(70,71,86,95)*.

Microalbuminuria is a well-known predictor of kidney impairment *(103)*. The excretion rate of plasma albumin in urine has been widely used to assess increased glomerular membrane permeability in many pathological conditions *(104–106)*. A subclinical increase in urinary albumin excretion rate is a powerful predictor of the later development of persistent proteinuria and renal failure. The upper level of albumin excretion in a healthy population under resting condition is 20 μg/min. Figure 1 shows the values obtained under different conditions of oral Cr supplementation in healthy subjects. None of the 52 subjects show any increase of albumin excretion when compared with a placebo investigation or a control population *(70,71,86,95)*. Thus, it may be stated that the glomerular membrane permeability is not affected by these different loads of Cr monohydrate supplementation in healthy subjects.

Anecdotally, a recent publication of Groeneveld et al. *(107)* on the long-term (310 d) Cr supplementation (10 g/d) in 57 patients with the neurodegenerative disease amyotrophic lateral sclerosis may also be reported. Long-term Cr supplementation did not lead to an increase of plasma urea levels or to a higher prevalence of microalbuminurea (<20 μg/min).

3.3.1. Animal Studies

Because Cr supplementation raised concern regarding its effect on the kidney, Edmunds et al. *(108)* decided to use an animal model to

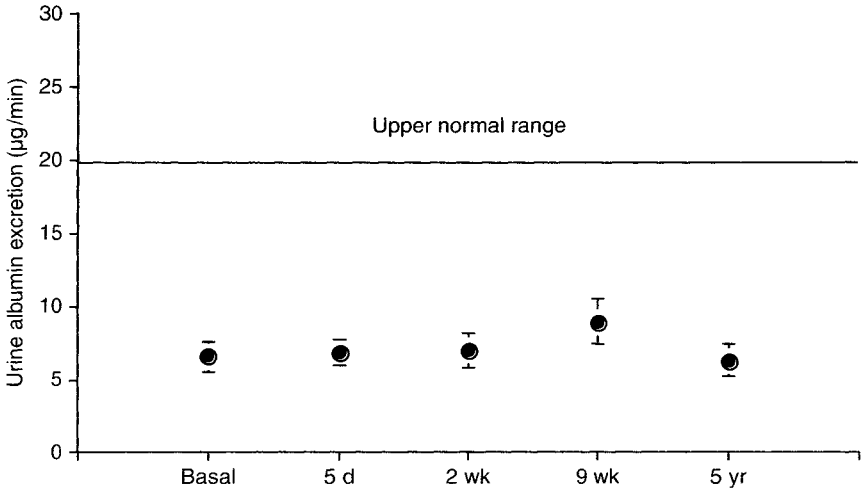

Fig. 1. Urine albumin excretion rate, before and after Cr supplementation *(70,71, 86,95,139)*.

investigate the renal disease progression in Han:SPRD-cy male and female rats. The Han:Sprague-Dawley Renal Disease-cy rat is a well-documented and accepted animal model of inherited renal cystic disease that resembles human autosomal dominant polycystic kidney disease. The authors reasoned that if Cr supplementation affects the kidney in any way, these alterations would be more detected in an animal model that has shorter life-span than humans. Four-week old rats were supplemented daily with 0.4 g Cr/kg body wt during 5 wk. Edmunds et al. *(108)* recorded that Cr supplementation resulted in increased disease progression and worsened renal function in the animal model of kidney disease. They concluded that Cr should be used with particular caution in humans with or at risks for renal disease.

These results on inherited renal cystic disease in rats were not confirmed by another team of nephrologists who investigated normal adult rats submitted to Cr monohydrate (2% wt/wt) during 4 wk *(109)*. Rats were allocated to four experimental groups:

1. Sham-operated, normal diet;
2. Sham-operated, Cr diet;
3. Renal failure (two-thirds nephrectomized), normal diet;
4. Renal failure (two-thirds nephrectomized), Cr diet.

The authors measured serum Crn and urea, 24-h urinary albumin excretion, and GFR. Their study could not demonstrate any deleterious

effect of Cr supplementation on kidney function in normal rats or in the animal model with pre-existing moderate renal dysfunction.

Recently, Ferreira et al. *(110)* investigated the effect of Cr supplementation (2 g/d/kg body wt during 10 wk) on renal function in endurance trained rats (treadmill at 12 m/min during 1 h/d). They observed a 40% reduction in resting renal blood flow and GFR with Cr vs control and no modification for the 24-h urinary protein excretion. The treadmill exercise itself had no effect on these kidney parameters but the additional Cr load enhanced them by 15%, without modification on protein excretion. The authors concluded that Cr alone induced an important and significant reduction of both renal plasma flow and GFR. Their results contrast with those of Taes et al. *(109)* who used three different method of GFR in addition to albumin excretion rate. More surprisingly, Ferreira et al. did not find any modification of either renal blood flow or GFR induced by exercise itself. Again, as said by Tarnopolsky *(67)* and Kreider *(59)*, because healthy mice nor rats experienced renal or other tissue pathological changes after long-term Cr supplementation, there is no specific reason to believe that Cr adversely affect renal function or health outcomes.

3.3.2. Human Nephropathies

In 1998, Pritchard and Kalra introduced the first case of kidney damage induced after Cr supplementation *(1)*. The 25-yr-old man mentioned in this study presented a focal segmental glomerulosclerosis, 8 yr ago, with frequently relapsing steroid-response nephrotic syndrome. He had required treatment with cyclosporin, a certified nephrotoxic drug, for the last 5 yr to minimize nephrotic relapses. As a soccer-trained individual this man started loading himself in mid-August 1997 with 5 g Cr monohydrate three times per day for 1 wk and then a maintenance dose of 2 g/d that he had been taking for 7 wk. His GFR dropped by 50%. The GFR evidenced a kidney impairment, which was gradually restored to normal value 1 mo after stopping the Cr supplements.

Another case report of interstitial nephritis was published by Koshy et al. *(111)* in a patient having absorbed 20 g of Cr/d for 4 wk. This previously healthy man presented a 4-d history of nausea, vomiting, and bilateral flank pain. The patient was hospitalized with a serum Crn concentration of 2.3 mg/100 mL (normal upper range limit: 1.5 mg) and a urine protein excretion of 472 mg/d (normal upper range limit: 150 mg). A renal biopsy revealed acute focal interstitial nephritis and acute tubular injury. After stopping the Cr supplements his renal function subsequently became normal. This is an anecdotal case out of thousands of regular Cr consumers. Nevertheless, it emphasizes the recommendation to be tested regularly for urinalysis (*see* Heading 4).

Table 11
Renal Dysfunction and Cr Supplements in Humans

Gender	*Age (yr)*	*Sport*	*Original*	*Dose, time (g/d, wk, mo)*	*Consequences*	*References*
Man	25	Soccer	Nephritic syndrome	15, 1 wk	Focal segmental glomerulo-sclerosis	*1*
Man	20	Active	Healthy	20, 4 wk	Interstitial nephritis	*111*
Man	27	Active	Healthy	?, ?	Acute renal failure	*194*
Man	27	Active	Healthy	?, ?	Acute renal failure	–
Man	28	Active	Healthy	?, ?	Acute renal failure	–
Man	20	Weight lifting	Healthy	2, 7 mo	Focal segmental glomerulo-sclerosis	*195*
Man	28	Baseball	Healthy	?, 30 d	Medullary sponge kidney	*196*

d, day; wk, week; mo, month.

Five more anecdotal cases (abstract reports) were introduced in the recent literature (Table 11). However, being modestly critical, these reports are dubious despite the diagnosis of acute renal failure. Either, the individual doses and duration are not reported for three individuals or the remaining two individuals might have consumed additional other unknown substances (steroids?).

Thus, the absence of controlled values does not allow to seriously conclude that Cr supplementation is an inducer of kidney impairment in healthy subjects.

Eventually, Cr supplementation has been administered in dialyzed patients by Kirschbaum (2000, personal communication) without any side effects on blood chemistry.

3.4. Mutagenicity and Carcinogenicity Risks of Excess Cr Supplementation

3.4.1. Methylamine and Formaldehyde Production

Based on the excellent and extended review by Wyss and Kaddurah-Daouk on Cr and Crn metabolism *(112)*, the French Food Agency (AFSSA) claimed unequivocally that excess consumption of Cr and Crn might induce derived carcinogenic and mutagenic compounds, which could put athletes and consumers of exogenous Cr at risk (evaluation of risks induced by Cr consumer and truth on allegations related to sport performance or increase in muscle mass. http://www.afssa.fr).

Indeed, the excess conversion of Cr to sarcosine may result in cytotoxic agents such as methylamine *(112)*. The latter has been found to be deaminated by semicarbazide-sensitive amine oxidase (SSAO, EC 1.4.3.6) to produce formaldehyde and hydrogen peroxide *(113)* (Fig. 2). Under special conditions, methylamine and formaldehyde are two well-known cytotoxic agents, the presence of which can be revealed by urine analyses *(113–116)*. Formaldehyde has the potential to cross-link proteins and DNA, leading to cytotoxicity and carcinogenic effects in cells *(117,118)*. The toxic aldehyde is related to different pathological conditions such as vascular damage, diabetic complications, and nephropathies.

In 2000, Yu and Deng *(119)* administrated a single dose of Cr (50 mg/kg) to mice, which did not seem to alter the urinary methylamine excretion. However, indirect selective inhibition of SSAO activity dramatically induced a fivefold increase in methylamine excretion. The authors concluded that chronic administration of a large quantity of Cr can increase the production of formaldehyde, which might potentially cause serious unwanted side effects on healthy athletes. This conclusion was amplified by the AFSSA, which led the French government to ban any official buying of Cr.

An old German publication showed that exercise practice (one man after a strenuous ski racing) induced a 2.5-fold increase in the urinary excretion of methylamine *(120)*. The authors argued that all conditions associated with creatinuria (such under supplementation during muscular exertion) implicate an increased excretion of methylamine. Of course, in these old days, scientists were not aware of the eventual potential risks of aldehyde formation in human tissues. However, looking at the original publication it was pointed out that the authors used an aliquot of urine obtained after exertion, without any information on the urine output.

Thus, recently 20 male young healthy subjects who were daily supplemented with 21 g Cr monohydrate during 14 d were investigated. Before and after Cr supplementation 24-h urine was collected and Cr,

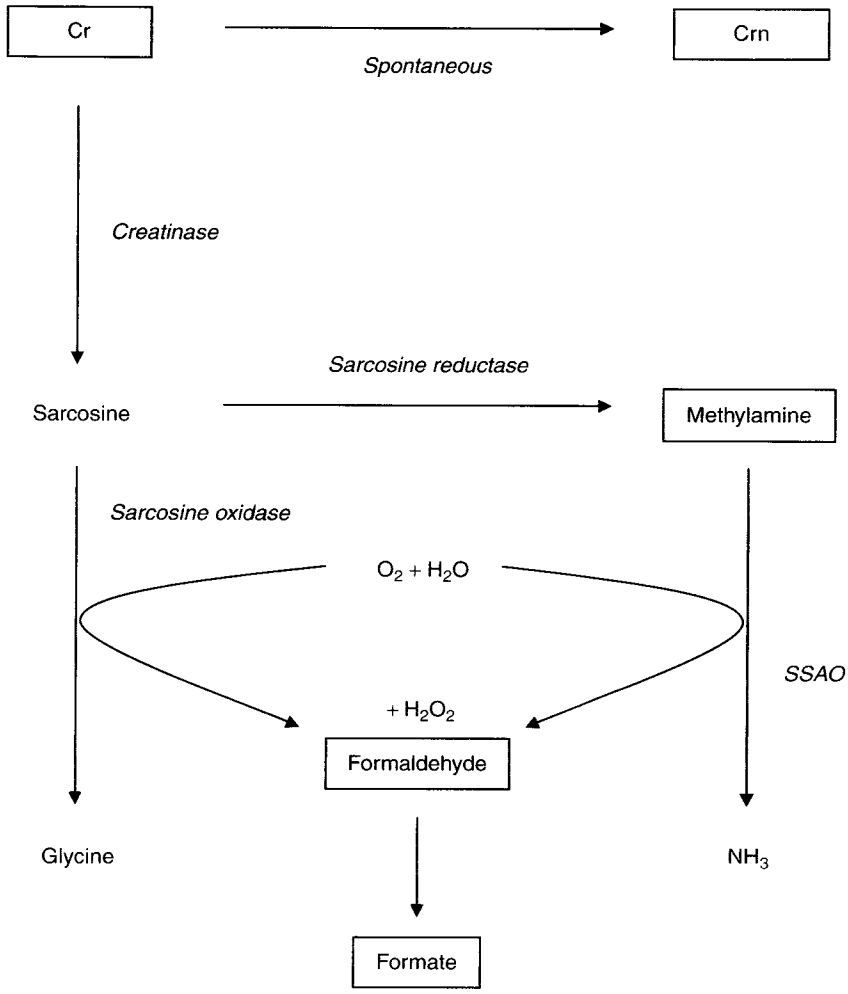

Fig. 2. Schematic pathways of Cr and Crn degradation in the human body. All products and metabolic steps are not shown. Compounds that are framed have been assayed in urine before and after Cr supplementation. SSAO, semicarbazide amine oxidase *(95)*.

Crn, methylamine, formate, and formaldehyde was determined. Table 12 includes the modifications of urine excretion of formaldehyde, formate, and methylamine before and after Cr supplementation. Twenty-four hour urine output of methylamine and formaldehyde increased 9.2- and 4.5-fold, respectively ($p < 0.001$), after Cr feeding with no increase in formate excretion. After Cr feeding, there was no correlation between

Table 12
Mean Values (± SEM) of Urine Contents Before and After Cr Supplementation, 21 g/d During 14 d[a]

	Before Cr	*After Cr*
Methylamine (mg/24 h)	0.69 ± 0.06	6.41 ± 1.45[b]
Formaldehyde (μg/24 h)	64.78 ± 16.28	290.4 ± 66.3[b]
Formate (mg/24 h)	12.46 ± 1.04	14.16 ± 1.84
Albumin (mg/24 h)	9.78 ± 1.93	6.97 ± 1.15

[a] From ref. *95*.
[b] $p < 0.001$ between values before and after Cr supplementation.

plasma Cr and urine methylamine ($r^2 = 0.025$, P = NS) or formaldehyde ($r^2 = 0.017$, P = NS).

The results from the investigation indicate that short-term oral Cr feeding in healthy subjects enhances the mechanisms leading to the conversion of Cr to sarcosine and then to methylamine, the latter one giving rise to formaldehyde. The conversion of formaldehyde to formate should be rather rapid in cells, the latter one representing indirectly the production of the former substrate *(121)*. Using rat and mice models, Yu and Deng *(114,119)* demonstrated that in vivo deamination of methylamine produces formaldehyde and hydrogen peroxide, which are both recognized as cytotoxic substances. Consequently, these authors hypothesized that chronic administration of large quantities of Cr as an ergogenic supplement would increase the production of methylamine and subsequently formaldehyde, both being potentially cytotoxic in renal glomerula *(114,119)*. The results support this hypothesis in humans.

Despite the 9.2-fold increase in methylamine urine excretion induced by Cr ingestion, this level did not reach the normal upper limit values from healthy humans, up to 35 mg/d (mean + 3 SD) *(115)*. After Cr supplementation, urine formate excretion remains below the upper range (14–20 mg/d) reported in healthy subjects *(122–124)*. However, under Cr supplementation, the urine excretion of formaldehyde has been increased 4.5-fold of the basal rate.

Because Cr is transformed to sarcosine by microbial enzymatic reactions *(112)*, it is likely that methylamine is formed in the intestine and is therefore potentially damageable for the integrity of the intestinal epithelium. Methylamine is toxic to human endothelial cells and forms patch-like lesions *(125)* and even kidney damage *(113)*. In mammals, SSAO activity has been found in various tissues associated to vascular system *(126,127)*. Therefore, it is likely that the deamination of methylamine

occurs in circulation. It could also be speculated that this flooding of methylamine in blood, together with SSAO, might produce formaldehyde, which favors microangiopathy in the renal glomeruli *(116,127)*.

The subjects consumed a total amount of 280 g of Cr monohydrate over 14 d without any modification of glomerular membrane permeability as assessed by their albumin urine excretion rate (9.78 ± 1.93 mg/24 h before Cr; 6.97 ± 1.15 mg/24 h after Cr). The upper limit of healthy humans is 25 mg/24 h. Albuminuria has long been known to be associated with specific renal abnormality, and is now recognized as an early test for vascular endothelial damage *(128)*. Despite the fact that formaldehyde and methylamine excretion rates were increased respectively to 4.5- and 9.2-fold after Cr supplementation in the subjects, there was no detectible consequence of glomerulonephropathy (Table 11). In this context, it has been shown, at least in rats, that formaldehyde administration in drinking water supplied *ad libitum* during 2 yr can produce specific carcinogenic effects on various organs and tissues *(129)*. This raises the question of the duration of the supplementation. In a previous study, no adverse effect was observed of a long-term (up to 5 yr) Cr supplementation in humans *(70)* (Fig. 1).

Even if systematic deleterious effect could not be observed, it cannot not exclude that a systematic production of low extra doses of cytotoxic agents never induce any nephropathy incidences. Clearly, epidemiological data are required to evaluate potential risks over a larger cohort of individuals. But in terms of results of the present investigation, caution should be applied. Kidney function of the patients and healthy subjects supplemented with Cr on a regular basis should be systematically monitored throughout the ingestion period.

To conclude, the investigation shows that short-term, heavy load oral Cr supplementation stimulates the production of an excess of methylamine and formaldehyde in urine of healthy humans. Even though the production of cytotoxic agents has no apparent effect on the kidney function of volunteers in this study, long-term and epidemiological data are essential to assess whether Cr supplementation is harmless in all healthy individuals under all conditions.

3.4.2. Induction of Carcinogenic and Mutagenic Amino-Imidazo-Azaarene Formation

The review of Wyss and Kaddurah-Daouk *(112)* reported that the processing of foods, in particular frying and broiling of meat, is associated with the generation of mutagenic and carcinogenic substances, namely the amino-imidazo-azaarenes products that shall be named the "heterocyclic amines (HCA)" for simplicity. These substances, which are

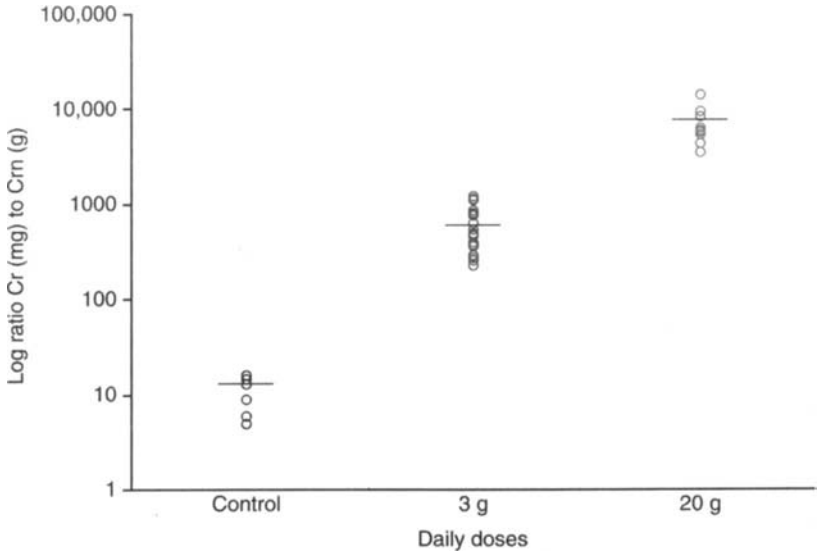

Fig. 3. Urinary excretion of Cr and Crn, expressed as the ration of Cr (mg) to Crn (g) in a urine sample by control individuals and study participants who ingested 3 or 21 g Cr monohydrate per day. The horitontal bars represent the geometric means of each population. A clear separation is observed between nonconsumers and consumers *(62)*.

numerous and classified into five groups (*see* ref. *112* for details), are formed during cooking in the presence of sugar and amino acid, depending on the cooking time and temperature (>250°C) *(130,131)*. Maximal mutagen yield is achieved by mixing Cr or Crn with amino acid and sugar with a molar ratio of 1:1:0.5 *(112,132)*. Crn rather than Cr is likely to be the actual precursor of the HCA mutagens *(112)*. Among the HCA compounds, imidazo-quinoxaline, 8-methyl-imidazo-quinoxaline, 4,8-dimethyl-imidazo-quinoxaline, and imidazo-pyridine are the most important mutagens and together contribute to about 80% of the mutagenicity. HCA are formed at high temperature, during frying or broiling of meat (barbecue effect!) and then at low concentration, so low that it is questionable whether they represent any significant cancer risk. Moreover, at 37°C, HCA formation from Cr or Crn most likely does not occur. Therefore, it would seem very unlikely at present to attribute any cancer risk to oral Cr supplementation *(112)*.

The negative opinion on oral Cr supplementation seem to be purported carcinogenic effect of Cr. Based on current knowledge, the probability that nitrosation products of Cr are formed in the stomach to any significant extent is close to zero *(133)*. A very recent short publication

by Derave et al. *(134)* supports this conclusion. These authors investigated in a double-blind, placebo-controlled study the urinary excretion of *N*-nitrososarcosine after 1-wk high dose (20 g/d) and 20-wk low-dose (5 g/d) Cr supplementation in healthy humans. They concluded that Cr ingestion does not increase the urinary excretion of the carcinogen *N*-nitrososarcosine.

The identification of HCA in human urine is not an easy procedure. The analytical methods involved solid-phase extraction and quantification by combined liquid chromatography-tandem mass spectrometry to identify the major HCA in urine *(135–138)*. Nevertheless, one will have to quantify this potential hypothetic risk to definitively exclude unproved allegation still present in nonscientific publications or media.

4. PRACTICAL CONCLUSIONS

The purpose of the present review was to present data and conclusions on the potential side effects of oral Cr supplementation in healthy individuals. Despite papers and editorials published in sports media, there are no real incidents of muscle cramps, gastrointestinal discomfort, or liver impairment after regular load of oral Cr.

There is neither apparent kidney dysfunction when healthy individuals consume oral Cr monohydrate with the usual daily amounts (20 g for 5 d, <10 g afterwards). The few renal incident remain anecdotal. Even if there are no health risks induced by oral Cr supplementation it sounds safe to remain cautious when this substance is administrated on a chronic basis. The excess Cr ingestion is still a burden to be eliminated mostly by the kidney. Regular checkups should be the elementary tactic to follow the potential dysfunction, which could appear with some individuals less prone to compensate any homeostasis imbalance. Blood chemistry for liver enzymes, urea, Crn should be investigated regularly (once a year). The analysis of urinary albumin excretion rate (<20 μg/min) appears to be the most simple, inexpensive, accurate test to assess any early incident of kidney impairment. Should this happen under resting condition, i.e., after 20 h of physical activity, further investigations need to be done by a nephrologist *(139)*.

It is quite easy to determine the Cr consumers when looking at the Cr/Crn ratio from a sample of urine (Fig. 3). This ratio is always less than 40 (mg Cr/g Crn) in nonconsumers and higher than 150 for individuals consuming at least 2 g of Cr/d. Thus, it is believed that healthy subjects are not confronted with health risks when consuming reasonable amounts of oral Cr monohydrate.

Nevertheless, it is advised that Cr supplementation should not be used by a person with pre-existing renal disease or those with a potential risk for renal dysfunction (diabetes, hypertension, and reduced GFR).

Great care should also be taken as far as the purity of exogenous Cr supplements is concerned. Analytical tests must prove their unique nutraceutical composition as safety is not assured in most preparations.

5. CONCLUSION

Doubtful allegations and adverse effects of Cr supplementation have been released from either press media or scientific publications. The present chapter has tried to separate the wheat from the chaff by looking for experimental evidences. One of the purported effects of oral Cr supplementation is to increase muscle mass to improve performance. A review of literature reveals a 1–2.3% increase in total BM after short-term (<10 d) or medium-term (>10 d), respectively. This increase is more focussed to FFM, which might gain a mean 3.3% after several weeks of exogenous Cr load. This increase in FFM is more specifically attributed to skeletal muscle mass changes with a mean 6.2% increase after Cr supplementation. Bioimpedance techniques showed that this increase in muscle mass by Cr is partially because of some water retention in the intracellular compartment (mean + 4.3%) whereas the remaining increase was supposed to be dry mass, such as protein. There is indirect evidence that Cr supplementation induces skeletal muscle protein increase in vitro and in growing cells and animal models. However, experimental investigation in healthy subjects failed to demonstrate any modification of muscle protein synthesis and breakdown under Cr supplementation. Clearly, exercise and amino acid intake are much stronger stimuli for protein synthesis measured over a period of a few hours.

Anecdotal reports from athletes have been released on muscle cramp incidences and gastrointestinal complaints during Cr supplementation, but these few incidences remain rare and not necessarily linked to Cr itself. Despite several allegations from scientific and press media, liver (enzymes, urea) and kidneys (glomerular filtration and albumin excretion rates) are keeping their functionality in healthy subjects supplemented with Cr, even during several months. However, it is advised that Cr supplementation should not be used by a person with pre-existing renal disease or those with a potential risk for renal dysfunction (diabetes, hypertension, and reduced GFR).

Mutagenicity and carcinogenicity potential effects (production of HCA) induced by Cr supplementation have been claimed by a French sanitary agency (AFSSA), which might put consumers at risk. Even if

there is a slight increase (within the normal range) of urinary methylamine and formaldehyde excretion after a heavy load of Cr (20 g/d), without any incidence on kidney function, the search for the HCA excretion remains a future task to definitively exclude the unproved AFSSA allegation.

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