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Consequences of one-week creatine supplementation on creatine and creatinine levels in athletes' serum and urine

Summary

We explored the washout period of creatine (Cr) after repeated ingestions of high doses of exogenous Cr. Ten athletes ingested daily, in a randomized double-blind study design, 30 g of exogenous Cr (n = 5, Cr-group) or a placebo (n = 5, Pl-group). Serum and urine samples were collected 1) before supplementation (BEFORE), 2) after one week Cr supplementation (AFTER), and 3) one week later without supplementation (LATER). The Cr and creatinine (Crn) concentrations in serum (sCr, sCrn) and in multiple spots urine (uCr, uCrn) were measured. We observed a significant rise ($p < 0.01$) in sCr, uCr and sCrn between BEFORE and AFTER supplementation in Cr-group, as well as a significant difference between Cr-group and Pl-group. Body weight increased significantly (+1.5 kg), but relative body fat (%fat) was unchanged. After the washout period in LATER Cr-group, sCr and uCr decreased to low residual values. No loss of body weight occurred during this period. In contrast, sCrn and uCrn returned to baseline values. In conclusion, regular uptake of high doses of exogenous Cr affects both Cr and Crn concentrations in serum (sCr: 14 folds; sCrn: 1.2 folds) and urine (uCr: 140 folds; uCrn: 1.5 folds). An abuse of Cr is therefore mostly spilled over in urine. Surprise drug tests, such as doping controls, happening during the period of Cr supplementation can reveal an important increase in Cr and Crn concentrations, although subjects stopped suddenly Cr loading. The discernible effect of Cr supplementation on these values disappeared within one week.

Key words:
Creatine loading, creatinine, non-enzymatic conversion, wash out, serum, urine, human, doping, sport nutrition

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Introduction

Since the discovery of creatine (Cr) in 1832 by Chevreul, many scientists have studied the metabolism and the function of this molecule largely used by athletes [1, 2]. Many comprehensive studies on Cr supplementation and reviews on this topic have been published [3–9].

Briefly, Cr pool in man is approximately 1.7 g/kg, most of which being located in skeletal muscle. The averaged turnover for an adult male is ~2 g/day. Cr is naturally ingested with food (meat, fish, milk) or is endogenously produced from arginine, glycine and methionine in kidney and liver [1, 3, 9–12]. Muscle fibers can store a high Cr concentration (from approximately 120 to a maximum limit of 160 mmol/kg dry muscle). The enzyme Creatine-kinase (CK) converts Cr into phosphorylated form called phosphocreatine (PCr). This easy releasable energy stored in phosphate bond constitutes the basic mechanism supporting many cellular energetic activities such as muscular contraction, cell growth and protein synthesis [7, 13–17]. The end product of Cr and PCr metabolism is a cyclic stable compound called creatinine (Crn) that will be released in blood and then filtered by the kidney and excreted in urine [18–32].

Because no evidence of direct toxicity has been observed following chronic or acute Cr supplementation, many studies were performed with relative high doses of exogenous Cr [9].

When chronic Cr supplementation was combined with daily hard exercise, the Cr content in exercised muscles was enhanced. Several authors also reported that the intracellular Cr pool could be substantially increased, in particular when the initial Cr concentration is low [5, 6, 9].

Studies using prolonged Cr loading have generally shown an ergogenic effect on anaerobic performances such as high-intensity short-duration exercises, but not for endurance-type aerobic exercises [1, 3–5, 9, 32]. In most of these Cr supplementation studies, a moderate increase in body weight was observed, explained by some authors by water retention [1, 9, 17].

Consequently, Cr became a fashion compound for athletes attempting to increase their anaerobic performance or muscular mass. Although no evidence of toxicity was demonstrated, many questions remain open about the changes in body fluid concentrations in response to large doses of ingested Cr, and this could be important for doping tracking or for foreseeing the potential side effects of long term Cr intake.

Harris, Söderlund and Hultman [5] have shown that a peak in plasma Cr concentration occurred after a single dose of Cr ingestion. We recently observed that a single high dose of Cr intake leads to a Cr maximum concentration in plasma after 2 hours (50 folds increased). Despite this substantial decrease, the Cr level in plasma remains significantly higher than basal level 6 h after ingestion [18]. It was concluded, in evidence, that ingested Cr directly increases Cr in plasma and affects the level of intracellular total Cr.

In this study we explored the short-term (6 days) consequences of high dose Cr supplementation (30 g/day) on the concentration of Cr and Crn in plasma and urine. We were also interested in the measurement of the Cr persistence in plasma and urine after stopping supplementation. This is a practical issue since the dynamic of washout is of major importance for drug tests.

Methods

Subjects

Ten healthy male athletes, students in physical education, were selected to participate in our study. They gave their informed consent in accordance with the Ethical Standards of the 1964 Declaration of Helsinki. All the subjects were Japanese citizens and were moderately-to-well trained in endurance or resistance exercise. Their mean anthropometric characteristics were as follows for the test group: age 24 (sd 2) years, body weight 68 (sd 6) kg, height 1.73 (sd 0.02) m, BMI 22.4 (sd 2.0) $kg \cdot m^{-2}$, fat mass 12 (sd 3) %, lean body mass 59 (sd 6) kg, and VO_2max = 57 (sd 3) $ml \cdot kg^{-1} \cdot min^{-1}$. For the control group, the values were: age 23 (sd 1) years, body weight 69 (sd 11) kg, height 1.74 (sd 0.07) m, BMI 22.8 (sd 2.0) $kg \cdot m^{-2}$, fat mass 13 (sd 4) %, lean body mass 60 (sd 7) kg, and VO_2max = 56 (sd 3) $ml \cdot kg^{-1} \cdot min^{-1}$. The fat mass and lean body mass were estimated with air displacement plethysmographic method (Bodpod system, Life Measurement Instruments, Concord, CA). The VO_2max was measured during a classical progressive bicycle ergometer test (Monark Model).

Experimental protocol

The subjects were separated in two randomized experimental groups. After a first visit for complete information and anthropometric measurements, each subject was asked to come to the laboratory in fasting state at 8:00 a.m. for blood sampling and urine collection (BEFORE). Then each subject received a personal bottle with a beverage solution to start supplementation. Each beverage solution (cacao, milk powder and sugar) contained 30 g pure creatine monohydrate (Soledor SA, Switzerland) completely dissolved in 1000 ml hot water for the test group (Cr-group). For the control group (Pl-group) an identical beverage without Cr was given. The study design was double-blinded. The subjects were asked to drink daily the full bottle in several fractions starting from morning to evening. They had to come every day to the laboratory to bring back the emptied bottle and take a new one for the following day. During 6 days the Cr-group ingested 30 g Cr per day. On the morning of the last day, the subjects were called at random and asked to come directly to the laboratory for blood sampling and starting 24 h spot urines collection (AFTER) as it could be done in official sport events. Later, after a wash-out period of 7 days without Cr supplementation, we collected once again blood and urine in the same experimental conditions (LATER). Note that for a drop-out reason, no LATER measurements were performed with the subject JS.

Analytical methods

Blood samples were taken from a venous catheter inserted into an antecubital vein. Each blood sample was transferred into hermetic vacuum vials and kept in ice during collection time. Centrifugation was done just after the last sample was collected. Serum and plasma samples were conserved at –80 °C and analyzed within one week. Cr concentration was determined by enzymatic method (SRL Inc., Japan), and Crn using the alkaline picrate method (SRL Inc., Japan). Cr and Crn concentrations were measured in the serum and in the urine. We were interested to evaluate a practical method for Cr detection tests, thereby we decided to measure metabolites concentration in spot urine. Because monitoring the water consumption could not be realistic during doping controls with competitive athletes, we decreased the spot-to-spot variation by collecting during one day numerous urinary-spots. The number of samples was variable, depending on voiding frequency. By using this protocol, a reasonable approximation of average Cr and Crn concentrations in 24 h urine was obtained in real competition conditions.

		1) BEFORE Mean	sd	$P_{(1-2)}$		2) AFTER Mean	sd	$P_{(2-3)}$		3) LATER Mean	sd	$P_{(3-1)}$		2-way ANOVA	
Serum Cr [mg/dl]	Cr-group (n=5)	0.23	0.03	0.005	**	3.31	1.22	0.02	*	0.35	0.03	0.02	*	p < 0.0001	****
	Pl-group (n=5)	0.30	0.08	0.5	ns	0.28	0.05	0.3	ns	0.30	0.08	0.9	ns	p > 0.05	ns
	$P_{(Cr\text{-}group/Pl\text{-}group)}$		0.09	ns			0.0005	***			0.3	ns			
Urine Cr [mg/dl]	Cr-group (n=5)	5.57	3.86	0.009	**	789.83	361.07	0.03	*	18.95	7.76	0.1	ns	p < 0.001	***
	Pl-group (n=5)	5.16	1.37	0.03	*	2.19	1.34	0.8	ns	2.39	0.81	0.04	*	p < 0.01	**
	$P_{(Cr\text{-}group/Pl\text{-}group)}$		0.8	ns			0.001	***			0.002	**			
Serum Crn [mg/dl]	Cr-group (n=5)	1.20	0.14	0.003	**	1.46	0.17	0.006	**	1.23	0.15	0.3	ns	p < 0.05	*
	Pl-group (n=5)	1.02	0.04	0.02	*	0.94	0.05	0.4	ns	0.96	0.05	0.07	ns	p > 0.05	ns
	$P_{(Cr\text{-}group/Pl\text{-}group)}$		0.03	*			0.0002	***			0.008	**			
Urine Crn [mg/dl]	Cr-group (n=5)	174.46	70.70	0.3	ns	257.18	74.92	0.4	ns	186.43	44.41	0.9	ns	p > 0.05	ns
	Pl-group (n=5)	178.42	24.30	0.3	ns	152.78	32.12	0.6	ns	156.20	26.13	0.3	ns	p > 0.05	ns
	$P_{(Cr\text{-}group/Pl\text{-}group)}$		0.9	ns			0.02	*			0.2	ns			
Body weight [kg]	Cr-group (n=5)	67.20	6.50	0.02	*	68.58	6.98	0.7	ns	68.80	8.51	0.09	ns	p > 0.05	ns
	Pl-group (n=5)	65.32	7.28	0.3	ns	65.50	7.07	0.1	ns	65.66	7.02	0.2	ns	p > 0.05	ns
	$P_{(Cr\text{-}group/Pl\text{-}group)}$		0.7	ns			0.5	ns			0.6	ns			
Body Fat [%]	Cr-group (n=5)	10.72	1.81	0.7	ns	10.38	2.48			(not measured)				p > 0.05	ns
	Pl-group (n=5)	12.86	2.69	0.3	ns	12.60	2.86			(not measured)				p > 0.05	ns
	$P_{(Cr\text{-}group/Pl\text{-}group)}$		0.2	ns			0.2	ns							

Table 1: Serum & urine creatine (Cr) and creatinine (Crn) concentrations, as well as body weight and relative body fat: 1) before, 2) at the end of one week exogenous creatine supplementation, and 3) after one week washout period in the creatine and placebo groups (*: $P \leq 0.05$; **: $P \leq 0.01$;***: $P \leq 0.001$; ns: $P > 0.05$).

Statistical analysis

Analysis of variance (ANOVA) was used to analyze the variation of urine and serum concentrations between BEFORE, AFTER and LATER. Paired two-tailed student t-test was used to compare differences within the same group ($P_{(1-2)}$: BEFORE vs AFTER; $P_{(2-3)}$: AFTER vs LATER; $P_{(3-1)}$: BEFORE vs LATER), and unpaired t-test was used to compare the difference among experimental groups (Cr-group vs Pl-group).

Results

The changes in serum and urine Cr and Crn concentrations in response to 30 g/day Cr loading for one week, are shown in Table 1. The sCr concentration increased by 14 folds (+1300%; $p < 0.01$) as compared to the baseline value (BEFORE). The rise in uCr concentration was substantial since it was 140 folds greater (+14000%; $p < 0.01$) than the baseline value. Regarding the Crn response to Cr loading, the increase was modest both in serum (+20%; significant, $p < 0.005$) and urine (+50%; non-significant, except when compared with the Pl-group, $p < 0.05$).

During the washout period (LATER), uCr, sCrn, uCrn were not significantly different from the preload baseline (BEFORE), whereas sCr was still slightly higher ($p < 0.05$). It is interesting to note that the uCr washout baseline concentration remained significantly elevated in the Cr-group when compared to the Pl-group ($p < 0.005$).

Cr administration induced a significant increase in body weight averaging 1.5 kg ($p < 0.05$); following the washout period, the gain in body weight was maintained at a similar level. In contrast, averaged body composition was unchanged.

Individual changes in serum and urine Cr and Crn concentrations in response to Cr loading are shown in Figure 1. A large interindividual variability in response was observed for both Cr and Crn.

An overview of the results for both groups, expressed as the net difference between the situations BEFORE vs AFTER, and BEFORE vs LATER is shown graphically in Figure 2.

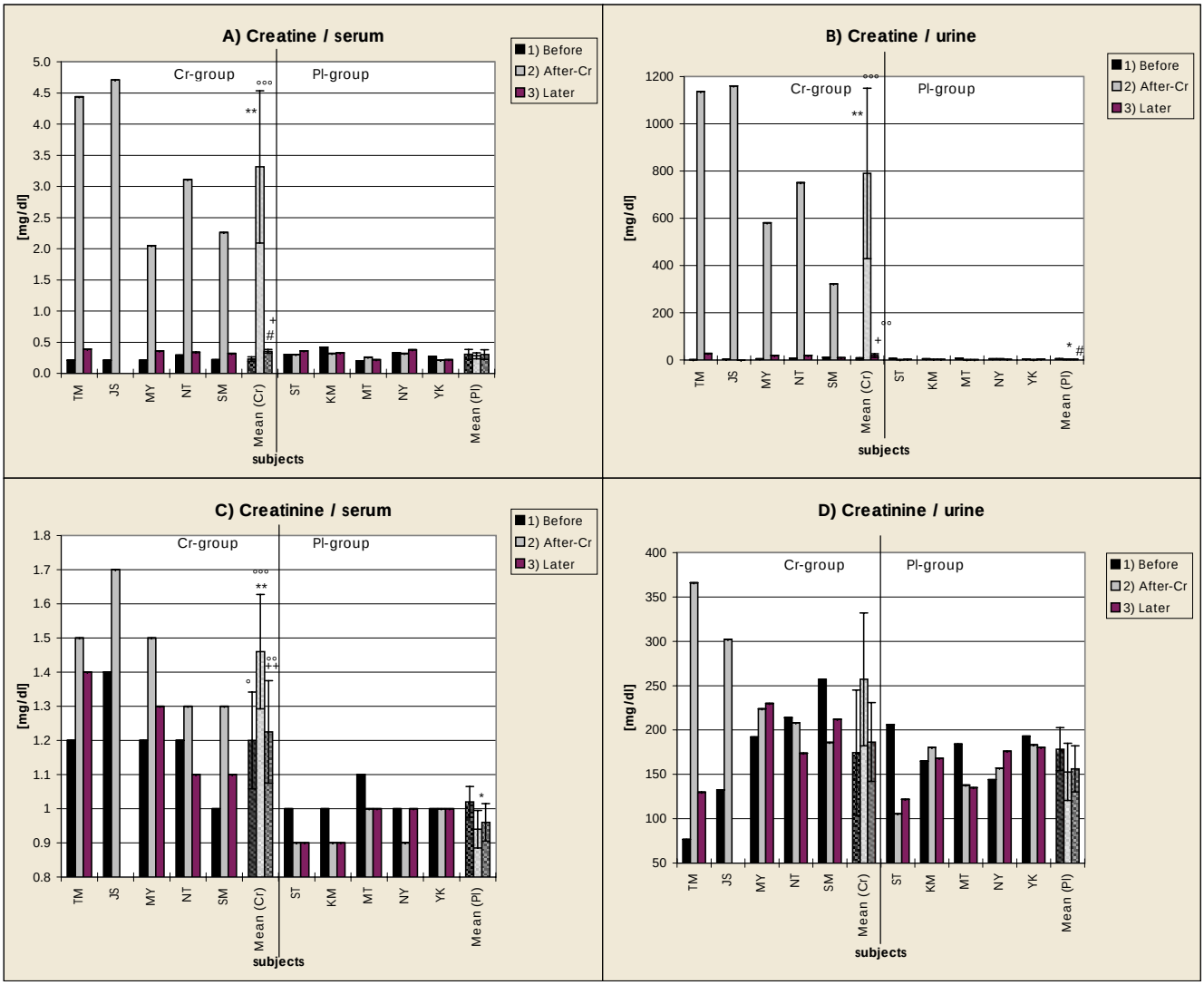

Figure 1:
Individual and averaged results consecutive to creatine (Cr) supplementation: 1) before supplementation (left bar), 2) after one week 30 g/day creatine (Cr-group) or placebo (Pl-group) supplementation (central bar), and 3) after one week washout period (right bar). A) Creatine concentration in serum. B) Creatine concentration in 24h-urine. C) Creatinine concentration in serum. D) Creatinine concentration in 24h-urine. Significant differences (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$) over time for a same group were analyzed by paired two-tailed t-test (*: Before vs After; +: After vs Later; #: Before vs Later), and significance (o: $p < 0.05$; oo: $p < 0.01$; ooo: $p < 0.001$) between Cr-group and Pl-group were analyzed by unpaired two-tailed t-test.

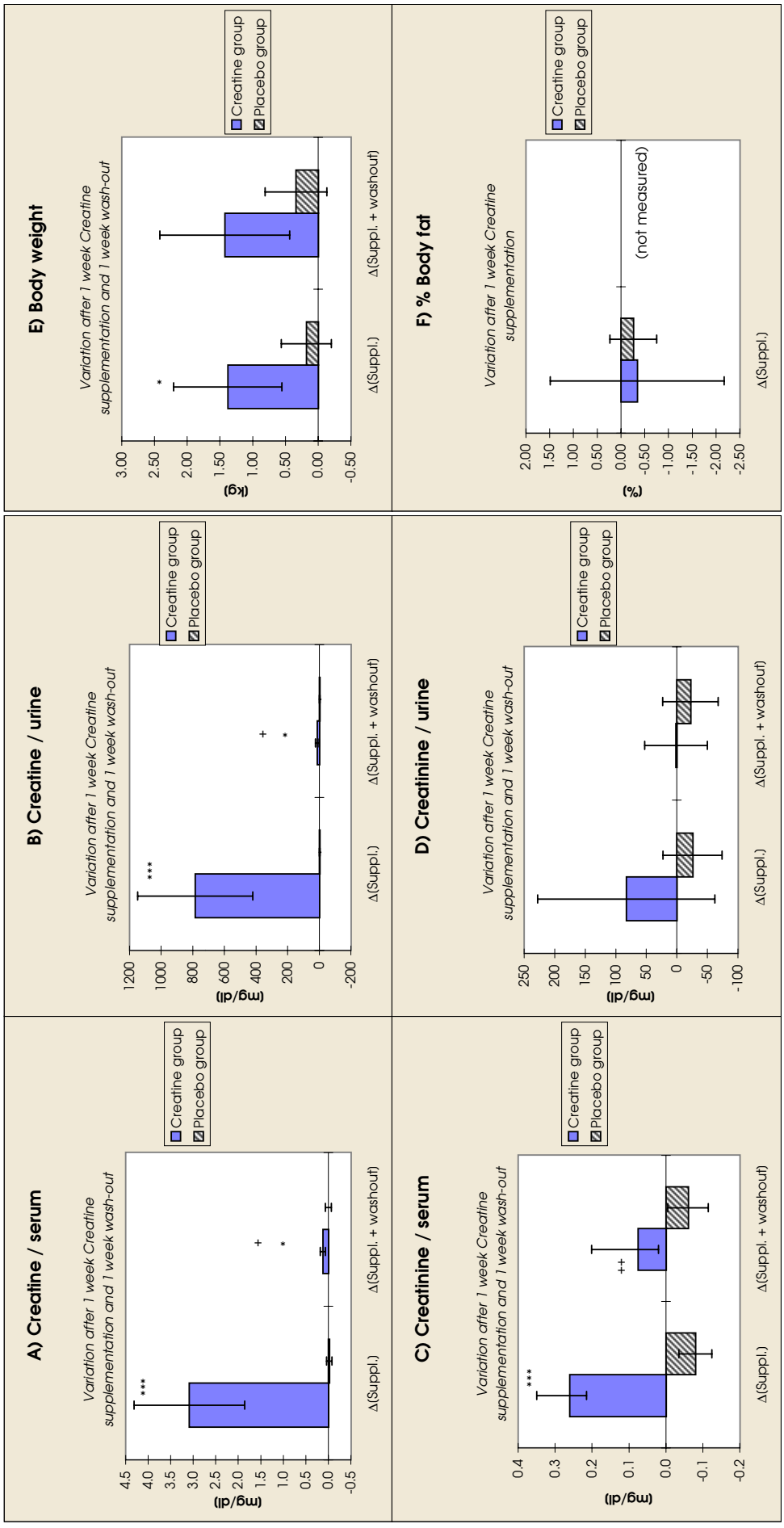

Figure 2:
Averaged net differences consecutive to one week Cr supplementation period (Δ[Suppl.]), or resulting from one week Cr supplementation and one week washout period (Δ [Suppl.+washout]), for creatine (left bar) vs placebo group (right bar). A) Creatine concentration in Serum. B) Creatine concentration in 24h-urine. C) Creatinine concentration in serum. D) Creatinine concentration in 24h-urine. E) Body weight. F) Relative body fat. Significant differences (+: $p < 0.05$; ++: $p < 0.01$; +++: $p < 0.001$) between Δ[Suppl.] and Δ[Suppl.+washout] were analyzed by paired two-tailed t-test, and significance (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$) between Cr-group and Pl-group were analyzed by unpaired two-tailed t-test.

Discussion

In a previous study we reported the serum concentrations of Cr and Crn during 6 hours following an acute ingestion of 20 g Cr [18]. The present study complements this previous work by reporting the effect of high dose supplementation on serum Cr and Crn concentrations over a longer duration and after one week of wash out period.

The results indicate that after the ingestion of 30 g Cr per day for 6 days, a significant increase in serum and urine concentrations occurred for sCr, uCr, sCrn (Fig. 2). Body weight increased slightly and then remained constant in accordance with previous studies [9, 17, 33–35], but no significant effect on body fat was observed.

After the washout period, Cr concentration in serum and urine of Cr-group was still significantly higher than Pl-group, but not as compared to BEFORE.

Poortmans et al. [30] reported in a Cr supplementation study involving 5 subjects (who ingested 20 g/day Cr for 5 days) a significant effect on arterial level of Cr, enhancing its uptake within the muscle compartment. The authors also indicated that a massive urinary excretion of exogenous Cr occurred (about 60% of the oral load), with no apparent sign of glomerular hyperfiltration. Hengelhardt et al. [32] have investigated the alterations in Cr and Crn concentrations following low dosages (6 g/day for 5 days) and reported significant increases in serum and urine Cr and Crn concentrations. Vandenberghe et al. [4] reported similar conclusions following the ingestion of 20 g Cr per day for 3 days, but no significant increase following 5 g Cr per day after 10 weeks supplementation. More recently, Kamber et al. [35] reported a 150% and a 640% increase for respectively sCr and uCr, and a 15% increase for both sCrn and uCrn concentrations, following the ingestion of 20 g Cr per day for 5 days.

The present research used a relative high dosage, and underlines the serious alterations of Cr and Crn levels that can be measured on tested athletes. We should keep in mind that 30 g Cr is dosage usually used by an important number of uninformed athletes (young athletes, bodybuilding,…). The average concentration of Cr in urine reached in our test was very high (Table 1), i.e. comparable to the usual concentration of urea (900 mg/dl) which is the main nitrogenous compound in urine. In the perspective of unexpected drug testing, such a concentration (obtained after a supplementation corresponding to that used by athletes) is very easy to detect and difficult to mask, even in the case of a single spot-urine collection.

It is interesting to calculate the ratio between urine and serum Cr concentration before vs after Cr supplementation: during baseline (BEFORE) there was a ratio of 24 folds whereas it reached a substantially higher value of 239 folds AFTER supplementation. This indicates a huge amount of exogenous Cr excreted in urine, which is in accordance with the previous reports [18, 30, 31, 35]. This demonstrates a large waste in Cr ingested, and mainly a heavy extra-workload performed by the kidney. It can be hypothesized that such a load taken chronically could constitute a potential danger (such as an increase in uremic toxins, damages on renal functions, diabetic complications, nephropathy,...), especially in case of patients with renal insufficiencies [36–48]. A long-term overuse of Cr would therefore not be recommended for athletes, before a rigorous safety profile concerning exogenous Cr has been established. Complete toxicological investigations, suggested also by other authors in recent studies [29–31, 37, 38, 49, 50], are needed to follow the potential effect of a long term high dose Cr supplementation on renal function.

It should be noted that at the end of the washout period (LATER), the ratio urine/serum was still higher than the baseline value and this ratio averaged 54 folds, i.e. more than double as compared to BEFORE. What are the practical implications of these results? Even during planned drug tests, where athletes can voluntarily stop the Cr ingestion few days before, this could be a potential technique to detect Cr supplementation. However, more experiments are needed to validate this approach.

The significant increase in Crn level is obtained by the spontaneously non-enzymatic degradation of Cr at a low pH, and has probably only two different origins: 1) a first production of Crn during the absorption of exogenous Cr in the stomach and the gut [18], and 2) the second origin from the spilling over of Crn from intracellular Cr in muscle tissue [3, 5, 6], which concord with previous specific studies [3, 5, 6, 18, 40, 46].

Conclusion

In conclusion, one-week high dose creatine loading results in a rise in serum creatine accompanied with an important rise in urinary creatine excretion. This indicates that at such high doses of creatine uptake, most of the ingested creatine is spilled over in urine. In parallel, serum creatinine concentration increased significantly; therefore we can strongly consider the hypothesis that the gastrointestinal conversion of creatine to creatinine was enhanced after repetitive ingestions of exogenous creatine. Regarding the non-enzymatic conversion of creatine to creatinine, our previous study investigating a single acute ingestion of creatine complements the present results. The effect on serum and urine concentrations of short term high dose creatine loading disappears within approximately one week that can be stand as the minimal indicated washout period. This has implications for doping detection in athletes: creatine can be easily detected in urine if the drug test takes place during the supplementation period or even one day after the last creatine ingestion, but become difficult to pick-up one week later.

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