Effect of Oral Creatine Supplementation on Urinary Methylamine, Formaldehyde, and Formate

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ABSTRACT

POORTMANS, J. R., A. KUMPS, P. DUEZ, A. FOFONKA, A. CARPENTIER, and M. FRANCAUX. Effect of Oral Creatine Supplementation on Urinary Methylamine, Formaldehyde, and Formate. *Med. Sci. Sports Exerc.*, Vol. 37, No. 10, pp. 1717–1720, 2005. **Purpose:** It has been claimed that oral creatine supplementation might have potential cytotoxic effects on healthy consumers by increasing the production of methylamine and formaldehyde. Despite this allegation, there has been no scientific evidence obtained in humans to sustain or disprove such a detrimental effect of this widely used ergogenic substance. **Methods:** Twenty young healthy men ingested 21 g of creatine monohydrate daily for 14 consecutive days. Venous blood samples and 24-h urine were collected before and after the 14th day of supplementation. Creatine and creatinine were analyzed in plasma and urine, and methylamine, formaldehyde, and formate were determined in 24-h urine samples. **Results:** Oral creatine supplementation increased plasma creatine content 7.2-fold ($P < 0.001$) and urine output 141-fold ($P < 0.001$) with no effect on creatinine levels. Twenty-four-hour urine excretion of methylamine and formaldehyde increased, respectively, 9.2-fold ($P = 0.001$) and 4.5-fold ($P = 0.002$) after creatine feeding, with no increase in urinary albumin output (9.78 ± 1.93 mg·24 h^{-1} before, 6.97 ± 1.15 mg·24 h^{-1} creatine feeding). **Conclusion:** This investigation shows that short-term, high-dose oral creatine supplementation enhances the excretion of potential cytotoxic compounds, but does not have any detrimental effects on kidney permeability. This provides indirect evidence of the absence of microangiopathy in renal glomeruli.
Key Words: CREATININE, KIDNEY, ALBUMIN, URINE, CYTOTOXIC COMPOUNDS

Creatine monohydrate supplementation is widely consumed by competitive athletes and sport leisure participants in attempt to enhance performance, delay fatigue, and improve recovery (22). Short-term (3 months) beneficial effects have also been described in patients suffering from myopathies (10). Two cases of renal dysfunction subsequent to creatine intake have been reported (9,17). However, previous studies from our laboratory have found no deleterious health effects on kidney function in healthy humans submitted to short-, medium-, and long-term supplementation of oral exogenous creatine (13–16).

The conversion of creatine to sarcosine results in cytotoxic agents such as methylamine (23). The latter has been found to be deaminated by semicarbazide-sensitive amine oxidase (SSAO, EC 1.4.3.6) to produce formaldehyde (26) (Fig. 1). Methylamine and formaldehyde are two well-known cytotoxic agents, the presence of which can be revealed by urine analyses (4,12,25,26).

Based on a review paper by Wyss and Kaddurah-Daouk (23), a French Agency (AFSSA) (1) claimed unequivocally that excess consumption of creatine and creatinine might induce derived carcinogenic and mutagenic compounds that could put athletes and consumers of exogenous creatine at risk (http://www.afssa.fr).

To shed light on those allegations, we investigated the appearance of methylamine, formaldehyde, and formate, the main metabolite of formaldehyde, in urine of healthy humans who received oral creatine supplementation.

METHODS

Twenty healthy male subjects, aged 24.1 ± 1.3 yr (mean ± SEM), volunteered and gave their free consent to the following protocol, which was approved by the medical ethic committee of the Faculty of Medicine (Free University of Brussels). For 14 d, the subjects consumed 21 g of creatine monohydrate (Flamma, Italy) daily, divided into three doses of 7 g each (morning, noon, evening), dissolved in water or fruit juice. The purity of creatine monohydrate has been tested by gas chromatography (99.9%) and found to be devoid of any detectible level of sarcosine as tested by enzymatic reaction. Energy intake was recorded by a food questionnaire over seven continuous days. Most foods were weighed before cooking and eating. Dietary intakes were

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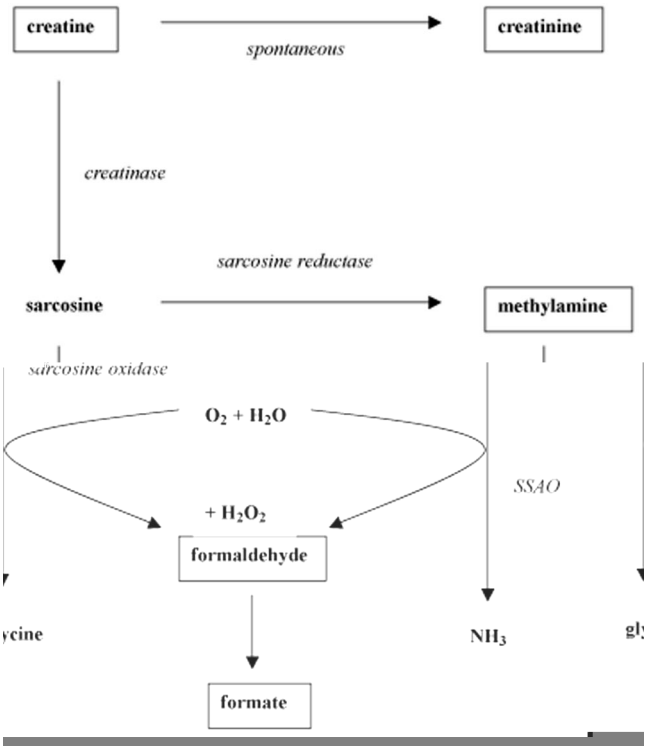

FIGURE 1—Schematic pathways of creatine and creatinine degradation in the human body. All products and metabolic steps are not shown. Compounds that are framed have been assayed in urine before and after creatine supplementation. SSAO, semicarbazide amine oxidase.

assessed by Prodiet 5.1 (Proform, France), professional software that displays the nutrient analysis of any food or combination of selected foods. After that period of food stabilization (mean 3353 kcal, 54% carbohydrate, 31% fat, and 15% protein), the subjects were asked to remain on their usual diet for 2 wk. Twenty-four-hour urine collection was obtained before creatine intake and at the end of creatine supplementation. Venous blood samples were drawn after an overnight fast before creatine feeding and at the end of creatine supplements. All samples (urine and plasma) were stored at −20°C until analyses were done.

Creatine and creatinine were determined in plasma and urine using an enzymatic colorimetric test PAP (Boehringer Mannheim-Roche, Germany). Formaldehyde concentrations in urine were determined by high-performance liquid chromatography by derivatization of 2,4-dinitrophenylhydrazine (4). Formate concentrations in urine were assayed by an enzymatic method using formate dehydrogenase and NAD^+ (6). Urine methylamine was determined by a stable isotope dilution gas chromatography-mass spectrometry method. We developed this from a gas chromatography procedure that used derivatization with isobutyl chloroformate (11). Albumin concentration in urine was determined by an immunochemical technique using specific monoclonal antiserum (Turbiquant, Behring). To allow for urine volume intra- and interindividual variation, urinary values were expressed as the 24-h excretion rate.

When the values followed the Gaussian distribution (Kolmogorov–Smirnov test), the concentration differences before and after creatine feeding were tested by the Student's *t*-test. For data that was not normally distributed, the *t*-test was applied after logarithmic transformation. The results are reported as means and SEM. The statistical significance was fixed at $P \leq 0.05$, two tailed.

RESULTS

Table 1 shows the data obtained on creatine and creatinine concentrations in plasma and urine samples before and after creatine feeding. After an overnight rest following the last intake of creatine, there was a significant increase in venous plasma creatine level (7.2-fold of the basal level) and there was no effect on plasma creatinine level. Creatine urine excretion rates were also highly elevated (141-fold of the basal level) after creatine feeding with no significant changes of the creatinine output. Approximately 77% of the daily dose of ingested creatine was released in the final urine. The albumin urine excretion rate was not affected by creatine supplementation (Table 1).

Table 1 also includes the modifications of urine excretion of formaldehyde, formate, and methylamine before and after creatine supplementation. Twenty-four-hour urine output of methylamine and formaldehyde increased 9.2- and 4.5-fold, respectively ($P < 0.001$), after creatine feeding with no increase in formate excretion. After creatine feeding, there was no correlation between plasma creatine and urine methylamine ($r^2 = 0.025$, P = not significant (NS)) or formaldehyde ($r^2 = 0.017$, P = NS).

DISCUSSION

The results of the present investigation indicate that short-term, high-dose oral creatine ingestion in healthy subjects enhances the mechanisms leading to the conversion of creatine 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 representing indirectly the production of the former substrate (3). Using rat and mice models, Yu and Deng demonstrated that *in vivo* deamination of methylamine produces formaldehyde and hydrogen peroxyde, which are both recognized as cytotoxic substances (4,24). Moreover, the inhibition of SSAO, the enzyme that converts methylamine into formaldehyde, enhances the urine output of methylamine

TABLE 1. Mean values (± SEM) of plasma and urine contents before and after creatine supplementation.

	Before Creatine	After Creatine
Plasma		
Creatine (μmol·L^{-1})	73.1 ± 7.8	526.9 ± 81.4*
Creatinine (μmol·L^{-1})	97.2 ± 12.5	72.5 ± 10.9
Urine		
Creatine (g·24 h^{-1})	0.11 ± 0.02	15.66 ± 2.06*
Creatinine (g·24 h^{-1})	1.86 ± 0.14	2.22 ± 0.14
Albumin (μg·24 h^{-1})	9.78 ± 1.93	6.97 ± 1.15
Methylamine (mg·24 h^{-1})	0.69 ± 0.06	6.41 ± 1.45*
Formaldehyde (μg·24 h^{-1})	64.78 ± 16.28	290.4 ± 66.3*
Formate (mg·24 h^{-1})	12.46 ± 1.04	14.16 ± 1.84

* $P < 0.001$ between values before and after creatine supplementation.

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after creatine feeding in mice (4,24). Consequently, these authors hypothesized that long-term administration of large quantities of creatine as an ergogenic supplement would increase the production of methylamine and subsequently formaldehyde, both being potentially cytotoxic in renal glomeruli (4,24). Our results support this hypothesis in humans.

Despite the 9.2-fold increase in methylamine urine excretion induced by creatine ingestion, this level did not reach the normal upper limit values from healthy humans, up to 35 mg·d^{-1} (mean ± 3 SD) (12). After creatine supplementation, urine formate excretion remained below the upper range (14–20 mg·d^{-1}) reported in healthy subjects (2,7,19). However, with creatine supplementation, the urine excretion of formaldehyde increased 4.5-fold of the basal rate. It has been claimed that formaldehyde is a mutagenic and genotoxic agent that reduces the rate of DNA synthesis (20); however, in human, besides our present results, there are not yet available data on the upper limit of the urinary level of formaldehyde that would indirectly support a mutagenic effect *in vivo*.

Because creatine is transformed to sarcosine by microbial enzymatic reactions (23), it is likely that methylamine is formed in the intestine and is therefore potentially harmful to the integrity of the intestinal epithelium. Methylamine is toxic to human endothelial cells and forms patchlike lesions (27) and even causes kidney damage (26). In mammals, SSAO activity has been found in various tissues associated with the vascular system (5,8). Therefore, it is likely that the deamination of methylamine occurs in the circulation. It could also be speculated that this flooding of methylamine in the blood, together with SSAO, might produce formaldehyde favoring microangiopathy in the renal glomeruli (8,25).

Our subjects consumed a total amount of 294 g creatine monohydrate during 14 d without any modification of glomerular membrane permeability as assessed by their albumin urine excretion rate (9.78 ± 1.93 mg·24 h^{-1} before creatine; 6.97 ± 1.15 mg·24 h^{-1} after creatine). The upper limit in healthy humans is 25 mg·24 h^{-1}. Albuminuria has long been known to be associated with specific renal abnormality and is now recognized as an early test for vascular endothelial damage (18). Despite the fact that formaldehyde and methylamine excretion rates were increased, respectively, 4.5- and 9.2-fold after high-dose of creatine supplementation, there was no detectable consequence of glomerulonephropathy. 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 (21). This raises the question of the duration of the supplementation. In a previous study, we did not observe any adverse effect of a long-term (up to 5 yr) creatine supplementation in humans (14). Moreover, the present study has only addressed 2-wk supplementation with high-dose creatine (21 g·d^{-1}). The more standard procedure of oral creatine is based on 2–5 g·d^{-1} after an initial 5 d at a higher dose. Thus, there is reason to believe that lower doses of creatine supplementation would reduce the impact on urinary methylamine and formaldehyde excretion. However, even if systematic deleterious effects could not be observed, it cannot be excluded that systematic production of low extra doses of cytotoxic agents never induces any occurrence of nephropathy. Clearly, epidemiological data are required to evaluate the 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 creatine on a regular basis should be systematically monitored throughout the ingestion period.

In conclusion, short-term, high-dose oral creatine supplementation in young healthy subjects increases urinary excretion of methylamine and formaldehyde within the normal range for a healthy population. In the conditions of the current study, increased urinary methylamine and formaldehyde excretion did not result in impaired renal function. Further studies are needed to evaluate whether long-term creatine supplementation, indeed, is harmless in all individuals.

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