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RECENT ADVANCES IN DOPING ANALYSIS (8)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

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A.GOTZMANN, B.KOMANNS, A.ADIB, M.BREDEHÖFT, W.SCHÄNZER:
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A. Gotzmann, B. Komanns, A. Adib, M. Bredehoeft, W. Schänzer

Creatine - A Doping Substance ?

Institute of Biochemistry, German Sport University Cologne, Carl-Diem-Weg 6, 50933 Köln, Germany

Introduction

Results of numerous scientific investigations have provided evidence of potential effects of creatine as an ergogenic aid. During short duration of maximal intensity exercise, ATP resynthesis is nearly exclusively due to anaerobic degradation of phosphocreatine.

Creatine phosphate + ADP $\leftrightarrow$ creatine + ATP $\Delta G^0 = -12{,}6$ KJ/mol

Creatine [(α-methylguanido)acetic acid] is produced by the human body and is also present in different concentrations in several types of food (meat, fish, milk, etc.).

Today creatine is also available as substance (creatine monohydrate) and as a diet supplement. In addition, it is widely used by athletes in various sports. Several publications reported a positive effect of creatine supplementation on the physical performance. The recommendation for use of this supplement vary between various publications and manufacturers. A loading dose of 20-30 g /day up to 6 days is recommended followed by a much lower daily dose up to 28 days.

To quantify creatine and creatinine in human urine for routine doping analysis, a fast and precise HPLC-UV method was determined. Data of urinary creatine concentration in routine samples from German athletes (n = 605, in competition and out-of competition tests) and a control group (n = 99) were collected. These data were compared to urinary creatine concentrations of an excretion study with creatine and experimentally with high creatine containing diet.

Experimental

- **Creatine excretion study**:

  A female, 27 years, 65 kg, administered once 3 x 6.3 g/day (Σ 18.9 g/day) creatine orally [Performance enhancer CREATINE FUEL®]. This amount was equivalent to 0.3 g/kg body weight/day. Urine samples were collected one day before, during, and one day after the experiment.

- **Creatine containing diet**:

  3 volunteers in groups 1-3, and 5 volunteers in group 4 consumed

500 g beef (creatine approximately 2.5 g)	[group 1]
500 g fish (herring - creatine approximately 3.2-5 g)	[group 2]
500 g shrimps (creatine approximately 0 g)	[group 3]
'average diet': 150 g chicken breast (creatine ???), vegetables and potatoes	[group 4]

Creatine concentrations in diets were calculated based on values reported in the literature. Three urine samples were collected before, and up to 22 hours after the experiment.

Sample preparation

The native urine was diluted 1:5 with deionized water before injection into the HPLC system. Samples containing more than 2000µg creatine per ml of urine were reanalyzed after dilution of 1:10 with deionized water.

HPLC parameter

A Hewlett Packard liquid chromatograph 1090, series II, with diode array detector and automatic injection system was used for the analysis. Ammonium sulfate, 0.01 molar, pH: 7.5 [A] and acetonitrile (gradient analyzed, J. T. Baker, Deventer, NL) [B] were used as solvents. A 100% of A was held for 4.5 min to elute creatine, followed by a column cleaning step with 25% B. The overall run time was 10 min. The injection volume was 5µl, the diode array detector was set to 246 nm with a bandwidth of 4 nm. A C18 reversed phase column (NUCLEOSIL 120-5 RP18 250 x 4 mm; Macherey & Nagel, Düren, Germany) was utilized for this procedure. The creatine concentration in urine was calculated against an external standard.

Results

The evaluation of 605 routine samples from doping analysis (male, female, in competition tests and out of competition tests of various types of sports from different German Sports Federations) was conducted. Table 1 shows the results of the analysis. By comparison, the creatine concentration of a control group where the athletes declared not to have taken any creatine containing supplements, were estimated. The highest creatine concentration analyzed in this group was 627µg/ml urine.

Table 1: Creatine concentration ranges in 619 routine urine samples from doping control and 99 samples of a control group, analyzed by HPLC/UV detection.

Creatine in urine [µg/ml]	Routine samples [n]	Routine samples [%]	Routine samples* [n]	Control group [n]	Control group [%]
< 100	475	78.5	3	92	92.9
101-500	93	93.9	3	5	98.0
501-1000	12	95.9	2	2	100
1001-2000	14	98.2	4	-	
2001-3000	5	99.0	0	-	
3001-4000	1	99.2	0	-	
> 4001	5	100	2	-	
Σ	**605**		**14**	**99**	

* Creatine use was declared by the athlete

After administration of creatine, (3 x 6.3g/day) the highest concentrations estimated were 11053µg/ml (240 min after the first dose), 10933µg/ml (225 min after the second dose) and 6327µg/ml (545 min, overnight, after the third dose) respectively (see also Fig. 1).

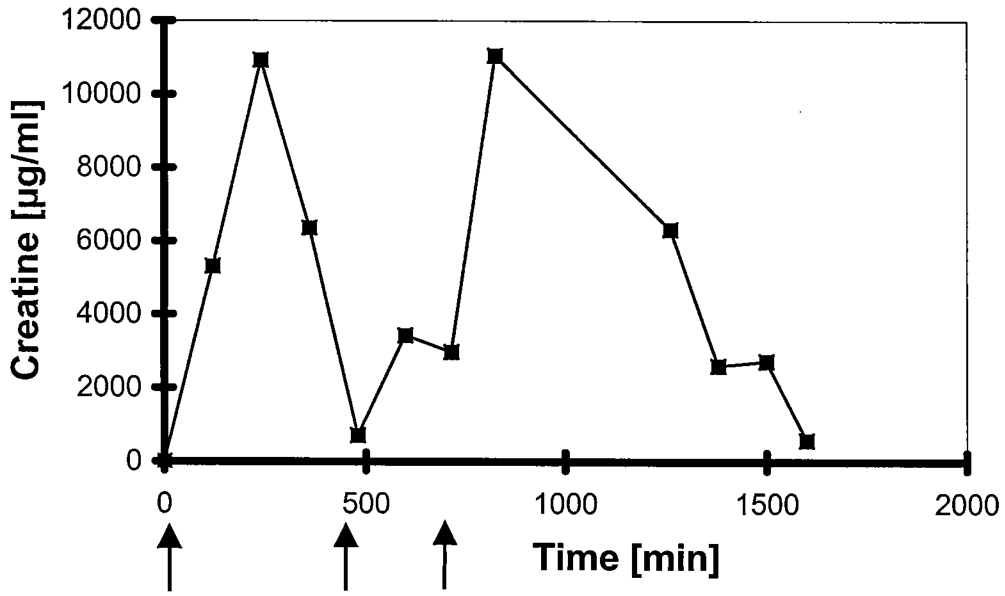

Figure 1: Urinary creatine concentration of a female volunteer after administration of 18.9g creatine (3 x 6.3g/day). The arrows indicate the time of application.

After consumption of 500g beef, 500g shrimps, 500g herring and 'average diet' at lunch time, the highest concentrations after administration have been estimated and are shown in table 2 (see also figure 2-5).

Table 2. Highest creatine concentration in urine after uptake of different types of food

Food consumption	**Volunteer 1** Creatine [µg/ml urine]	**Volunteer 2** Creatine [µg/ml urine]	**Volunteer 3** Creatine [µg/ml urine]	**Volunteer 4/5** Creatine [µg/ml urine]
Beef	1330	67	80	-
Herring	415	23	66	-
Shrimps	51	62	27	-
'Average diet'	204	< 10	142	96 / 224

Discussion

Results of numerous investigations have revealed beneficial results of creatine intake and subsequent increase in physical performance in sports, whereas others have not. A differentiation between different types of workload is necessary. There is virtually no effect of creatine on physical performance in long duration, aerobic performance type activities.

Supplementation of creatine should be avoided where it does not produce the desired benefit and it should not be consumed in excessively supra-large and potentially harmful non-physiological doses. [1-4]

In doping analysis, it is obvious that athletes from several types of sports use creatine products. Only in a few cases, it is marked on the official doping control form under the section of declared medications.

The intention of this study was to assess a cut-off-level to distinguish between normal creatine excretion, creatine excretion after creatine containing diet, and extensive creatine supplementation during the so called 'loading phase'.

Side effects of creatine supplementation may cause an increase in body weight, diarrhea and muscular problems (cramps). Cases of renal dysfunction associated with creatine supplementation were also reported. Nothing is known about the effect of creatine supplementation on the cardiovascular system. Longitudinal studies of creatine use were not available until now [4-7].

According to the Rules of Ethics (CHAPTER II, Article 2) of the medical commission of the International Olympic Committee (IOC) doping is defined as "the use of an expedient (substance or method) which is potentially harmful to athletes' health and/or capable of enhancing their performance (..)." and "the presence in the athlete's body of a prohibited substance or evidence of the use thereof or evidence of the use of a prohibited method."

This definition requires a ban of supplementation of creatine: it is performance enhancing, it is potentially harmful, and most importantly, creatine is easily detectable in routine doping analysis. A cut-off-level of 1 µg creatine / ml urine can be recommended, on the basis of the data of the control group. It should be noted that increased consumption of meat before the competition can affect this level.

Athletes should also be aware of creatine products since creatine supplements may be contaminated with prohormones, which may lead to a positive doping case for anabolic steroids. Some commercially available products were identified as contaminated. [8,9]

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Appendix

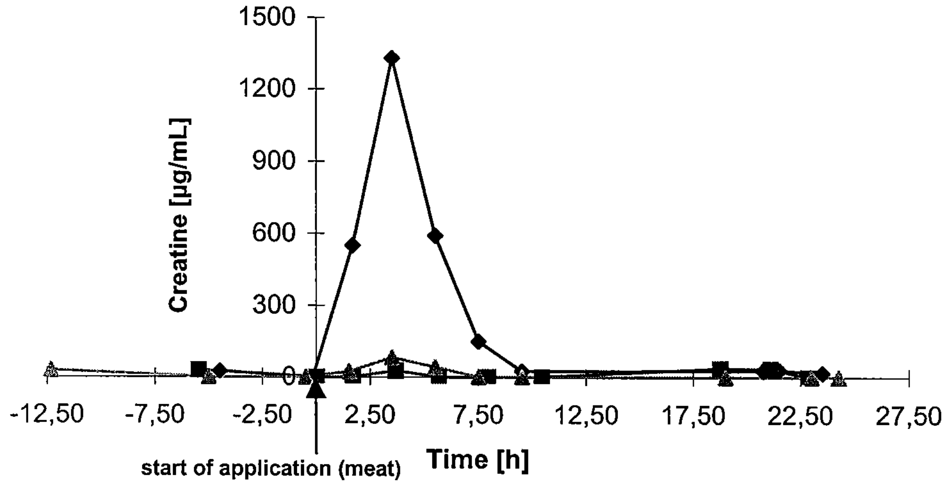

Figure 2: Urinary creatine concentration prior and after the consumption of 500g of beef (group 1, n = 3)

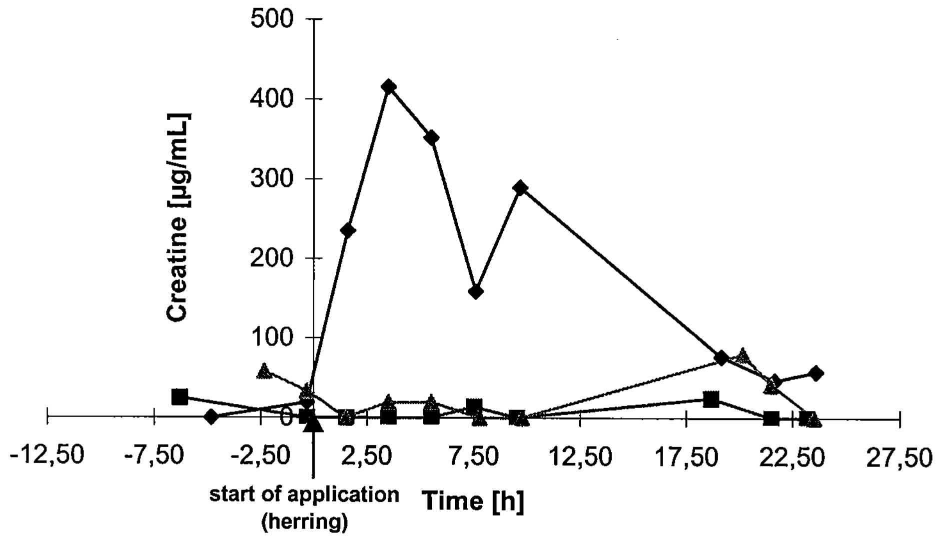

Figure 3: Urinary creatine concentration prior and after the consumption of 500g of herring (group 2, n = 3)

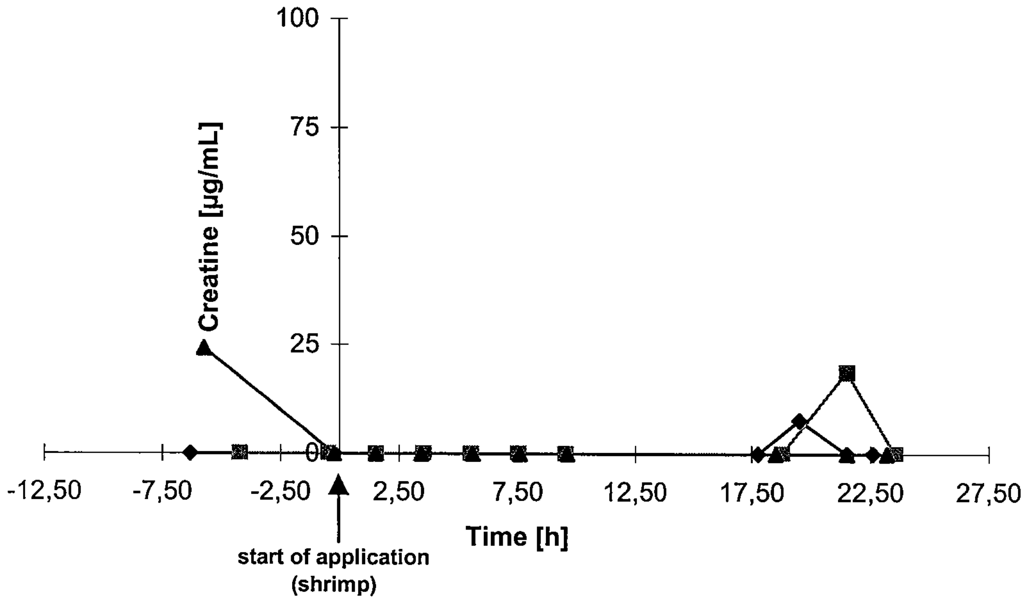

Figure 4: Urinary creatine concentration prior and after the consumption of 500g of shrimps (group 3, n = 3)

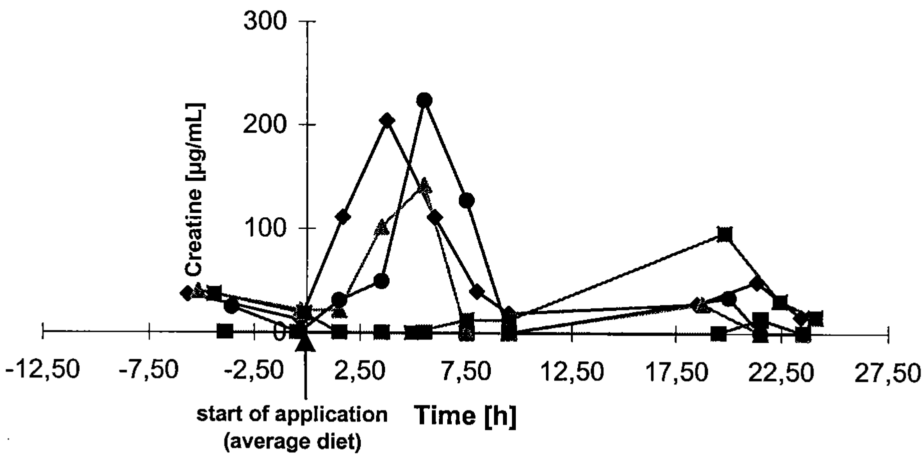

Figure 5: Urinary creatine concentration prior and after the consumption of 'average diet' (group 4, n = 5)