

# URINARY CREATINE AT REST AND AFTER REPEATED SPRINTS IN ATHLETES: A PILOT STUDY

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**ABSTRACT:** Creatine plays a key role in muscle function and its evaluation is important in athletes. In this study, urinary creatine concentration was measured in order to highlight its possible significance in monitoring sprinters. The study included 51 sprinters and 25 age- and sex-matched untrained subjects as a control group. Body composition was measured and dietary intake estimated. Urine samples were collected before and after standardized physical exercise. Creatine was assessed by gas chromatography mass spectrometry. Basal urinary creatine (UC) was significantly lower in sprinters than controls ( $34 \pm 30$  vs.  $74 \pm 3 \mu\text{mol}/\text{mmol}$  creatinine,  $p < 0.05$ ). UC was inversely correlated with body mass ( $r = -0.34$ ,  $p < 0.01$ ) and lean mass ( $r = -0.30$ ,  $p < 0.05$ ), and positively correlated with fat mass ( $r = 0.32$ ,  $p < 0.05$ ). After acute exercise, urinary creatine significantly decreased in both athletes and controls. UC is low in sprinters at rest and further decreases after exercise, most likely due to a high uptake and use of creatine by muscles, as muscle mass and physical activity are supposed to be greater in athletes than untrained subjects. Further studies are needed to test the value of urinary creatine as a non-invasive marker of physical condition and as a parameter for managing Cr supplementation in athletes.

**KEY WORDS:** athlete, creatine, exercise, physical performance, sprinter

## INTRODUCTION

Creatine (Cr) is a naturally occurring amino acid-like compound provided by the diet and synthesized in the body mainly in the liver and kidney [30]. Cr is then transported to tissues by a membrane creatine transporter (SLC6A8) [30]. In humans, over 95% of the body Cr content is stored in skeletal muscle, where Cr or more specifically phosphocreatine (PCr) plays a major role in a muscle's ability to perform and maintain short duration, high intensity exercise [2]. In athletes competing in speed and strength sports, the demand for ATP is elevated during exercise due to the nature of effort. An energy substrate required for ATP regeneration is PCr [9]. The importance of the 'phosphagen system' lies in the extremely rapid rates at which it can re-synthesize ATP [2]. Hence, PCr hydrolysis does not depend on oxygen availability, nor necessitate the completion of several metabolic reactions to buffer energy at the onset of exercise and during anaerobic intense muscle contraction [2,6]. Clearly, the energetic capacity of this system is dependent on the availability of PCr. In this regard, several studies have shown that Cr supplementation increases total Cr skeletal muscle content [12,26], and enhances performance during high intensity exercise [8,19,28] as well as resistance train-

ing [4,27]. Most studies investigating Cr status in athletes have focused on muscle Cr content [9,13,24]. It is obvious that measurement of Cr in muscle is highly invasive as it requires muscle biopsy and its estimation using nuclear magnetic resonance is costly [7]. In this regard, no study has looked at urinary Cr in athletes although urine is easily and safely accessible. We hypothesized that the measure of urinary Cr could be of interest in athletes. Thus, we conducted this study to explore urinary Cr status in sprint athletes and untrained subjects at rest and after exercise, and to test the significance of this parameter as a possible marker of physical performance.

## MATERIALS AND METHODS

**Subjects.** Fifty-one volunteer sub-elite young sprinters (30 males and 21 females; sex-ratio, 1.4; age,  $17 \pm 1.6$  years), living and train-

Non-standard abbreviations

Cr, creatine; GC/MS, mass spectrometry coupled with gas chromatography; SLC6A8, creatine transporter protein; TEE, total energy expenditure

**TABLE I.** SPRINTER CHARACTERISTICS (MEAN±SD)

	Males (n=30)	Females (n=21)
100 m sprint athletes	12	8
200 m sprint athletes	10	8
400 m sprint athletes	8	5
Performance*	92 ± 5.1	82.8 ± 7.4

Note: \* Performance compared to world record

ing at the National Center for Elite Athletes (Tunis, Tunisia), participated in this study (Table 1). All athletes trained 20 to 24 hours a week. The average of practice of sprint training was  $4 \pm 2.6$  years. Exclusion criteria were subjects who had taken Cr supplements within the 3 months prior to the experiment and those who were taking nutritional supplements at the time of the study. Sprinters' records were assessed during their participation in the Zitouna meeting (Tunis, June 2009). Physical performance for each athlete was expressed by the percentage of his own record compared with the gender-specific world record for the specialty.

Twenty-five volunteer healthy students (14 males and 11 females; sex-ratio, 1.2; age,  $17 \pm 1.4$  years) who had never been engaged in any regular sport training were selected as the control group. Controls had only attended school physical education classes (less than 3 hours, weekly). The experimental protocol was approved by ethical committees of Rabta Hospital and all participants and their parents/guardians gave their written informed consent to participate in the study. The study protocol was in accordance with the ethical principles laid out in the 2008 revision of the Declaration of Helsinki.

#### Study protocol

##### Anthropometric measurements

Body mass and height were measured with the subjects barefooted and lightly clothed. Waist circumference was measured by a trained dietician with non-extensible tape midway between the lowermost rib margin and the iliac crest. Body composition was measured only in sprinters using foot-to-foot bio-electrical impedance analysis (Model TBF 300, Tanita Inc., Tokyo, Japan) [3] allowing the estimation of body fat, lean mass and total body water.

##### Nutritional inquiry

Values for nutrient intakes were obtained using a 3-day food record. Records were processed using the professional Bilnut program (Nutrisoft, Cerelles, France) and the food composition tables published by the Tunisian National Institute of Statistics in 1978. In order to estimate energetic needs, total energy expenditure (TEE) was calculated based on the equation of Black et al. [5] as follows:  $TEE = \text{basal metabolic rest} * \text{physical activity level}$ . For the athletes, the energy expenditure corresponding to the sport type according to McArdle [20] was added to the TEE value.

#### Exercise protocol

A standardized exercise, described and validated by Skare et al. [23], was performed by sprinters and controls in order to investigate the effect of physical exercise on urinary Cr excretion. The exercise consisted of 6 maximal bouts of 60 m sprints separated by 5 min rest periods. Five minutes rest has been shown to be sufficient to totally restore muscle Cr stores [2].

#### Sample collection and analytical methods

All subjects were called for urine collection before exercise and one hour after the completion of exercise. During this period, subjects were not allowed to eat, but were allowed to drink. A sample of 100  $\mu\text{L}$  of urine was collected from all sprinters and untrained subjects. Urine samples were immediately transferred to the laboratory at 4°C, then stored at -20°C until analysis (within 3 months). Urinary creatinine was determined using the Jaffe method [17]. Urinary Cr was determined by mass spectrometry coupled gas chromatography (GC/MS) as described by Nasrallah et al. [21]. Briefly, 100  $\mu\text{L}$  of urine is mixed with 100  $\mu\text{L}$  of 2-phenylbutyric acid (internal standard), 50  $\mu\text{L}$  of saturated sodium bicarbonate solution, 600  $\mu\text{L}$  of toluene and 50  $\mu\text{L}$  of hexafluoro-acetylacetone. The mixture is heated at 80°C for 2 h then is allowed to cool. The toluene phase is separated by centrifugation at 3000 rpm for 5 min. From the upper toluene phase 400  $\mu\text{L}$  are transferred dried under nitrogen flow. The dried residue is dissolved in 50  $\mu\text{L}$  of bis-trimethylsilyl trifluoroacetamide (BSTFA) and 50  $\mu\text{L}$  of chloroform for 30 min at 65°C and then 1  $\mu\text{L}$  is injected in a Hewlett Packard® gas chromatograph 7890A coupled to a HP 5975C mass selective detector (Agilent Technologies, Inc., Loveland, CO, USA). Chromatographic separation is achieved on a GC ultra 2 non-polar capillary column, 25 m in length, 0.2 mm in internal diameter and 0.33  $\mu\text{m}$  in film thickness (Agilent Technologies) with helium as the carrier gas. The instrument is operated under electronic impact ionization. The temperature of the transfer line is 270°C and the mass spectrometer source temperature is 250°C. The specific Cr ion selected has  $m/z$  239 and 258. The method sensitivity was 20  $\mu\text{mol} \cdot \text{L}^{-1}$ . The inter-assay (n=10) imprecisions (CVs) were 6.4% and 6.9% at concentrations of 289 and 4909  $\mu\text{mol} \cdot \text{L}^{-1}$ , respectively.

#### Data analysis

The statistical analyses were performed using the SPSS version 11.5 software package (SPSS Inc., Chicago, IL, USA). Differences between groups were compared by ANOVA test or Kruskal-Wallis test for continuous variables and chi-squared test for categorical variables. Receiver operating characteristic (ROC) curves were plotted and areas under curve (AUC) were calculated to assess basal and post-exercise urinary Cr in relation to athletic activity. The association between continuous variables was tested by Pearson correlation analysis. All probabilities were two-tailed and p values < 0.05 were regarded as significant.

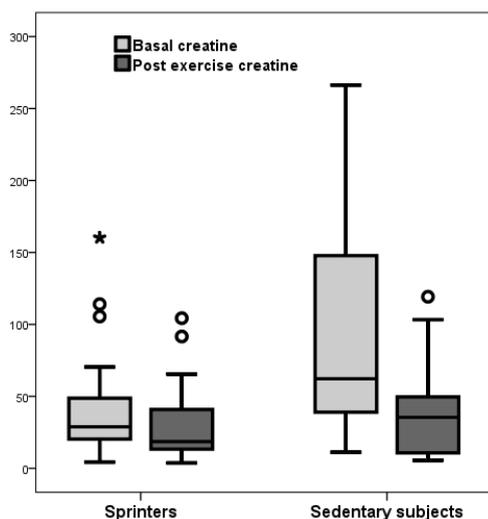
**RESULTS**

Table 2 shows the main demographic, anthropometric and nutritional parameters in athletes and untrained subjects. Sprinters and controls did not differ according to age, sex ratio or BMI. Impedance measures showed that sprinters were properly hydrated and the percentage of fat was high in females. Dietary inquiry revealed that total energy, protein and carbohydrate intakes in sprinters were markedly lower than recommended dietary allowances.

**TABLE 2.** DEMOGRAPHIC, ANTHROPOMETRIC AND NUTRITIONAL PARAMETERS MEASURED IN THE ATHLETES AND CONTROL UNTRAINED SUBJECTS

	Sprinters (n = 51)	Untrained subjects (n=25)
Age (years)	17.4±1.63	16.9±1.44
Weight (kg)	62.9±4.63	61.2±5.83
BMI (Kg·m <sup>-2</sup> )	21.2±1.94	21.2±2.61
Fat mass (%)	17.6±5.39	-
	M:14.0±3.2	
	F: 22.4±3.8	
Lean mass (%)	82.4±5.39	-
Body total water (%)	59.5±5.57	-
Energy intake (Kcal·day <sup>-1</sup> )	2499±619 <sup>a</sup>	2261±556
Carbohydrate intake (g·Kg weight <sup>-1</sup> ·day <sup>-1</sup> )	5.10±1.20 <sup>a</sup>	5.81±2.11
Protein intake (g·Kg weight <sup>-1</sup> ·day <sup>-1</sup> )	1.12±0.32 <sup>a</sup>	1.21±0.41
AP/VP	1.31±0.50	1.25±0.25
Fat intake (g·Kg weight <sup>-1</sup> ·day <sup>-1</sup> )	1.42±0.43	1.62±0.61

Note: Values are expressed as means ± SD; AP, animal proteins; VP, vegetal proteins; a values are significantly lower (P<0.01) than the recommended dietary allowances (calculated from specific total energy expenditure).

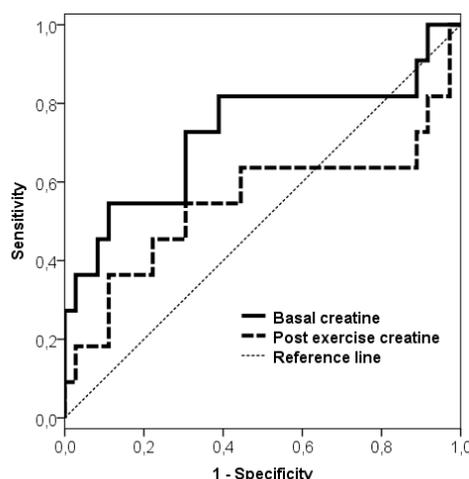


**FIG. 1.** BASAL AND POST-EXERCISE URINARY CREATINE CONCENTRATIONS ( $\mu\text{mol} \cdot \text{mmol}$  CREATININE) IN SPINTERS AND UNTRAINED CONTROLS (BOX PLOT).

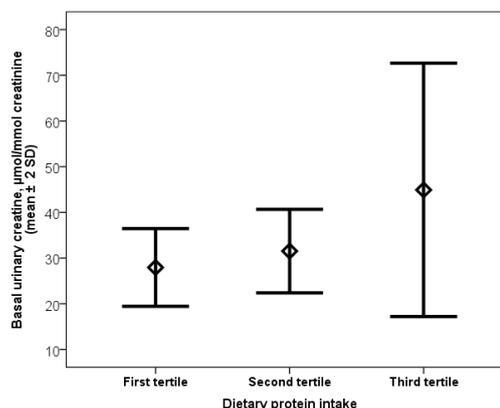
Note: The bottom and top of the box are the first and third quartiles (interquartile range or IQR) and the band inside the box is the median. The ends of the whiskers are the lowest and the highest values still within 1.5 IQR. Outlier values within 3 IQR are displayed as circles and those outside are displayed as stars.

Basal urinary Cr concentrations were significantly lower in sprinters than controls ( $34 \pm 30$  vs.  $74 \pm 73 \mu\text{mol} \cdot \text{mmol}^{-1}$  creatinine;  $p < 0.05$ ). Compared to respective basal levels, post-exercise urinary Cr concentrations were significantly lower in both sprinters and controls; the decrease was significantly greater in untrained subjects (figure 1). ROC curves showed that basal urinary Cr [AUC (95% CI), 0.725 (0.523–0.927);  $p = 0.025$ ] but not post-exercise urinary Cr [0.523 (0.312–0.786);  $p = 0.633$ ] discriminated sprinters from untrained subjects (figure 2).

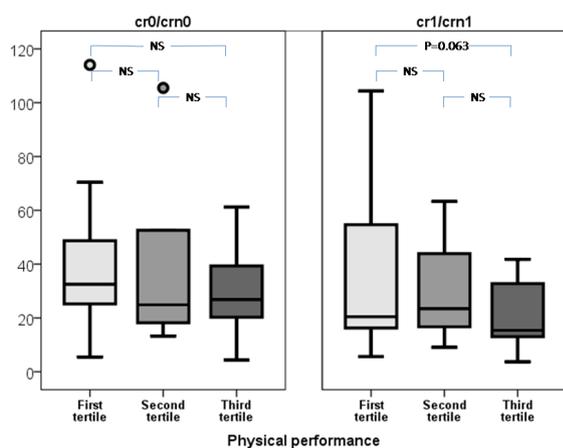
In sprinters, basal urinary Cr was inversely correlated to total body water ( $r = -0.324$ ;  $p = 0.028$ ) and lean mass ( $r = -0.307$ ;  $p = 0.038$ ), but positively related to fat mass ( $r = 0.310$ ;  $p = 0.036$ ). No significant correlation was observed with total energy, protein and carbohydrate intakes. Nevertheless, there was a trend towards increase of basal urinary Cr through protein intake tertiles (figure 3). Basal and post-exercise urinary Cr levels did not vary according to athletes' physical performance tertiles. However, the worst performing sprinters (first



**FIG. 2.** RECEIVER-OPERATING-CHARACTERISTIC (ROC) CURVES FOR BASAL AND POST-EXERCISE URINARY CREATINE ( $\mu\text{mol} \cdot \text{mmol}$  CREATININE) TO DISCRIMINATE UNTRAINED SUBJECTS AND SPINTERS. Note: Area-under-the-curve (AUC) (95% CI) was 0.725 (0.523–0.927) for basal urinary creatine and 0.523 (0.312–0.786) for post-exercise urinary creatine.



**FIG. 3.** BASAL URINARY CREATINE CONCENTRATIONS ( $\mu\text{mol} \cdot \text{mmol}$  CREATININE) BY TERTILES OF SPINTERS' DIETARY PROTEIN INTAKE



**FIG. 4.** BASAL (cr0/crn0) AND POST-EXERCISE (CR1/CRN1) URINARY CREATINE CONCENTRATIONS ( $\mu\text{mol} \cdot \text{mmol}$  CREATININE) BY TERTILES OF SPRINTERS' PHYSICAL PERFORMANCE (BOX PLOT).

Note: The bottom and top of the box are the first and third quartiles (interquartile range or IQR) and the band inside the box is the median. The ends of the whiskers are the lowest and the highest values still within 1.5 IQR. Outlier values within 3 IQR are displayed as circles and those outside are displayed as stars.

tertile of physical performance) showed a trend for significant ( $p=0.063$ ) higher post-exercise urinary Cr levels compared to the most efficient sprinters (third tertile) (figure 4).

## DISCUSSION

The present study showed that basal urinary Cr concentrations are significantly lower in sprinters compared to untrained subjects. Urinary Cr concentrations decreased after acute physical exercise in both groups. The decrease was more marked in untrained subjects. These findings may be explained by differences in muscle mass and metabolism. Since muscle mass and activity are greater in athletes, their muscular Cr uptake and consumption are higher compared to untrained subjects. Therefore, athletes seem to excrete smaller amounts of Cr in urine. Indeed, urinary Cr concentrations were found to be inversely related to lean mass that mainly reflects muscle mass. In accordance with these data, studies showed that Cr supplementation was accompanied by a significant increase in body weight, lean tissue and body water, but a decrease in fat mass [10,15,16,18,26,29]. To date, no study has examined urinary Cr concentration in sprinters. However, some studies have investigated Cr and phosphocreatine in sprinters' muscles [11,24]. Spencer et al. [24] reported that maximal sprint exercise of 10 to 12.5 seconds induced approximately 40 to 70% depletion of muscle phosphocreatine stores. Hirvonen et al. [13] also showed that faster sprinters deplete greater amounts of muscle phosphocreatine than slower sprinters during a 100-m sprint. The present study data suggest that practice of regular high-intensity short-term ('anaerobic') exercise influenced Cr metabolism not only during exercise but also at rest. A possible metabolic adaptation to sprint exercise has been shown to be an increased expression/activity of

membrane Cr transporter protein (SLC6A8) [22]. In accordance, Brault et al. [6] demonstrated that variations in Cr uptake rate reflected changes in SLC6A8 protein content/activity. SLC6A8 protein content is reduced with elevated intra-muscular Cr concentrations and would be elevated as intra-muscular Cr decreases.

Urinary Cr excretion may also be influenced by diet. Indeed, Arias et al. [1] showed that the urinary creatine:creatinine ratio increases after high protein intake. It was also demonstrated that Cr uptake into muscles is better when Cr is consumed with a high carbohydrate diet [14,11].

This study showed that total energy, protein, and carbohydrate intakes of the studied sprinters are markedly inferior to their estimated daily needs. Low carbohydrate intake causes deviation of protein from plastic functions to energetic purposes and then amplifies protein insufficiency [25]. Thus, reduced urinary Cr excretion in these sprinters may also result, at least in part, from deficient dietary intake. Protein insufficiency would result in reduced Cr synthesis and muscle storage. As a consequence, almost all available Cr is taken up by muscles and only a small amount is excreted in urine. Such data suggest that urinary Cr might be used as a marker for protein dietary intake. This hypothesis should be tested in future studies.

This study failed to establish a clear relationship between urinary Cr and physical performance. However, a low urinary Cr level may point toward a deficient diet, which stands for insufficient conditioning. Arias et al. [1] showed that diet influences urinary Cr excretion. They reported a significantly higher urine Cr/Crn ratio in healthy volunteers after a meal based on beef or oily fish as compared to eggs, pasta or salad. Deficient diet in these sprinters would have influenced urinary Cr concentrations and interfered in their relationship with physical performance. Thus, whether urinary Cr reflects the level of conditioning should be tested in well-nourished sprinters. Urinary Cr excretion may also be affected by the hydration status. In this study, urine samples were collected in June when the temperature averaged 30°C, which could potentially influence the hydration status of sprinters. Such an effect was attenuated by adjustment of urinary Cr to urinary Crn excretion.

The study has some limitations. Firstly, the sample size is relatively small, especially for controls. In reality, urinary Cr analysis by mass spectrometry coupled with gas chromatography is time-consuming and costly. Moreover, the constraint of practice of sprint exercise followed by urine collection limited the number of controls. Secondly, dietary intake the day before Cr analysis was not controlled, which could have resulted in the large dispersion of urinary Cr concentrations in both sprinters and controls. Finally, the personal record:world record ratio may not be a reliable marker for physical performance as it could be affected by diverse factors such as stress or lack of experience in these young athletes.

Urinary Cr concentration was found to be high (similar to controls) in two sprinters who were later recognized as having taken Cr supplements and were then excluded from the study (not included in the 51 subjects). These observations raise the option of using urinary Cr

to monitor Cr supplementation in athletes. It could be suggested that supplementation is provided while rates are low and stopped when they reach a normal steady state. Further studies are necessary to answer this question.

## CONCLUSIONS

Urinary Cr excretion at rest and after exercise is lower in sprinters than untrained subjects. These data suggest that urinary Cr could inform on the level of muscle activity and probably on diet adequacy. The study showed no relationship between urinary Cr and physical performance. Further studies should be undertaken in well-nourished sprinters to test whether urinary Cr is helpful to evaluate physical conditioning and also to test its usefulness in managing Cr supplementation.

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## Competing interests

The authors declare no conflicts of interest.

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