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Prevalence of Caffeine use in urine samples analyzed during 2010-2012

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Abstract

Actually caffeine is not a prohibited substance but it is included in the Monitoring Program by the World Anti-Doping Agency (WADA). The present work shows the prevalence of caffeine found in urine samples analyzed in competition during 2010-2012 from Venezuela, Mexico and Cuba. Most urine samples had urine caffeine concentrations below 6 µg/mL (limit of WADA monitoring program) but 7 % of urine samples (n=2154) exceeded the former threshold of caffeine doping (12 µg/mL) and 14 % were higher than 6 µg/mL. Cycling was the sport with more prevalence in caffeine abuse (14.2 ± 19.3 µg/mL) followed by weightlifting (7.2 ± 10.4 µg/mL) and aquatics sports (6.5 ± 10.3 µg/mL). In conclusion, endurance sports showed the highest urine caffeine concentrations and its use is in increment.

Introduction

Caffeine is an stimulant of the central nervous system that increase the attention and concentration capacities [1]. Caffeine has been shown to enhance several different modes of exercise performance including endurance [2], high-intensity team sport activity [3], and strength-power performance [4]. Since 1984 it was included in the prohibited list by the International Olympic Committee at a threshold level of 15 µg/mL and in 1985 the threshold was set at 12 µg/mL. Actually caffeine is not a prohibited substance but it is included in the Monitoring Program by WADA at 6 µg/mL. The aim of the present work was to investigate and to describe the use of caffeine by athletes in urine samples analyzed in competition during 2010-2012. A method to quantify caffeine by gas chromatography with nitrogen/phosphorous detector was revalidated.

Experimental

Method Validation: Method was validated according to ISO/IEC 17025 (specificity, linearity, limits of detection and quantification, carryover, recovery, inter and intra-assay precision and robustness).
Sample preparation: 5 mL of urine sample were spiked with 25 µL of internal standard (diphenylamine 1000 µg/mL). After that, 500 µL of KOH 5 M and 3 g of sodium sulphate were added. Liquid-liquid extraction under alkaline condition was performed by adding 2 mL of tert-butyl methyl ether. 100 µL of the organic phase was transferred to the vial and 3 µL was injected in the GC/NPD.
Instrumentation: Gas chromatograph 6890 (Agilent Technologies) with nitrogen/phosphorous detector. The GC was equipped with a fused capillary column HP Ultra-2 (12 m x 0.2 mm x 0.33 µm). Temperature program: Initial temp. 90 °C increased to 300 °C at 20 °C/min. Held for 3 min. Carrier gas was helium at a flow of 1 mL/min. Injection volume 3 µL at 280 °C. Hydrogen and air flow was 3 mL/min. and 80 mL/minute, respectively.

Results and Discussion

For this study, it was analyzed 4885 samples collected in competitions. A total of 2154 samples contained caffeine, most of them from Venezuela, Mexico and Cuba. The numbers of samples with concentrations greater than 6 µg/mL were 4.4, 5.3 and 9.2 % in 2010, 2011 and 2012 respectively even when the number of samples analyzed in competition were similar

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(2010: 1717 samples; 2011: 1545 samples; 2012: 1623 samples).
Although most of them showed caffeine concentration between 1-2 µg/mL, 156 samples (7.2 % of samples) showed caffeine concentration greater than 12 µg/mL, the former WADA cutt-off before its removal from the Prohibited List. The data obtained showed an increment in caffeine consumption. From this total of sample (156), 17 samples corresponds to 2010, 35 to 2011 meanwhile 104 samples corresponds to 2012. Figure 1 represents the distribution (in number of samples) of all urine samples with caffeine in relation to caffeine concentration measured.
Caffeine concentrations was compared in 10 sports. Table 1 shows data obtained after application of ANOVA test (Tuckey HSD). Cycling was the sport with more prevalence in caffeine abuse (14.2 ± 19.3 µg/mL) followed by weightlifting (7.2 ± 10.4 µg/mL) and aquatics sports (6.5 ± 10.3 µg/mL). In summary, in the last three years have changes the utilization of this substance by athletes. Endurance sports showed the highest urine caffeine concentrations and its use is in increment. Our results are in contrast with other previously published works, where the use of caffeine in sports during 2004-2008 changed minimally after its removal from the WADA prohibited list [5,6].
Validation: Linearity at 5 concentration levels (1, 3, 6, 12, 16 µg/mL) was R^2=0.9999. The intra and inter-assay precision (at 16 µg/mL) was 8.6 and 7.1 %, respectively. The value obtained for recovery was 102 %. The change of Na_2SO_4 by NaCl (robustness) was not suitable (recovery 67 %). The detection and quantification limits were 0.1 and 0.25 µg/mL, respectively. No interference was observed in the analysis of 20 different blank urines. No carryover was observed after injection of blank urine spiked at 16 µg/mL.

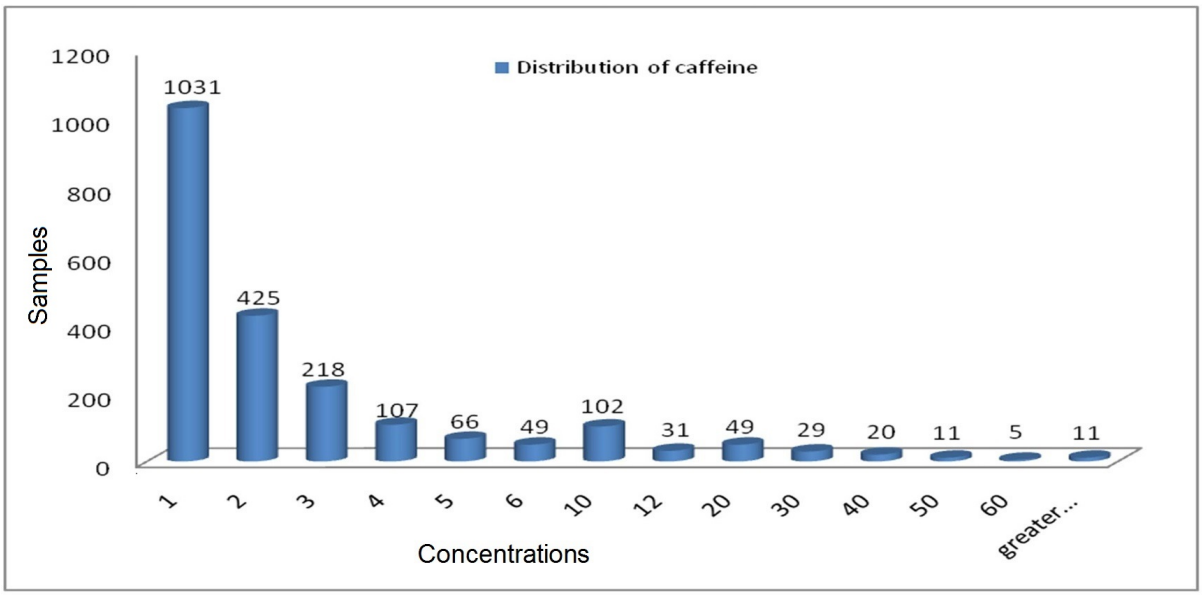

Figure 1. Distribution of urinary caffeine concentration

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X	Judo	Fencing	Karate	Football	Athletics	Basketball	Weightlifting	Gymnastic	Shooting	Cycling	Aquatics	Boxing	Baseball	Wrestling
media ± s.d	3.15 +- 5.39µg/mL	2.4 +- 2.48µg/mL	1.76 +- 1.68µg/mL	2.02 +- 4.39µg/mL	2.3 +- 4.44µg/mL	4.96 +- 6.49µg/mL	7.21 +- 10.41µg/mL	0.85 +- 0.96µg/mL	3.64 +- 8.78µg/mL	14.25 +- 19.31µg/mL	6.56 +- 10.35µg/mL	1.99 +- 5.73µg/mL	0.8 +- 0.67µg/mL	1.24 +- 0.73µg/mL
Wrestling										*				
Baseball						*	*			*	*			
Boxing						*	*			*	*			
Aquatics				*				*		*				
Cycling	*	*		*	*		*	*	*					
Shooting														
Gymnastic						*	*							
Weightlifting				*	*									
Basketball				*	*									
Athletic														
Football														
Karate														
Fencing														
Judo														
Comparison results between sports following ANOVA (Turkey HSD), * significant differences (a=0.05)														

Table 1. Variance analysis by sports

Conclusions

The statistical analysis showed an increment in caffeine abuse with prevalence in endurances sports such as cycling, weightlifting and aquatics ports. From 2154 samples with caffeine, 7 % showed urine concentration greater than 12 µg/mL and 14 % greater than 6 µg/mL. Endurance sports showed the highest urine caffeine concentrations and its use is in increment. Caffeine abuse has incremented in the last years.

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