Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (12)

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Sport und Buch Strauß, Köln, 2004

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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (12). Sport und Buch Strauß, Köln (2004) 27-35

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Caffeine use in sports: an overview before the removal from the doping list

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Introduction

Caffeine (1,3,7-trimethylxanthine) is, according to pharmacological criteria, the most common drug of abuse used in the world today. Besides several sources including chocolate and energy drinks, the main source of caffeine is coffee. A complete list of the caffeine content in several drinks and chocolate products has previously been published [1].
In addition, caffeine is also present in several over-the-counter medications, such as cold remedies, diuretics, weight loss products and wake-up pills in doses varying from 30 to 100 mg [2]. Some of these products are referred to as natural health products and contain guarana (*Paullinia cupana*), a South American plant which has shown to contain caffeine [1]. Also, numerous nutritional supplements, like energy drinks, promoted for their performance enhancing and stimulating effect contain caffeine. According to the European regulations, caffeine can be added to sport/energy drinks up to levels of 320 mg/l.
Often, caffeine is associated with central nervous system stimulation, diuresis and withdrawal effects.
Several reviews have discussed the influence of caffeine on performance [3-6]. Generally, caffeine tends to have a positive influence on endurance performance. It is also reported that strength athletes use caffeine in order to increase their performance although it is not clear whether caffeine has a positive influence on strength or not [7].
Because of the reported misuse of high doses, caffeine was listed as a doping agent in class I A "Stimulants" in 1984. The threshold level of 15 µg/ml was reduced to 12 µg/ml in 1985.
From January 1st 2004, caffeine is removed from the WADA list of prohibited substances. The reason is that caffeine is ubiquitous and present in a whole range of products incorporated in our daily food and beverages. WADA acknowledges that caffeine can enhance

performance but removes the drug from its list of prohibited substances because the ergogenic effect is said to be rather small and realised by the vast majority of athletes of whom most are caffeine users, and because it is not possible to distinguish social use from doping attempts. However, laboratories are asked by WADA to keep on monitoring caffeine concentrations. This could allow later comparison with results obtained before the removal of caffeine from the list of prohibited substances.

Therefore, an overview of the results of caffeine concentrations found in the Ghent laboratory before the removal of caffeine from the doping list is presented.

Experimental

Urine Analysis

All urine samples were analysed according to the procedure previously described by Delbeke et. al. [8]. Briefly, 100-120 mg of sodium chloride, 50 µl of internal standard (β-OH-ethyltheophylline 100 µg/ml, aqua bidest) and 100 µl of ammonium buffer (pH 9.5) were added to 1 ml of urine. Extraction was performed by rolling with 5 ml CH_2Cl_2-CH_3OH (9:1) for 20 minutes. After centrifugation, the organic layer was separated and evaporated under oxygen free nitrogen at 40°C. The residue was dissolved in 200 µl of mobile phase. 20 µl was injected on the chromatographic system. The method allowed separation of theobromine, theophylline, paraxanthine and caffeine.

The HPLC system consisted of a Model P4000 liquid chromatograph, a Model AS 3000 autosampler and a Spectra Focus forward optical scanning detector set at 275 nm, all from TSP (Fremont, CA, USA). The column was a Hypersil 5 ODS, 100x3 mm I.D., 5 µm (Chrompack, Antwerp, Belgium) with an appropriate precolumn (10x2 mm I.D., 40 µm, C_{18}). The loop volume was 20 µl. The mobile phase used was tetrahydrofurane-water (1:100, v/v) at a flow rate of 1.0 ml/min.

The method was validated according to ISO 17025. Therefore, an equal weighted linear calibration curve was constructed in the range from 0-20 µg/ml. Peak heights were used for quantification. Other parameters determined during the validation procedure were reproducibility, repeatability, selectivity and specificity

Caffeine concentrations

Urinary caffeine concentrations were used from samples originating from the Flemish Community, The Netherlands and several sports federations, including UCI, UEFA, IAAF and others, in the period from 1993 – 2002.
Totally 11361 samples were analysed and comprised in the statistical analysis. All samples originated from in competition controls.
The urine samples could be classified in approximately 90 sport categories.

Statistical analysis

Based on the limit of quantification (LOQ) of 0.1 μg/ml, all concentrations below this LOQ were excluded from further statistical analysis. The resulting concentrations were divided into intervals of 0.25 μg/ml to create a caffeine distribution.
Because the data was not normally distributed, a non-parametric form of analysis was used to evaluate the data from the 11361 urine samples where the caffeine concentration was higher than the LOQ. All results were ranked numerically, the median value and the interquartile range (IQR) were determined as well as the far outside value defined as {(3xIQR)+Q3}, where Q3 is the 75^{th} percentile.
To evaluate the difference between sports, a log transformation was used. This allowed for the determination of an average concentration. Comparison of sports with more than 200 samples being analysed was done by an ANOVA using the Tuckey HSD test comprised in the SPSS software package (SPSS inc, Chicago, USA)

Results and discussion

Figure 1 shows the distribution of caffeine concentrations in athletes' urine specimens. Caffeine was not detected in 3002 or 26.42% of all samples analysed. Non parametric analysis of the data resulted in a median value of 1.23 μg/ml. The interquartile range was calculated to be 1.64 μg/ml. The far outside value, defined as {(3xIQR)+Q3}, was 7.2 μg/ml. Hence, any value exceeding this far outside value can be considered as extremely unusual [9]. Using this far outside value as a threshold level would have resulted in 158 positive urine samples instead of the 16 samples declared as positive with the threshold level of 12 μg/ml. In doping control great care should be taken that no false positives are reported. Therefore a safety margin should be applied between the level of abnormality and the decision limit. The former threshold level of 12 μg/ml seemed to allow for this precaution.

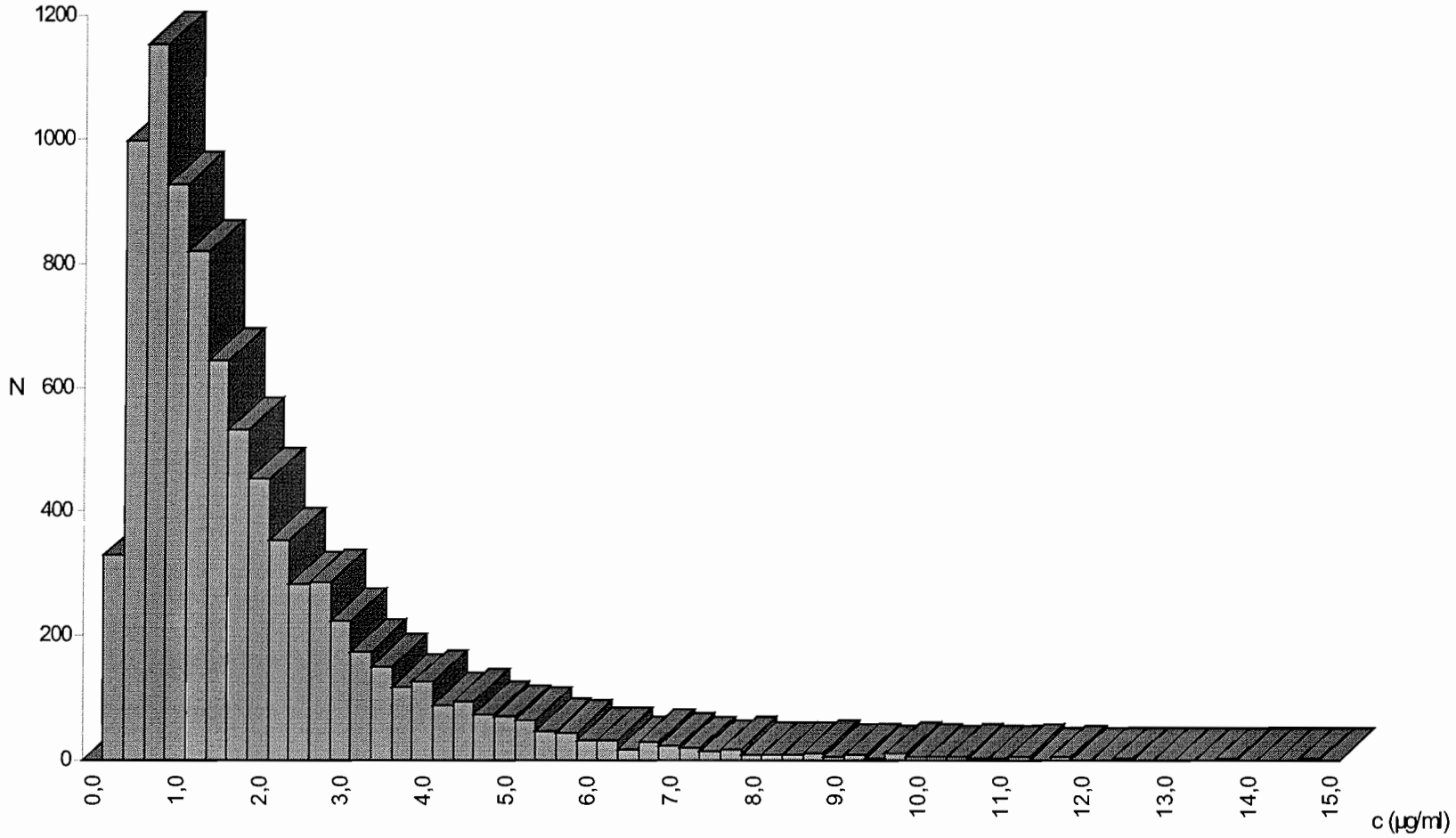

Figure 1: Distribution of urinary caffeine levels in the urine of 11361 athletes tested for doping control during the period 1993-2002. Caffeine concentrations <LOD and >15 µg/ml are not shown.

To evaluate the difference between sport categories a log transformation was used. This allowed the data to adapt in a Gaussian form (Figure 2). Again, all concentrations below the LOQ were excluded. This normal distribution allowed to determine an average concentration which was calculated as 1.22 µg/ml with a standard deviation of 2.45 µg/ml. This results in an apparent threshold level of 11.02 µg/ml (= average + 4xSD). Again, the threshold level of 12 µg/ml seemed reasonable.

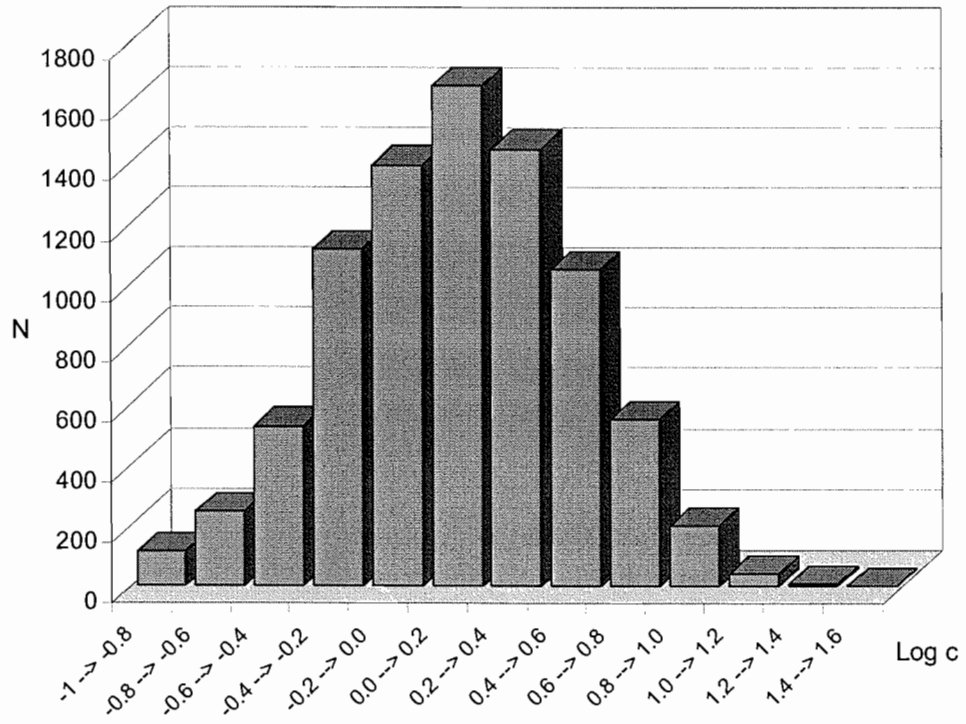

Figure 2: Distribution of log-transformed urinary caffeine concentrations of urine samples exceeding the LOQ of the analytical method.

Further analysis of the data was done to evaluate the difference between sport categories. Only those sports with more than 200 samples being analysed were included in this comparison. The resulting average concentrations, standard deviation, percentage of values below the LOQ and number of samples analysed for those sports are summarised in Table 1.

Table I: Average caffeine concentration and standard deviation after transformation, and percentage of values below LOD in sports with more than 200 samples analysed (1993-2002).

Sport	N	Average concentration (µg/ml)	Standard deviation (µg/ml)	Percentage below LOQ (%)
cycling	4344	1.34	2.44	22.17
athletics	643	1.06	2.62	32.50
swimming	215	0.90	2.37	45.12
soccer	1295	1.05	2.23	27.80
basketball	478	1.04	2.34	32.01
volleyball	641	1.20	2.39	20.90
bodybuilding	255	1.72	2.49	26.27

Large variations can be observed between sports, for example swimming and bodybuilding, as well as within each sport.

Results of the ANOVA test are summarised in Table 2. As can be seen, overall concentrations found in bodybuilding are significantly higher when compared to other sports including cycling and ball sports. This could be due to the fact that caffeine in combination with ephedrine is known as a fat burner. Several studies have demonstrated that ephedrine, particularly in combination with caffeine, is effective in promoting weight loss without increasing serious adverse events [10,11]. Numerous sites on the internet promote the caffeine-ephedrine-asperin stack (ECA-stack) for its fat burning effect.

Significantly higher concentrations were also found in cycling compared to most ball sports. Use of caffeine is very popular in the late stage of a race for its stimulating and ergogenic effects [12].

Table II: Comparison of mean urinary caffeine concentrations found in sports with N>200.

	cycling 1.32 µg/ml	athletics 1.03 µg/ml	swimming 0.9 µg/ml	soccer 1.04 µg/ml	basketball 1.03 µg/ml	volleyball 1.20 µg/ml	bodybuilding 1.72 µg/ml
bodybuilding 1.72 µg/ml	#	#	#	#	#	#	
volleyball 1.20 µg/ml	=	=	#	=	=		
basketball 1.03 µg/ml	#	=	=	=			
soccer 1.04 µg/ml	#	=	=				
swimming 0.9 µg/ml	#	=					
athletics 1.03 µg/ml	#						
cycling 1.32 µg/ml							

= no significant difference (α=0.05)

≠ significant difference (α=0.05)

As can be seen from Table 3 most positive case were detected in cycling, but taking into account the number of samples analysed this only counts for a small percentage. The largest percentage of positives are found in strength sports including bodybuilding, powerlifting and weightlifting with the highest concentration being detected of approximately 30 μg/ml in bodybuilding. This is in agreement with the significantly higher concentrations that were detected in this type of sport.

Table III: Number of positive samples and highest detected urinary caffeine concentration during the period 1990-2002.

Sport	Number of positives	Percentage positives	Highest Concentration (μg/ml)
Cycling	4	0.09	15.0
bodybuilding	3	1.18	29.9
powerlifting	3	2.05	16.9
volleyball	2	0.31	17.7
athletics	1	0.16	14.7
soccer	1	0.08	18.6
weightlifting	1	2.17	12.3
gymnastics	1	0.81	20.1

Conclusion

It can be concluded that analysis of 11361 urine samples, collected for doping control purposes, results in caffeine concentrations far below the former threshold level of 12 μg/ml. Looking at the results, one can wonder why caffeine was removed from the list of prohibited substances as the vast majority of athletes, of whom most are caffeine users, has a urinary caffeine concentration far below the threshold level of 12 μg/ml.

Caffeine concentrations found in strength sports, including bodybuilding, powerlifting and weightlifting, are significantly higher compared to other sports. Also a higher percentage of positives are found in those sports.

Deleting caffeine from the list of prohibited substances could result in an increased use of caffeine as the ergogenic effects of caffeine are well known. If the goal of drug testing is to prevent unfair advantage, to encourage ethical behaviour and to protect human health it will be important to keep on monitoring caffeine concentrations and to take appropriate actions if necessary

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