In: W Schänzer, H Geyer, A Gotzmann, U Mareck (eds.) Recent Advances In Doping Analysis (13). Sport und Buch Strauß - Köln 2005

W. Van Thuyne and F.T. Delbeke

Did caffeine use in sports change after the removal from the doping list ?

Doping Control Laboratory – Ghent University-UGent, Technologiepark 30B, B-9052 Zwijnaarde, Belgium.

Introduction

For many people caffeine, or 1,3,7-trimethylxanthine is a very common part of the diet. Besides the classical caffeine containing products such as cola drinks, chocolate, energy drinks and coffee, caffeine, just as theophylline is readily available as an over the counter drug in wake-up pills [1]. Also, several nutritional supplements promoted for performance enhancing and stimulating effects contain caffeine [2]. Some of these supplements comprise, besides caffeine, extracts of guarana (*Paullinia cupana*), a South American plant which has shown to contain caffeine [3].

Ingestion of caffeine to improve athletic performance is common use among athletes. A survey conducted in Canada revealed that 35% of 11-18 year-old students questioned, believed that caffeine could enhance their performance, and 25% admitted using caffeine to increase their athletic performance [4]. The effects of caffeine have previously been discussed. Caffeine has a positive influence on endurance performance [5-7]. An improve in short term exercise has also been observed, although reports on this subject are contradicting caused by the short nature of this type of exercise [8-12]. Caffeine is also used by strength athletes because of the popular belief that caffeine will increase maximal strength. Internet sites dedicated to strength athletes often promote the use of an ECA-stack (ephedrine-caffeine-aspirin) to increase maximal power. However, the effects of caffeine on strength are not yet scientifically proven.

Before January 1st 2004, caffeine was prohibited by the IOC and WADA. Urinary caffeine concentrations higher than the threshold level of 12 µg/ml resulted in an adverse analytical finding. From January 1st 2004 caffeine was removed from the WADA list of prohibited substances. In this study, results of monitoring caffeine concentrations in 4633 urine samples tested for doping control after the removal of caffeine from the doping list will be presented.

An evaluation will be made with results obtained before the removal of caffeine from the doping list [13].

Experimental

Urine analysis

All urine samples were analysed according to the procedure previously described by Delbeke et. al. [14]. 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 into 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. 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 2004.

Totally 4633 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

All data were ranked numerically and divided into intervals of 0.25 µg/ml to create a caffeine distribution. Concentrations below the LOQ of 0.1 µg/ml (N = 942) were considered as not detected. To evaluate the difference between sports a log-transformation of the concentrations was necessary. Therefore, all values below the LOQ were excluded. This allowed the determination of an average concentration with a standard deviation of the concentrations above the LOQ (N = 3691). Comparison of the most frequent tested sports in 2004 was done by a one-way ANOVA using the Tuckey HSD test comprised in the SPSS software package (SPSS 12.0, SPSS inc., Chicago, USA). Comparison of the results obtained before (N = 11361) and after the removal (N = 4633) of caffeine of the WADA doping list was done using an F-test to determine the variances, followed by an appropriate student t-test for the most abundantly tested sports in both periods.

Results and discussion

Figure 1 shows the distribution of caffeine concentrations in athletes' urine specimens. Caffeine concentrations were found up to 21 µg/ml. Percentages of caffeine values higher than 4 µg/ml, a caffeine concentration found in moderate to frequent coffee drinkers [15] are shown in Table 1. Higher percentages were found in cycling and strength sports compared to the overall percentage, and to values observed in other tested sports. Compared to the previously examined period, the overall percentage of samples with a urinary caffeine concentration higher than 4 µg/ml remained approximately the same while the percentage in cycling increased.

Because the data was positively skewed (cfr.: Fig 1), a log-transformation was used to adapt the data into the Gaussian form. All samples with a concentration below the LOQ of 0.1 µg/ml were excluded from this statistical analysis. These samples accounted for 20.33% of all samples analysed in 2004. Urine samples with caffeine concentrations below the LOQ in the previous examined period accounted for 26.42 % or 6 percent higher.

Six samples or 0.13% of all samples were found to contain a urinary caffeine concentration exceeding the former threshold level of 12µg/ml. This is comparable to the results obtained in the last decade.

The normal distribution allowed the determination of an average caffeine concentration and standard deviation which were calculated as 1.12 µg/ml and 2.68 µg/ml respectively. This resulted in an apparent threshold level of 11.84 µg/ml (= average + 4 x standard deviation).

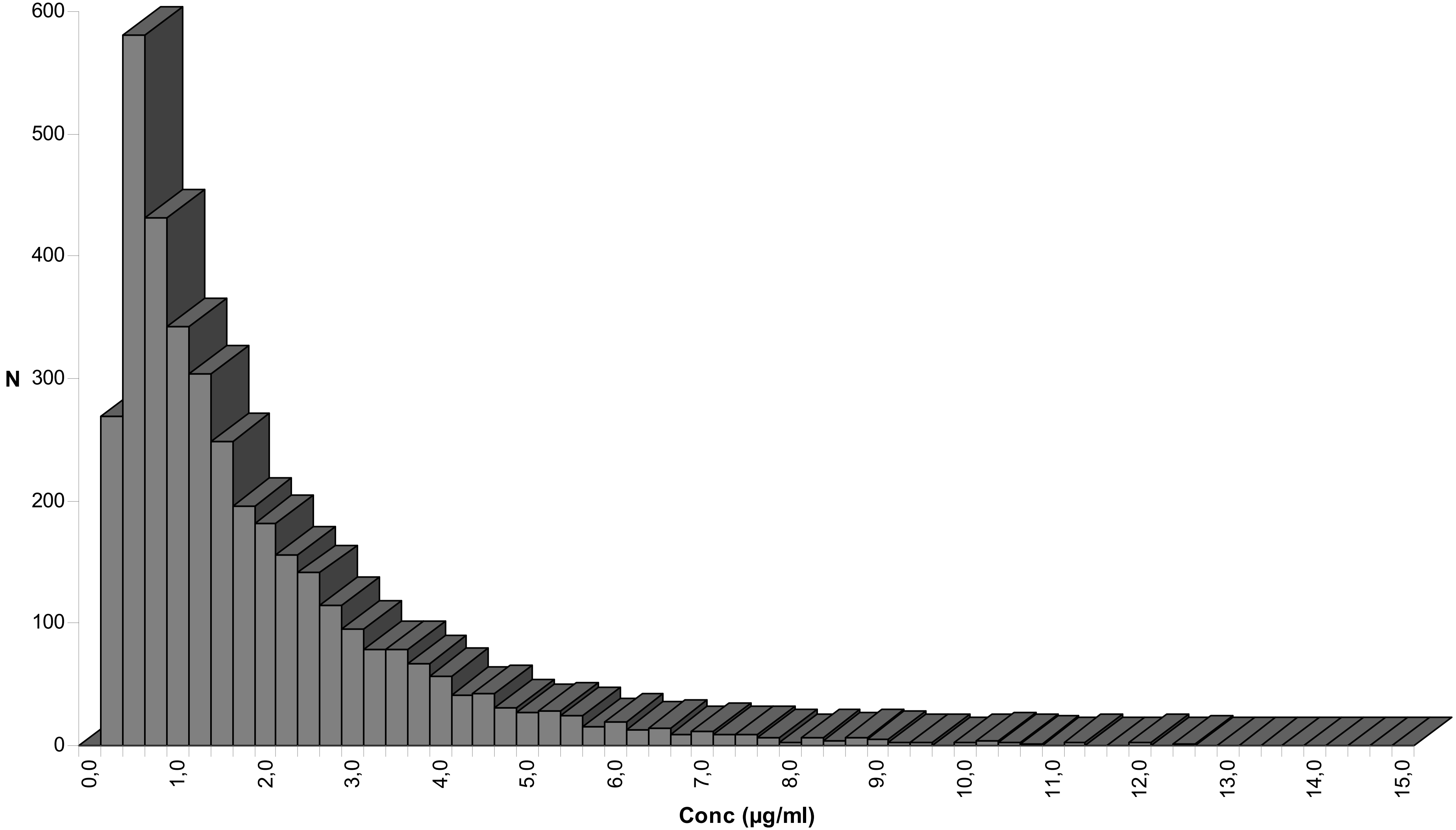

Figure 1: Distribution of urinary caffeine levels in the urine of 4633 athletes tested for doping control during 2004. Caffeine concentrations <LOD and >15 µg/ml are not shown.

Table 1: Percentage of samples with a urinary caffeine concentration ≥4 µg/ml in different sports in 1993-2002 and in 2004.

Sport	1993-2002	2004
Overall	7.01	7.55
Cycling	8.89	11.18
Athletics	6.07	4.76
Swimming	2.79	1.33
Soccer	3.32	4.79
Basketball	5.02	3.03
Volleyball	6.71	7.60
Bodybuilding	13.73	13.51
Power lifting	19.86	18.56

Results obtained before the removal of caffeine from the doping list indicated an average caffeine concentration of 1.12 µg/ml and an apparent threshold level of 11.02 µg/ml [13]. From these results it appears that the former threshold level of 12 µg/ml is an acceptable criterion to discriminate use from abuse.

Comparison of the resulting concentrations found in different sports in 2004 was done using an ANOVA test on the most frequently tested sports in both examined periods. Table 2 summarises the number of samples analysed, average caffeine concentration, standard deviation and percentage of samples with a concentration lower than the LOQ of 0.1 µg/ml. Large variations could be observed between sports (e.g.: Powerlifting and swimming) as well as within each sport (see standard deviation). Big differences could also be observed in the percentages of samples with a caffeine concentration below the LOQ.

Table 2: Average caffeine concentration and standard deviation after transformation, and percentage of values below LOQ in the most frequently tested sports (2004).

Sport	N	Average concentration (µg/ml)	Standard deviation (µg/ml)	Percentage below LOQ (%)
cycling	1467	1.40	2.60	16.84
athletics	357	0.95	2.74	20.73
swimming	75	0.45	2.24	48.00
soccer	438	1.00	2.53	18.95
basketball	165	0.83	2.41	29.70
volleyball	171	1.03	2.75	8.77
bodybuilding	37	1.35	2.99	27.03
Boxing	135	0.87	2.65	21.48
Judo	94	0.83	2.52	35.11
Motorsport	87	1.12	2.41	26.44
Handball	87	1.00	2.68	11.49
Gymnastics	76	0.72	2.53	34.21
Powerlifting	97	1.71	2.55	9.28

Results of the ANOVA test are summarised in Table 3. As can be seen, overall concentrations found in powerlifting are significantly higher compared to most other sports. This could be due to the fact that caffeine in combination with ephedrine is known as a fat burner. Indeed, several studies have demonstrated that ephedrine, particularly in combination with caffeine, is effective in promoting weight loss without serious adverse events [16,17]. 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, which could explain the popularity of caffeine in the late stage of a cycling race.

Table 3: Comparison of mean urinary caffeine concentrations found in frequently tested sports.

	Cycling	Athletics	Boxing	Judo	Swimming	Motor-sport	Soccer	Basketball	volleyball	handball	Body-building	Gymnastics	Power lifting
Power lifting	=	≠	≠	≠	≠	=	≠	≠	≠	≠	=	≠	
Gymnastics	≠	=	=	=	=	=	=	=	=	=	=		
bodybuilding	=	=	=	=	≠	=	=	=	=	=			
handball	=	=	=	=	≠	=	=	=	=				
volleyball	≠	=	=	=	≠	=	=	=					
Basketball	≠	=	=	=	≠	=	=						
Soccer	≠	=	=	=	≠	=							
Motorsport	=	=	=	=	≠								
Swimming	≠	≠	≠	=									
Judo	≠	=	=										
Boxing	≠	=											
Athletics	≠												
Cycling													

= no significant difference (α=0.05)

≠ significant difference (α=0.05)

Comparing caffeine concentrations in the examined sports in 2004 and the previous examined period are summarised in Table 4. Except for swimming and basketball, caffeine concentrations did not vary significantly between the two examined periods. Only for cycling caffeine concentrations increased after the removal from the doping list. The percentage of samples with a urinary caffeine concentration higher than the former threshold level also increases in cycling. There seems to be a general trend towards a lower percentage of samples with a urinary caffeine concentration below the LOQ in all monitored sports, except for swimming confirming the significant decrease in monitored average concentration.
Results obtained in bodybuilding are contradictory. The average caffeine concentration decreased in 2004, the percentage of samples below the LOQ increased but the percentage of positive samples according to the former threshold increased. A possible explanation could be the low number of samples analysed in bodybuilding in 2004.

Table 4: Comparison of the average concentration, percentage of samples below the LOQ and percentage of positive samples between 1993-2002 and 2004.

	Average Concentration		Percentage <LOQ		Percentage Positives	
	1993-2002	2004	1993-2002	2004	1993-2002	2004
Cycling	1.34	1.40	22.17	16.84	0.09	0.27
Athletics	1.06	0.95	32.50	20.73	0.16	0.28
Boxing	1.01	0.87	35.23	21.48	0	0
Judo	0.97	0.83	43.60	35.11	0	0
Swimming *	0.90	0.45	45.12	48.00	0	0
Motor Sport	1.14	1.12	39.33	26.44	0	0
Soccer	1.05	1.00	27.80	18.95	0.08	0
Basketball *	1.04	0.83	32.01	29.70	0	0
Volleyball	1.20	1.03	20.90	8.77	0.31	0
Handball	1.27	1.00	18.35	11.49	0	0
Bodybuilding	1.72	1.35	26.27	27.03	1.18	2.70
Gymnastics	0.59	0.72	68.29	34.21	0.81	0
Powerlifting	1.74	1.71	17.12	9.28	2.05	0

Conclusion

It can be concluded that the overall percentage of values below the LOQ has decreased in 2004. Nevertheless, the overall average caffeine concentration has remained approximately the same resulting in an apparent threshold level similar to the one calculated in the period 1993-2002. These apparent threshold levels approach the former threshold level of 12 µg/ml. Caffeine concentrations observed in individual sports have decreased after the removal of caffeine from the doping list, except for cycling and powerlifting. As for the period 1993-2002, urinary caffeine concentrations observed in those sports were significantly higher than in other monitored sports such as ball sports highlighting the frequently purported ergogenic effects of caffeine in those sports.

The final question remains whether the removal of caffeine from the doping list was justified or not. Caffeine concentrations did not increase after the removal from the WADA doping list, hence the misuse of caffeine has not increased. On the other hand, observed average caffeine concentrations show that the use of caffeine in the daily diet does not result in high concentrations. In other words, caffeine concentrations exceeding the former threshold level of 12 µg/ml can be identified as misuse. If the goal of drug testing is to prevent unfair advantage, encourage ethical behaviour and protect the health of athletes the position of caffeine on or off the doping list should be reconsidered.

References

[1] T. Graham, News on Sport Nutrition: Insider 6 (1998) 1.

[2] C.A. Haller, M.J. Duan, N.L. Benowitz, P. Jacob, Journal of Analytical Toxicology 28 (2004) 145.

[3] A.M. Davies, Food Surveillance 103 (1997) 1.

[4] Canadian Centre for Drug Free Sport, (1993) 1.

[5] T.E. Graham, J.W.E. Rush, M.H. Vansoeren, Canadian Journal of Applied Physiology-Revue Canadienne De Physiologie Appliquee 19 (1994) 111.

[6] C.J.D. Sinclair, J.D. Geiger, Journal of Sports Medicine and Physical Fitness 40 (2000) 71.

[7] M.A. Tarnopolsky, Sports Medicine 18 (1994) 109.

[8] F. Anselme, K. Collomp, B. Mercier, S. Ahmaidi, C. Prefaut, European Journal of Applied Physiology and Occupational Physiology 65 (1992) 188.

[9] K. Collomp, S. Ahmaidi, M. Audran, J.L. Chanal, C. Prefaut, International Journal of Sports Medicine 12 (1991) 439.

[10] K. Collomp, S. Ahmaidi, J.C. Chatard, M. Audran, C. Prefaut, European Journal of Applied Physiology and Occupational Physiology 64 (1992) 377.

[11] F. Greer, C. McLean, T.E. Graham, Journal of Applied Physiology 85 (1998) 1502.

[12] C.D. Paton, W.G. Hopkins, L. Vollebregt, Medicine and Science in Sports and Exercise 33 (2001) 822.

[13] W. Van Thuyne, F.T. Delbeke, in W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (Editors), Manfred Donike Workshop 22nd Cologne Workshop on Dope Analysis, Sport & Buch Strauβ, Cologne, 2004, p. 27.

[14] F.T. Delbeke, P. De Backer, Journal of Chromatography B 687 (1996) 247.

[15] F.T. Delbeke, M. Debackere, International Journal of Sports Medicine 5 (1984) 179.

[16] A. Astrup, L. Breum, S. Toubro, P. Hein, F. Quaade, International Journal of Obesity 16 (1992) 269.

[17] P.A. Daly, D.R. Krieger, A.G. Dulloo, J.B. Young, L. Landsberg, International Journal of Obesity 17 (1993) S73.