Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (5)

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

Sport und Buch Strauß, Köln, 1998

W. SCHÄNZER, A. GOTZMANN:
The Cologne Protocol to Follow-up Positive Caffeine Cases
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (5). Sport und Buch Strauß, Köln, (1998) 259-268

Wilhelm Schänzer und Andrea Gotzmann

The Cologne Protocol to Follow-up Positive Caffeine Cases

Institute of Biochemistry, German Sports University Cologne

Introduction

The use of caffeine has been banned first by the IOC in 1984 for the Olympic Games in Los Angeles. The IOC established a urinary threshold of 15µg/ml. Later this limit was reduced to 12 µg/ml of urine. Urine samples of athletes exceeding this level are considered as a doping offence. The reason to ban caffeine was the observation that athletes misused pharmaceutical preparations containing high dosages of caffeine to obtain stimulating effects in competition.

Pharmacology of caffeine

The main reason for the high popular use of caffeine containing beverages is the physiological effect of caffeine to increase mood and to decrease fatigue.
Caffeine, theophylline, and theobromine (Fig.1) are closely related methylxanthines.

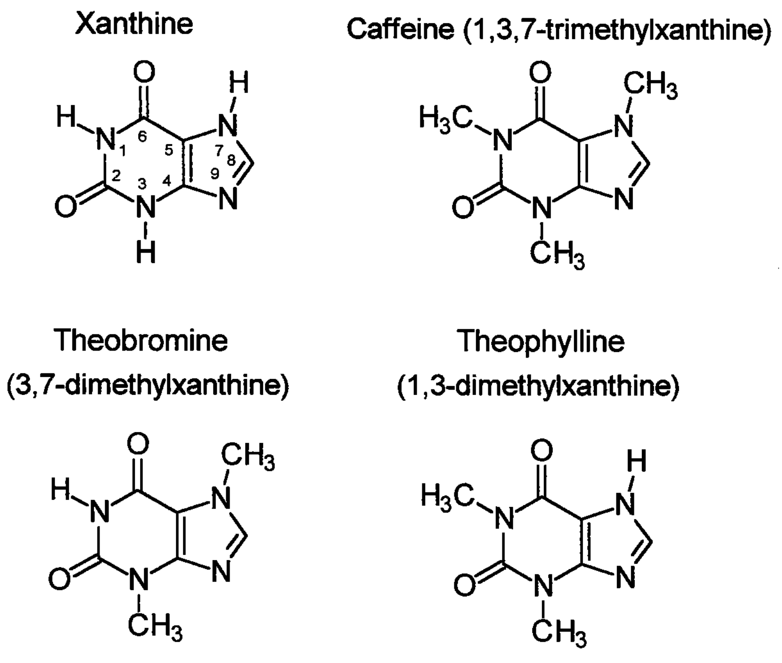

Fig.1 Structure formula of the principle methylxanthines

Methylxanthines are alkaloids which originate from plants occurring all over the world (Table 1).

Table 1 Sources of caffeine, theophylline, and theobromine

Source	Xanthine	Plant
Tea	Caffeine, and small amounts of theophylline and theobromine	Thea sinensis
Cocoa and chocolate	Theobromine and some caffeine	Theobroma cacao
Coffee	Caffeine	Coffea arabica and related spezies
Cola drinks	Caffeine (and addition of caffeine)	Cola acuminata

Caffeine, theophylline, and theobromine have similar pharmacological effects and are of therapeutic interest to treat asthma. They relax smooth muscle mainly the bronchial muscle. They further have stimulating effects on the central nervous system, the cardiac and skeletal muscle. On the kidney they have diuretic actions.

The physiological and pharmacological effects of caffeine are explained by their cellular actions as 1) inhibition of adenosine receptor, 2) inhibition of phosphodiesterase yielding an increase in intracellular cyclic-AMP concentration, and 3) increase in intracellular calcium concentrations (Table 2). The inhibition of adenosine receptor is actually discussed as the main effect of caffeine in doses which are pharamacologically used and are administered via caffeine containing drinks.

Table 2 Pharmacological effects of methylxanthines

	Concentration of caffeine [µMol] in plasma for			
	50% inhibition of adenosine receptors	50% inhibition of phosphodiesterase	50% release of intracellular Ca^{2+}	plasma concen-tration [µM]
Theophylline	15	400	3000	50*
Caffeine	30	400	3000	10**
Theobromine	120	700	3000	

* = 9 µg/ml. In asthma therapy a plasma concentration of 28-83 µM is intended to be obtained.
** = 1.9 µg/ml. This value is obtained after consumption of 1 cup of coffee (100 mg caffeine).
Plasma elimination half-times: Theophylline 8h, caffeine 3-7h, theobromine 9h.

Lit.: Forth, Henschler, Rummel, Starke: Pharmakologie und Toxikologie, 1996 [1]

Metabolism of caffeine

Caffeine is rapidly absorbed after oral administration. Caffeine as well as theophylline and theobromine are distributed after application into all body compartments with a volume of distribution between 0.4 and 0.6 l/kg. Caffeine is extensively metabolized and less than 5% of the administered substance is excreted unchanged via the kidney. Half-life of caffeine in plasma is between 3 and 7 hours [2]. Main metabolites of caffeine are 1-methylxanthine and 1-methyluric acid.(Fig 2).

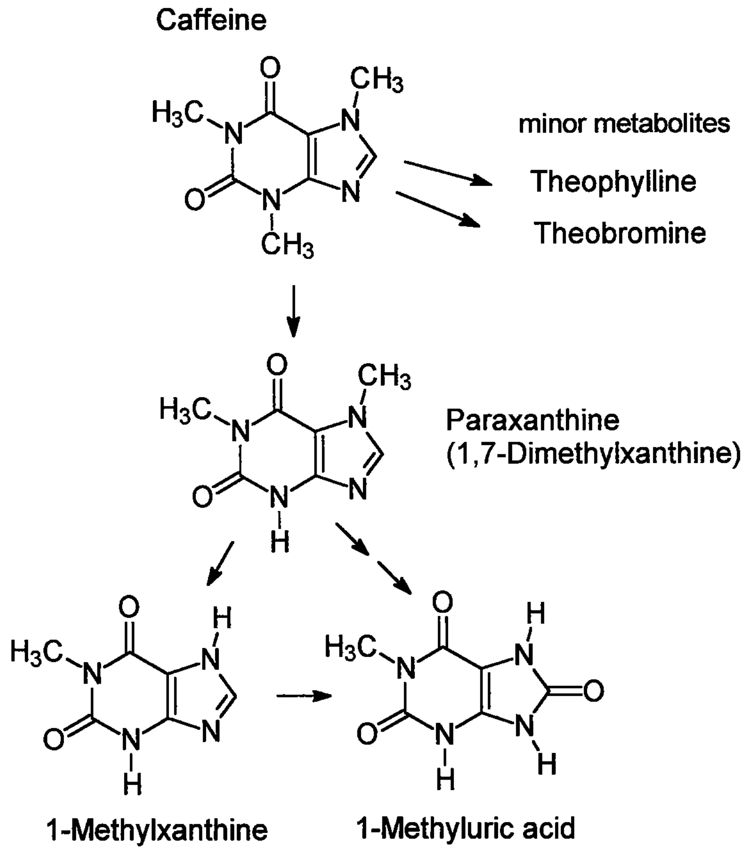

Fig.2 Metabolism of caffeine in human

There is a high inter individual variation in caffeine metabolism, based on genetic factors and influence of pharmacological active substances. This causes differences in the excreted amount of unchanged caffeine.

As sport federations established a concentration limit for caffeine the interesting question, whether this concentration can be influenced by the urine flow or not, had to be clarified. It was discussed that a high urine flow results in a low urine density which could lead to a reduced caffeine concentration. This would open the possibility to manipulate urine samples. For this purpose a correction of the caffeine concentration via the urine density was proposed. Investigations concerning the change of urinary caffeine excretion in correlation to the urine flow show that this assumption was wrong! The excretion of caffeine is highly correlated to the urinary flow as shown in Fig.3 and Table 3. An increased urinary flow with a reduced urinary density results in a higher caffeine excretion to such an extend that the urinary concentration is less influenced.

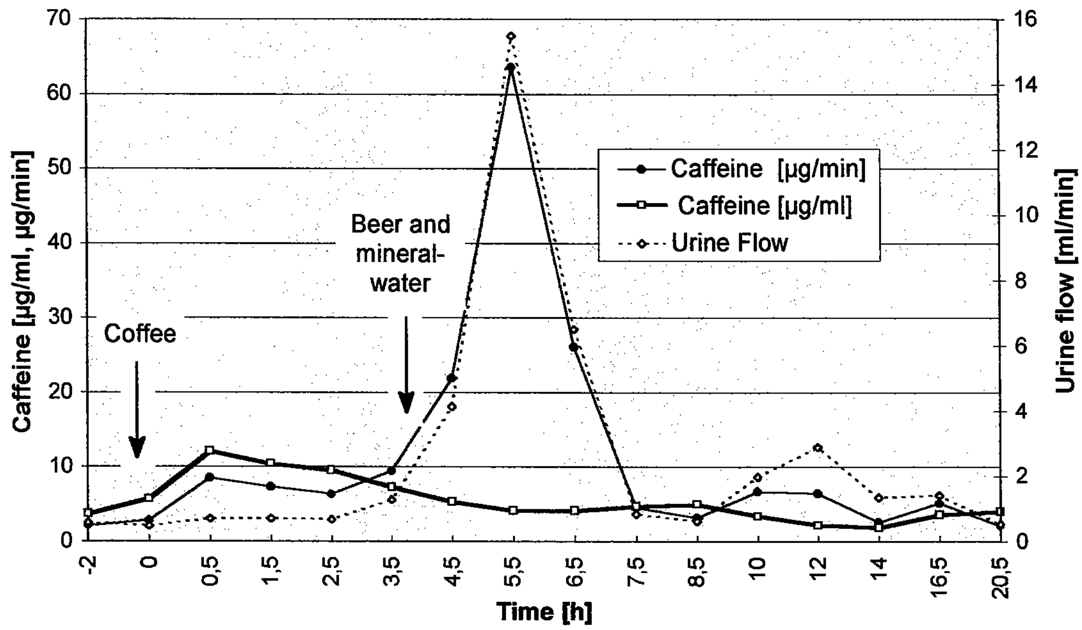

Fig 3. Influence of diuresis on the excretion of caffeine (Protocol of the study see Table 2)

Table 3 Influence of diuresis on the excretion of caffeine
(from 7:45 to 8:30 a total volume of 400 ml of freshly brewed coffee containing 353 mg of caffeine was consumed)

Time	Volume [ml]	Excr.-rate [ml/min] .	Density	pH-Value	Caffeine [µg/min]	Caffeine [µg/ml]
3:45-7:35	132	0.57	1.0176	5.48	2.10	3.7
7:35-8:35	29	0.48	1.0215	5.39	2.80	5.7
+ 1 h	421	0.70	1.0227	5.14	8.50	12.1
+ 2 h	42	0.70	1.0222	5.44	7.30	10.4
+ 3 h	40	0.67	1.0215	5.33	6.30	9.5
+ 4 h	77	1.28	1.0158	5.79	9.41	7.3
from 4- 4.5 h 1000 ml of beer (Früh Kölsch) was drunk						
+ 5 h	248	4.13	1.0049	5.79	21.90	5.3
from 5 - 5.5 h 750 ml of mineral water was drunk						
+ 6 h	930	15.50	1.0000	6.08	63.60	4.11
+ 7 h	392	6.50	1.0011	5.81	26.10	4.00
+ 8 h	51	0.85	1.0141	4.93	4.50	4.70
+ 9 h	38	0.63	1.0189	5.03	3.20	5.00
+ 11 h	238	1.98	1.0058	5.53	6.70	3.40
+ 13 h	347	2.89	1.0052	6.96	6.40	2.20
+ 15 h	162	1.35	1.0174	7.47	2.60	1.90
+ 18 h	255	1.42	1.0118	5.56	5.10	3.60
+ 23 h	156	0.52	1.0212	5.81	2.10	4.00

Caffeine consumption and urinary excretion

The limit of 12µg/ml of urine has been set based on results from caffeine determinations of real doping test samples and from excretion studies performed with caffeine. Unfortunately the data of these studies have never been published. As sports authorities do not insist on a total ban of caffeine, the question raised, how much caffeine in form of caffeine containing drinks, such as coffee or tea, one would have to ingest to produce a positive urine sample? Donike and Rauth [3] published that even for "strong" coffee drinkers the urinary caffeine concentration is somewhere between 3 and 4µg/ml and concluded that it is nearly impossible to reach the 12µg/ml level by coffee consumption. Van der Merve et. al. published in 1988 [4] administration studies with nine volunteers, who achieved 1000mg of caffeine in form of coffee, 600mg in form of tea and 160mg in form of Cola drinks. Maximum caffeine concentrations were reported for the 1000mg study with a mean of 10.64 µg/ml and a maximum value of 14,02 µg/ml, 600mg study: mean 8.16 µg/ml, maximum 11.95 µg/ml; 150mg study: mean 1.63 µg/ml, maximum 3.17 µg/ml. Delbeke [5] investigated urinary caffeine concentrations in cyclists and in coffee drinkers and showed that 1.4% of coffee drinkers had concentrations between 12.5 and 15 µg/ml and no concentration above 15µg/ml was detected.

The results of Delbeke confirm the statement that caffeine concentrations higher than 12 µg/ml of urine can be obtained by coffee consumption, and can lead to a positive doping test. Unpublished caffeine studies performed in our laboratory in 1994 showed also that in some indiviuals the urinary 12µg/ml level can be exceeded by the intake of "strong" coffee. Therefore recommandations for "normal" caffeine consumption should be made, or possible the limit of 12µg/ml should be increased. For example a single excretion study with a female athlete, who consumed 500ml of "strong" coffee, containing 875 mg of caffeine showed a maxium urinary caffeine concentration of **26.6 µg/ml** 1.5 to 2.5 h after the caffeine administration. When the caffeine intake was reduced to 290 mg the same person achieved a maximum urinary caffeine level of **13.3 µg/ml** 1.5 to 3 h after applcation.

In 1996 a female athlete was declared positive by the German Athletic Association (DLV) for caffeine misuse. The urinary caffeine concentration was 15.3µg/ml. The athlete denied that she had taken pharmaceutical caffeine preparations, but she had drunk a few cups of coffee during the competition. She assumed that the volume of coffee she had consumed would not cause a doping relevant urine sample. In this case we were interested whether a normal caffeine consumption of the athlete has lead to a positive urine sample or not. We decided to investigate this athlete to clarify if her caffeine metabolism shows a trend to a higher excretion of unchanged caffeine compared to other persons. For this purpose a precise amount of coffee was freshly brewed. The athlete drank 300 ml of this coffee with a total amount of 275-295 mg of caffeine within 30 min. Urine samples were collected before and after the coffee intake. Two studies were performed with the female athlete, one without drinking coffee, at least 12h before the test and in a second study the athlete did not changed her normal coffee consumption in the morning (Fig.4, Table 4 and 5).

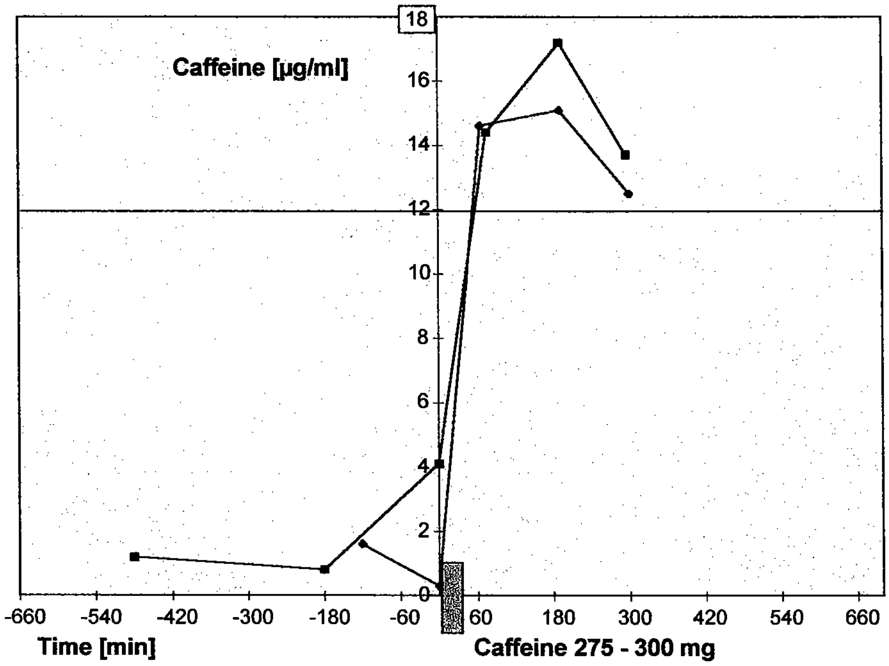

Fig.4 Urinary caffeine excretion after consumption of 270-300 mg of caffeine (Excretion study with a positive tested female athlete, see also Table 4 and 5)

Table 4 Caffeine excretion study with a caffeine positve tested female athlete, study 1, normal coffee consumption in the morning

Time	Urine volume [ml]	pH	Densitiy [g/ml]	Caffeine [µg/ml]
20:00-2:00	570	5.58	1.0068	**1.2**
2:00-7:00	198	5.94	1.0067	**0.8**
7:40 1 cup of coffee (100ml)				
7:00-10:00	173	6.75	1.0043	**4.1**
10:10-10:50 coffee (300 ml), 275 mg caffeine				
10:00-11:15	186	7.53	1.0040	**14.4**
11:15-13:10	96	6.74	1.0144	**17.2**
13:10-14:55	96	7.50	1.0167	**13.7**

Table 5 Caffeine excretion study with a caffeine positve tested female athlete, study 2, without coffee consumption in the morning

Time	Urine volume [ml]	pH	Densitiy [g/ml]	Caffeine [µg/ml]
20.30-8.00	699	6.47	1.01046	**1.6**
8.00-10.00	223	6.56	1.00530	**0.3**
10:0-10:4 coffee (300 ml), 296 mg caffeine				
10.00-11.05	187	7.17	1.00258	**14.6**
11.05-13.10	202	6.73	1.00839	**15.1**
13.10-15.00	67	5.18	1.01384	**12.5**

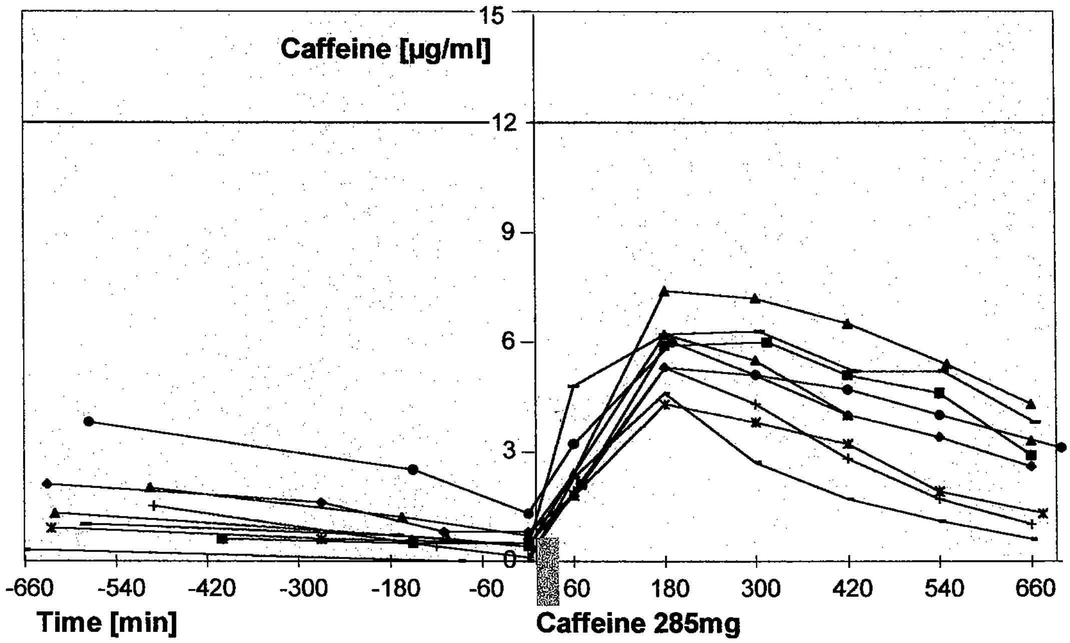

Fig.5 Caffeine excretion after administration of 285 mg of caffeine in 300ml of coffee, 9 volunteers without coffee consumption at least 12 hours before

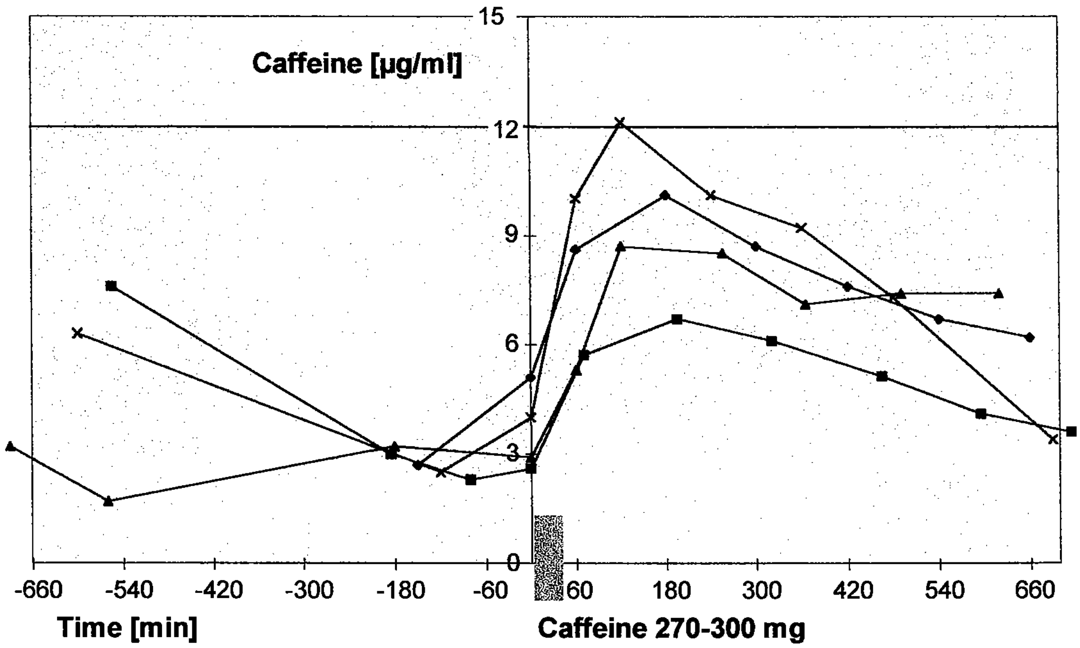

Fig.6 Caffeine excretion after administration of 275-295 mg of caffeine in 300ml of coffee, with coffee consumption 2-3 hours before the study

A similar study with the same applied amount of caffeine (270-300mg) was performed with 9 persons who did not use caffeine 12h before the coffee test and with 4 persons who did not change their normal coffee intake in the morning (Fig. 5 and 6).

All urine samples were analysed by HPLC by direct urine injections as described elsewhere [6]. The caffeine concentration was calculated against an external aqueous standard solution containing 20 µg/ml of urine or 12 µg/ml of urine respectively.

Whereas the female athlete (positive tested athelete) reached maximum urinary caffeine concentrations of 17.2 µg/ml (study 1) and 15.1 µg/ml (study 2 without caffeine consumption 12 h prior to the test), the study with 9 volunteers shows a maximum urinary caffeine concentration with an average of 5.7 µg/ml and a single maximum value of 7.4 µg/ml between 1 and 3 hours after the caffeine application. The study with 4 persons, who did not change their coffee consumption in the morning had an average maximum of caffeine concentration of 9.4 µg/ml and a single maximum concentration for one person of 12,1 µg/ml.

Summary

Caffeine is banned in competition with a quantitative limit of 12µg caffeine / ml of urine. This level can be exceeded when a high quantitative of caffeine containing beverages are consumed. It is impossible to lay down the exact amount of caffeine intake, which can be used not to pass the 12µg/ml limit. As caffeine metabolism can vary to a high extent between individuals athletes can come into conflict with the doping rule when their individual caffeine metabolism enables the excretion of a high percentage of unmetabolized caffeine.
To establish the individual caffeine metabolism, we recommend a caffeine study (application of 300 mg of caffeine) with the athlete.

References

1. Allgemeine und spezielle Pharmakologie und Toxikologie, 7.Aufl. (Hrsg.: Forth W. ,Henschler D., Rummel W. und Starke K.), Spektrum, Heidelberg, 1996.

2. Goodman and Gilman's The pharmacological basis of therapeutics. 9.Edition (Eds.: Hardmann J.G., Limbird l.E., Molinoff P.B., RuddonR.W. and Goodman Gilman A.), McGraw Hill, New York, 1996.

3. Donike M. und Rauth S.: Dopingkontrollen, 1.Aufl., Sport und Buch Strauß, Köln, 1992.

4. Van der Merwe P.J., Müller F.R., and Müller F.O.: Caffeine in sport. Urinary excreteion of caffeine in healthy volunteers after intake of common caffeine containing beverages. SAMJ 74 (1988) 163-164.

5. Delbecke F.T. and Debackere M.: Caffeine: Use and abuse in sports. Int. J Sports Med. 5 (1984) 179-182.

6. Gotzmann A. and Donike M: Quantitative caffeine determination by direct injection of urine. In Donike M., Geyer H., Gotzmann A., Mareck-Engelke U. (Eds.): Proceedings of the 10th Cologne Workshop on Dope Analysis 1992, Sport und Buch Struaß, Köln, 1993, 281-284.