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RECENT ADVANCES IN DOPING ANALYSIS (8)

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Report on the doping control during the 7th All Africa Games in South Africa

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Introduction

The 7th All Africa Games was held from 9 – 19 September 1999 in Johannesburg. The International Olympic Committee (IOC) accredited laboratory at the Department of Pharmacology, University of the Free State was asked by the Medical Commission (MC) of the Games to be responsible for the doping analyses during the Games. We were requested that results should be available within 24 hours for negative samples and 36 hours for positive samples.

Personnel

The laboratory was staffed by 9 persons. Besides the director, there were 4 senior analysts responsible for the instrumental analysis and interpretation of results as well as doing confirmatory analysis. Three temporary analysts were responsible for the extraction procedures up to the obtaining of the final extract to be analysed instrumentally. One laboratory assistant was responsible for cleaning. Thus, the responsibility of each analyst was unique and the quality-control chain easy to follow.

Collection of samples and transport

The collection of urine samples was done by the MC who used well-trained sampling officers. Samples were collected in Johannesburg and vicinity at the different sporting events and then couriered overnight to Bloemfontein by road. A strict chain of custody was followed to ensure maximum security of the samples.

Reception and pre-analysis

The samples arrived in the early morning at the laboratory. On arrival the seals of the carrybags were inspected, the code numbers noted and compared with those on the chain of custody forms. Then the A- and B- samples were unpacked, the code numbers noted and compared with those on the doping control collection (DCC) forms. Stickers with the laboratory numbers were sticked on the A- and B- sample containers as well as on the DCC forms. These coded numbers were used throughout the analytical process. The B-samples were stored immediately at -20°C in a lockable fridge. The volume, pH and specific gravity of the A-samples were measured and noted. The urine was then aliquotted for analysis for the different groups of banned substances.

Analysis of samples

The IOC Medical Commission (IOC-MC) classified banned substances according to its pharmacological properties and it consist of stimulants, narcotic analgesics, anabolic agents, diuretics and peptide hormones. Each of these group of substances requires its own special method of detection. Our screening methods were performed by following internationally accepted methods and consisted of 5 different procedures[1, 2]. Table 1 describes the analytical equipment employed in our laboratory to perform each procedure during the Games. All analytical instruments were ran with sufficient stability allowing sensitive and reproducable GC/MS analysis. Analysis of the whole batch of samples arriving on a particular day was started at about 07:00. At least one senior staff member was on duty in the laboratory overnight. Early the following morning the results of the batch of samples from the previous day were available and all negative results reported to the chairman of the Medical Commission of the Games. Confirmation and re-analysis of suspicious A-samples were achieved that same day.

Some quality-control strategies were followed to ensure maximum reliability of results. The samples analysed on a particular day also contained blank urine and control urine samples so that interbatch repeatability could be monitored. The composition of the control urine samples consisted of blank samples supplemented with known amounts of some of the banned substances of that group. Each operation in the laboratory was described in detail in written standard operating procedures.

Results

The laboratory received 406 samples (260 from males and 146 from females) during the 11 days of the Games. Table 2 reflects the number of samples analysed each day as well as the distribution of samples between sports. On most days more than 35 samples were analysed, the most being 55 samples on day 8.

Samples containing banned substances and reported to the Medical Commission of the Games are listed in table 3. Four of the 10 positives results were from blind control samples introduced at random by the Medical Commission to verify the proper functioning of the laboratory. The positive identification and interpretation of these results demonstrated the reliability of the analysis.

The ephedrine and norspeudoephedrine concentrations in urine samples 149 and 271, respectively, were quantified by high performance liquid chromatography[4] and were well above the limit set by the IOC (5μg/ml urine for ephedrine and norpseudoephedrine).

The IOC-MC rules regards a testosterone:epitestosterone (T:ET) ratio in urine higher than 6 to be a positive doping case but with the obligation to perform additional investigation to exclude the small percentage of people having high T:ET ratios as a result of physiologic or pathologic conditions. The T:ET ratios in urine were monitored during the Games and the highest observed value was 4.5 for males and 3.6 for females. The distribution of the T:ET ratios are given in fig. 1. The distribution for both males and females are skew with the most ratios in the interval 0.5 – 1 (adding of males and females). All the ratios were below 6. This distribution is in agreement with ratios previously reported for South Africa[3] and internationally[1, 2].

Conclusion

During the 7th All Africa Games held in Johannesburg in 1999, 406 samples were analysed in 11 days from 19 different sporting codes. The highest number of samples on one day were 55. The results were reported to thr Medical Commission within 24 hours for negative samples and 36 hours for positive samples, after arrival of the samples in the laboratory. 10

samples were reported to contain banned substances: 4 samples were control samples introduced by the Medical Commission and 6 were true positive samples from competitors. This 6 positive cases were 3 less than the 9 positive cases obtained at the 6th All Africa Games in Harare, Zimbabwe in 1995[5].

Acknowledgement

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6. Table 1 Instruments used for the analyses

INSTRUMENT	GROUP	NUMBER AVAILABLE
Gas chromatograph with nitrogen selective detector (NPD)	Stimulants	1
Gas chromatograph/mass selective detector (GC/MSD 5972)	Narcotic analgesics	1
Gas chromatograph/mass selective detector (GC/MSD 5973)	Anabolic agents	2*
Gas chromatograph/mass selective detector (GC/MSD 5972)	Diuretics (and confirmation of stimulants)	1
Gas chromatograph/tandem mass spectrometer (GCQ)	Confirmation of low concentrations anabolic agents	1
High performance liquid chromatograph	Quantification of ephedrines and caffeine	1
Automatic fluoressence analyser (IM_x from Abbott)	hCG	1

*One instrument kindly supplied to us by Chemetrix for the duration of the Games.

Table 2 Number of samples analysed distributed between sports and dates

SPORT	9/9	11/9	12/9	13/9	14/9	15/9	16/9	17/9	18/9	19/9	TOTAL
ATHLETICS						10	12	11	9	6	**48**
BASEBALL	*7*								*8*		**15**
BASKETBALL				4	6			4	4		**18**
BOXING			*5*	*3*	*4*	*5*			*7*		**24**
CYCLING								6		6	**12**
GYMNASTICS				*9*	*6*						**15**
HANDBALL		4					4		4		**12**
HOCKEY		*4*			*4*				*4*		**12**
JUDO		8	8	9	8						**33**
KARATE					6	10	6	7			**29**
NETBALL					4						**4**
SOCCER	6	4								4	**14**
SWIMMING		8	8	9		8	8	8			**49**
TABLE TENNIS				4					4		**8**
TAEKWANDO						8	8	8	8		**32**
TENNIS			8					4			**12**
VOLLEYBALL				4					6		**10**
WEIGHTLIFTING		9	11	12	6						**38**
WRESTLING					7		7	7			**21**
TOTAL	**13**	**37**	**40**	**54**	**51**	**41**	**45**	**55**	**54**	**16**	**406**

Table 3 Positive A-Samples analysed

DAY	SAMPLE NR	SUBSTANCES	EVENT
Day 1	1	Caffeine & ephedrine	Control
	2	Caffeine	Control
Day 5	127	Caffeine & Cocaine	Control
	128	Furosemide & ephedrine	Control
Day 6	149	Ephedrine	Weightlifting
	177	Nandrolone	Boxing
Day 7	258	Furosemide	Taekwando
	272	Norpseudoephedrine	Karate
Day 9	291	Stanozolol	Athletics
Day 10	342	Epitestosterone*	Volleyball

*Epitestosterone concentration higher than 200ng/ml. Should be handled as for high T:ET ratios.

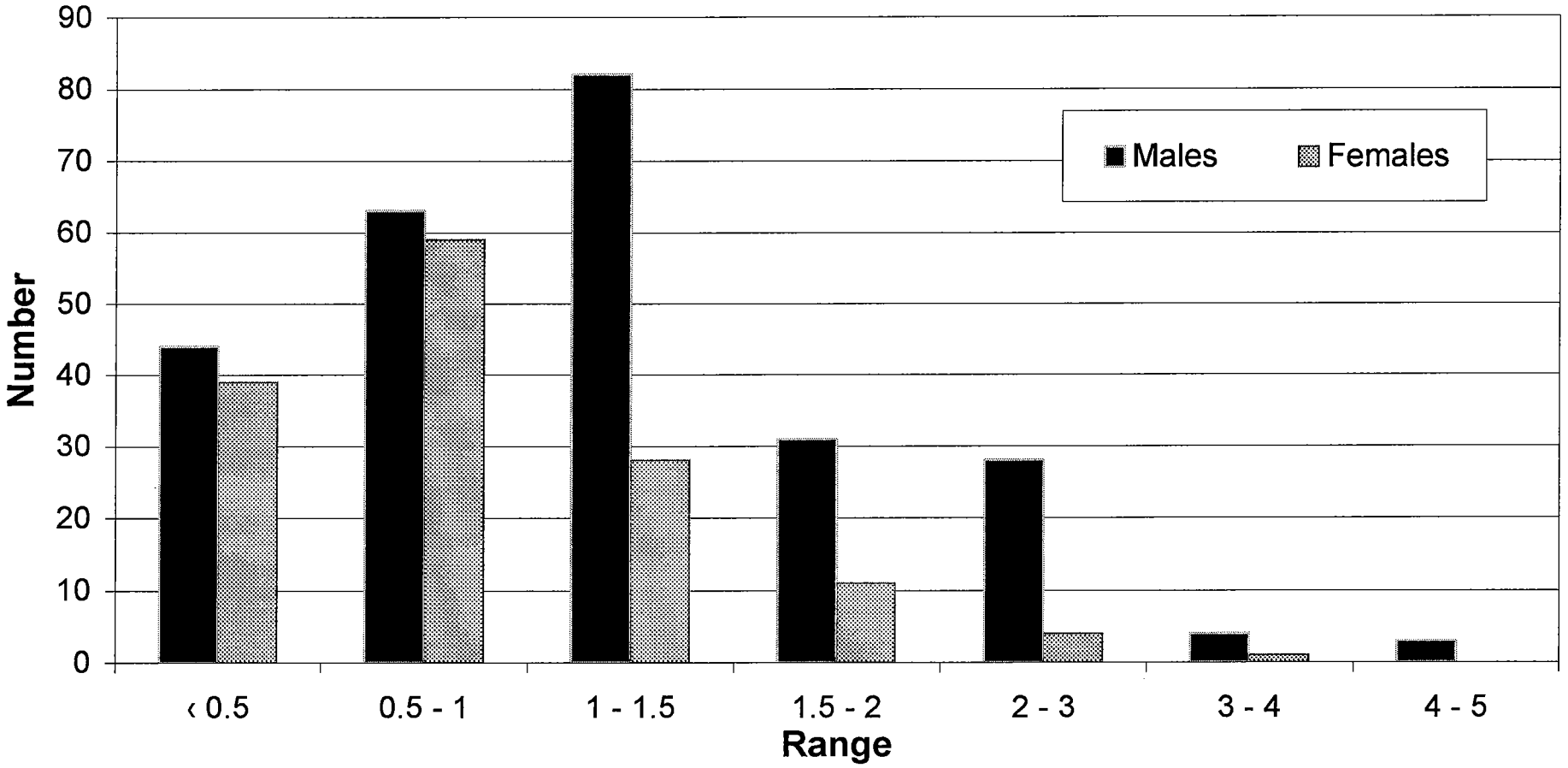

Fig 3: Distribution of T:ET ratios