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RECENT ADVANCES IN DOPING ANALYSIS (2)

M. Donike
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

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P. Hemmersbach, B. Bjerke, K. Birkeland, E. Haug:
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P. Hemmersbach, Bjerke, B., Birkeland K. , Haug, E.

Short Report from the Doping Analysis During the XVII Olympic Winter Games

Hormone Laboratory, Aker Hospital, N-0514 Oslo, Norway

Introduction

The XVII Olympic Winter Games were held at Lillehammer, Norway, from February 12 -27, 1994. The analysis of all doping control samples was performed at the IOC accredited laboratory in Oslo. It is located at the Hormone Laboratory, Aker Hospital in Oslo.

The aim of this short report is to focus on two aspects which were of some concern during the Games:

- Measurement of urine density during sample collection
- The use of beta-2-agonists

In addition we would like to give a short summary of the analytical work carried out during the games.

Summary of the doping analysis during the XVII Olympic Winter Games

In preparing for the games we found out that the challenges for the laboratory were mainly related to the following two facts:

- Analysing up to 50 samples a day and reporting latest 24 hours after sample reception
- Analysing blood samples taken for the first time during Olympic Games

During the 16 days we analysed a total of 529 urine and 59 blood samples. The distribution of the urine samples is shown in figure 1.

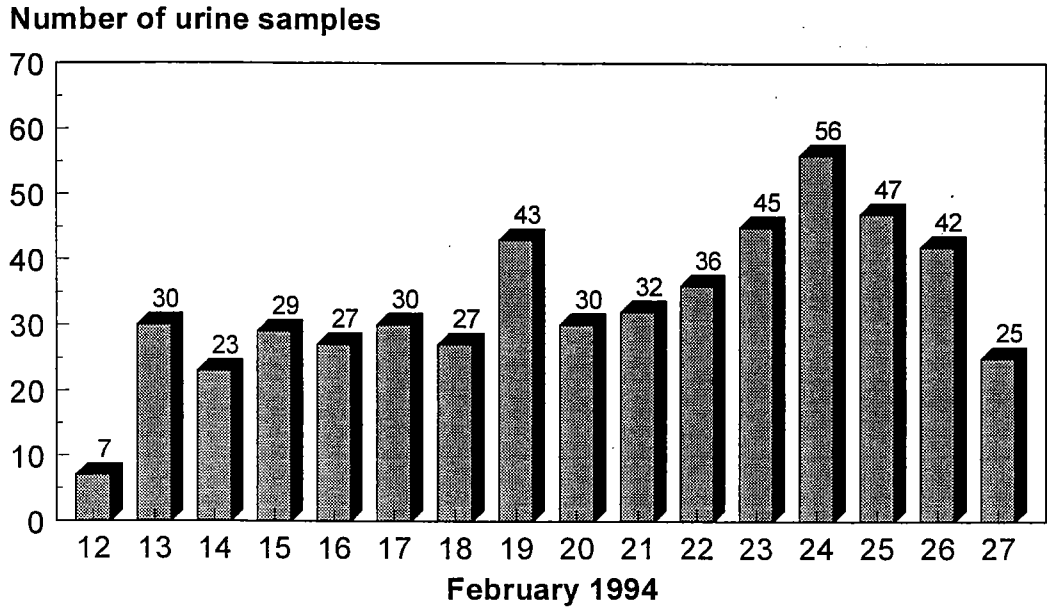

Figure 1 Daily distribution of urine samples.

While the urine samples were analysed for all classes of doping agents including those with certain restrictions[1], the IOC Medical Commission decided to analyse the blood samples only for the presence of non autologous blood.

To have sufficient analytical capacity during the games our instrument equipment had to be enlarged. This was done both by buying and loaning instruments. Table 1 gives an overview on the most important instrumentation.

3	GC-NPD (HP 5890)
7	GC-MSD (HP 5890/5970/5972)
1	GC-MS (HP5890/Finnigan SSQ 7000)
1	GC-HRMS (HP 5980/Finnigan MAT 95)
3	HPLC-UV (HP 1040/1050, PE LC 250, Spectra Physics
1	UNIX-server (HP 4920)
1	EMIT-system (Solaris
1	DELFIA-system (Wallac)

Table 1 Instrumentation - Doping analysis during the XVII Olympic Winter Games

A full report on the results of the analysis will be given later, but to finish this short summary report table 2 shows the analytical results reported to the IOC Medical Commission during the Games.

Amineptine	IOC test sample
Morphine	IOC test sample
Boldenone and furosemide	IOC test sample
Metandienone-M	IOC test sample
Nandrolone-M (ca. 2 ng/ml)	Noretisterone application
Stanozolol-M	IOC test sample
Lidocaine, 3x	
Cannabis	

Table 2 Analytical results reported to the IOC Medical Commission

Urine density measurements

To prevent the delivery of diluted urines caused by extraordinary big water intake, the measuring of the urine density during sample collection has been discussed for many years. Several countries and the IOC have introduced the regulation that the urine density should be greater or equal to 1.010 g/ml. If the density is lower, the athlete shall deliver a new sample. One problem with this regulation was the lack of precise and practical instrumentation for measuring the density on-site. The mainly used dip-sticks show a big imprecision (especially when the pH of the sample is elevated).

During the Winter Games in Lillehammer a urine refractometer (ATAGO URICON-PN, cat. no. 2721) was used during sample taking and showed excellent agreement with the measurements performed in the laboratory later on.

To answer the question about whether a lower limit of density should be set, both practical and analytical arguments should be taken into account . The laboratory don't want to receive diluted urines where the concentration of a doping agent has dropped below the identification limit. One could however compensate for this by analysing a larger aliquot of urine as long as a sufficient urine volume is available. To study the correlation between the excretion of

steroids and urine density we measured the densities of all urine samples when they arrived in the laboratory.

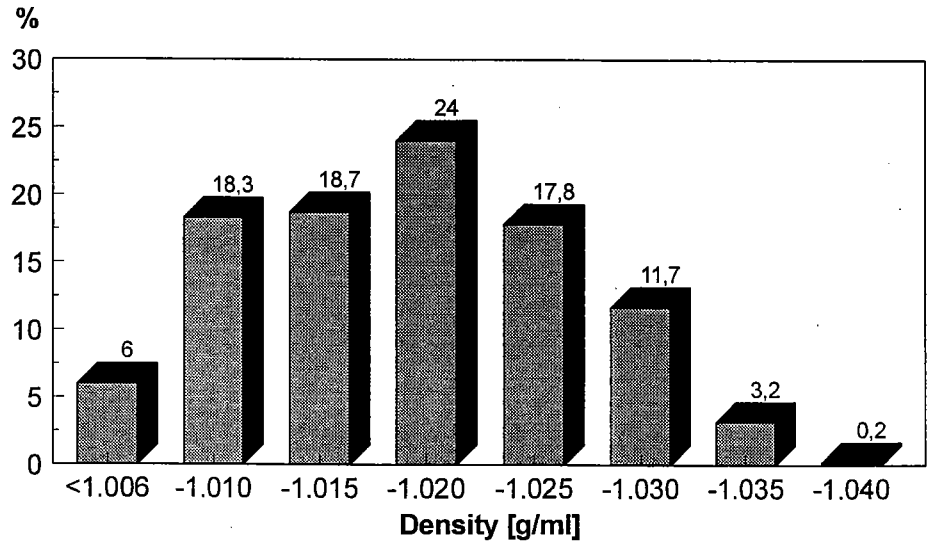

Figure 2 Olympic Winter Games 1994 - Densities of urine samples

Figure 2 shows the distribution of urine densities for all urine samples. There was no difference between the sexes.

When we looked at the correlation between the urine densities of all male athletes and the androsterone concentration in the urine we got a correlation coefficient of 0.80. The high positive correlation between these two parameters showed that a remarkable part of differences in the androsterone concentration was related to differences in urine density. This is shown even more clearly in figure 3, where the androsterone values are grouped according to the corresponding urine density.

The means of the grouped androsterone values are linearly correlated to the urine densities. These results confirm the idea of correcting the measure urine steroid concentration for low urine density[2] within the range 1.005 - 1.025 g/ml. If the laboratory works up four times the volume of a urine with a density of 1.005 g/ml , one will get about the same amount of androsterone as we would expect if the urine had a density of 1.020 g/ml. We obtained similar results when we correlated the concentrations of other endogenous steroids to the corresponding values for urine density. Consequently plotting the ratios of endogenous steroid concentrations grouped after urine density showed no differences for different densities (Figure 4).

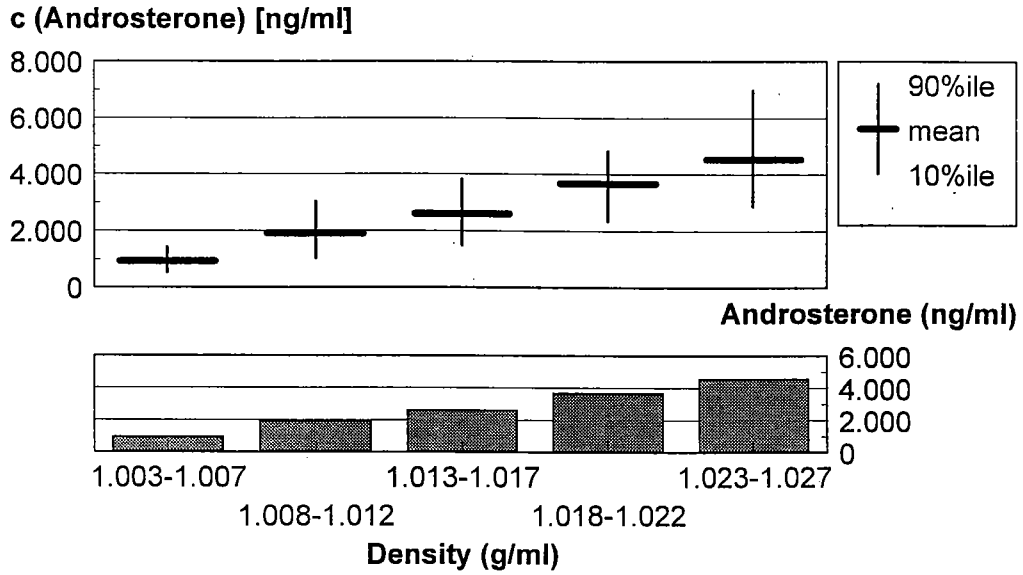

Figure 3 Mean level and 10-90%ile of androsterone concentrations in male athletes' urine according to urine densities.

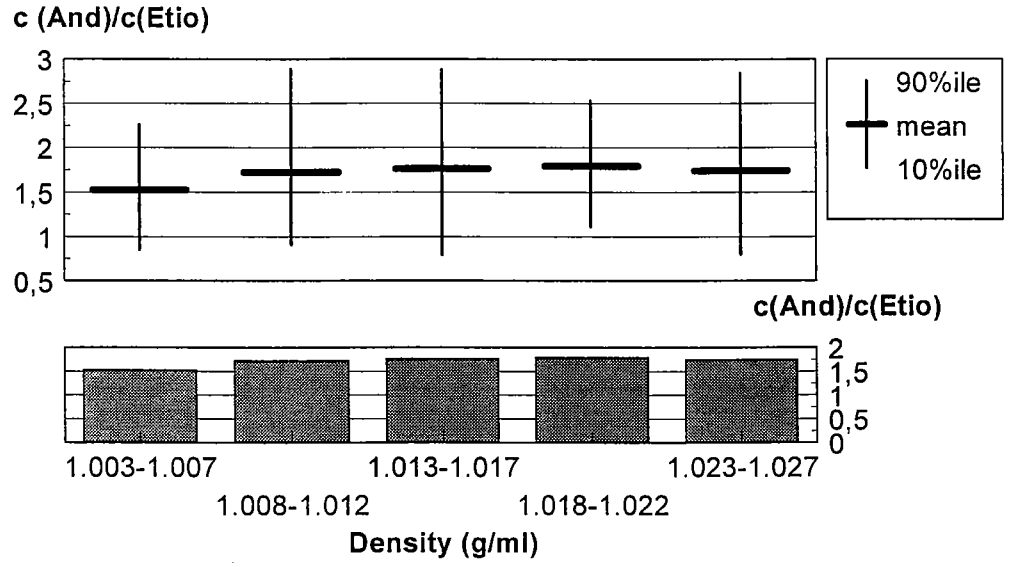

Figure 4 Mean level and 10-90%ile of the ratio c(androsterone)/c(etiocholanolone) in male athletes' urine according to differences in urine densities.

We also had the opportunity to analyse samples with different urine densities obtained from the same athlete. Figures 5 and 6 show some concentrations and ratios of endogenous steroids from one athlete, confirming our findings for the total number of samples.

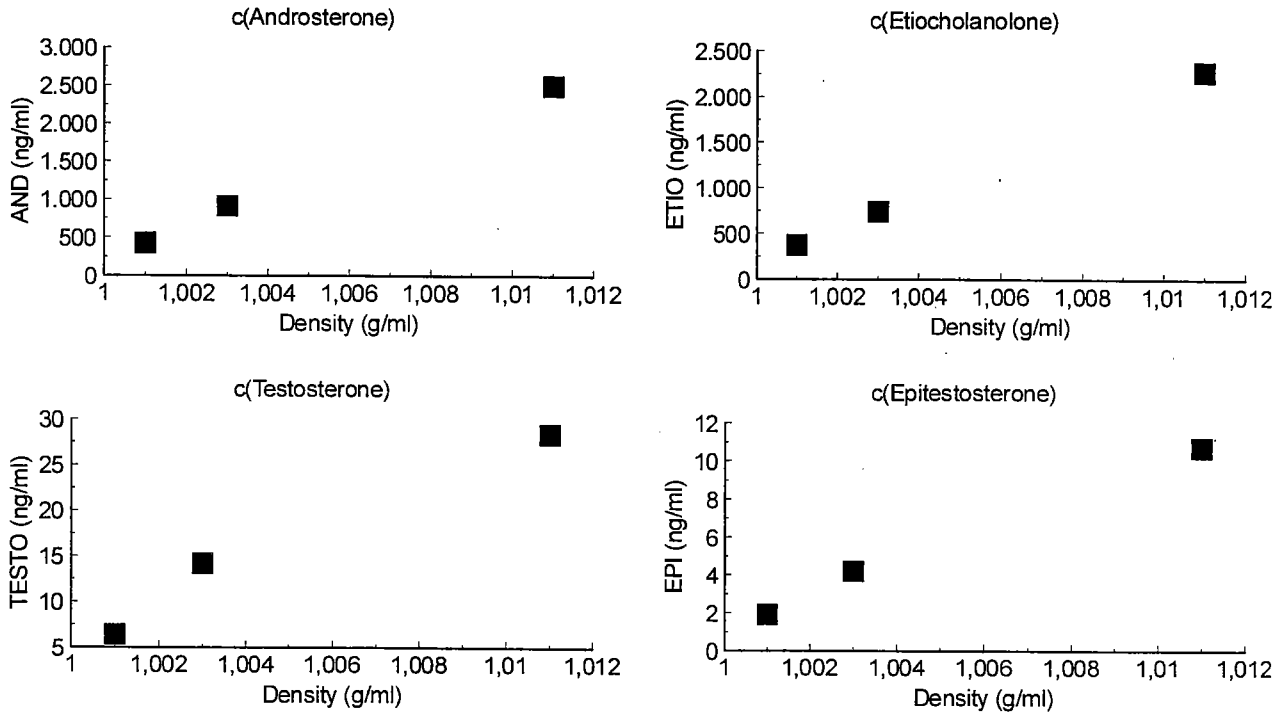

Figure 5 Steroid concentrations in different urine samples from the same athlete.

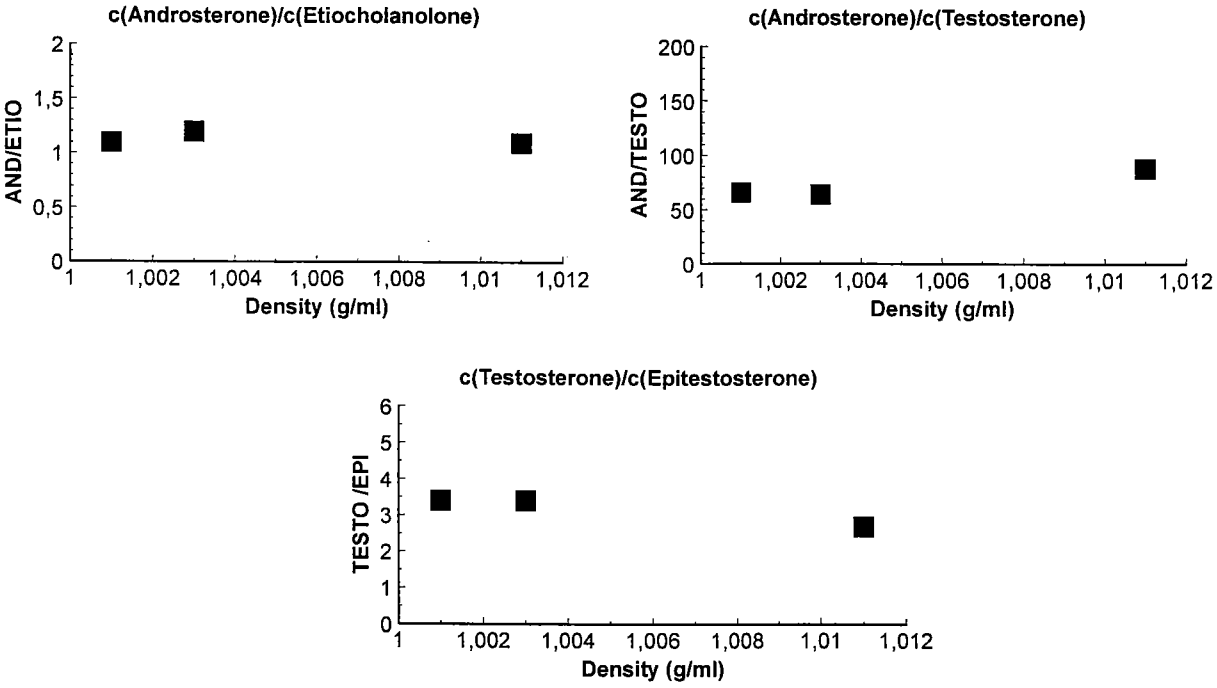

Figure 6 Steroid concentration ratios in different urine samples from the same athlete.

In conclusion,

- the introduction of urine density measurement at the sample collection site with a refractometer has significantly improved the correlation between on-site and laboratory measurements,
- the results from endogenous steroid quantification showed a good correlation with urine density measurements,
- the density limit for the rejection of control samples could be lowered to 1.007 or even 1.005 g/ml if sufficient urine volume is available.

Use of beta-2-agonists

The IOC Medical Commission has for the treatment of asthma and respiratory ailments with beta-2-agonists only allowed the use of salbutamol and terbutaline by inhalation[1]. During the Olympic Winter Games many questions have been raised concerning the treatment of exercise-induced asthma[3] with beta-2-agonists. Reports on an increasing use among cross-country skiers[4] and on anabolic effects of beta-2-agonists[5] are of some concern. Figures 7 and 8 show the number of athletes who declared the use of beta-2-agonists. The figures are based on the doping control schemes and not on the declarations made to the IOC Medical Commission. The analytical findings during the Olympic Winter Games are presented in figure 8.

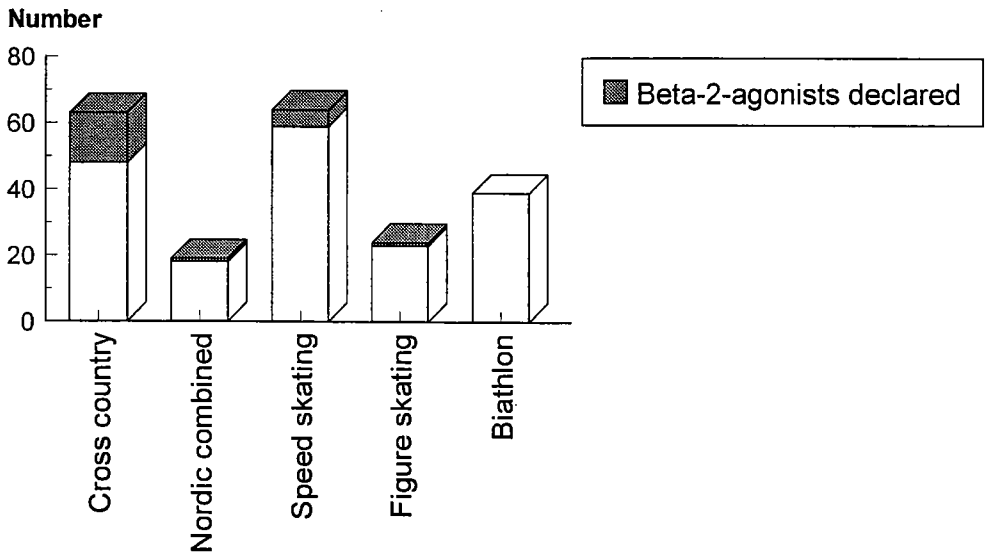

Figure 7 Beta-2-agonist use declared

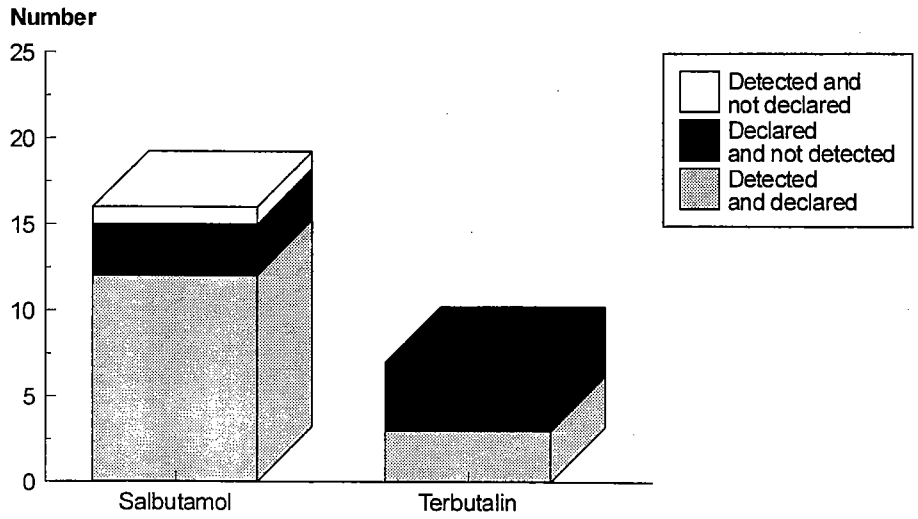

Figure 8 Beta-2-agonist use declared and detected during analysis.

The results show that the compliance between declarations and analytical findings is good. It might be of some interest that 24% of all cross-country skiers, who were tested during the games, declared the use of salbutamol or terbutaline while none of the biathletes did so. For the nordic combined, speed skating and figure skating events the percentage of β_2-agonist declarations were 5, 8 and 4%, respectively. The concentrations of salbutamol found ranged from 0.2 to 240 ng/ml.

Acknowledgement

For an excellent collaboration before and during the XVII Olympic Winter Games we would like to thank the IOC Medical Commission, the Norwegian Confederation of Sports and the Lillehammer Olympic Organising Committee.

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