Evaluation of elevated testosterone/epitestosterone values in athlete's urine samples

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by Christiane Ayotte

The use of prohibited substances by athletes has been controlled for nearly thirty years, mainly by conducting tests on urine samples provided in-competition or out-of-competition. The athlete found guilty of a doping offence has the right to appeal the decision through administrative procedures. Whenever an adverse finding is litigated, all aspects of the testing are challenged including in numerous instances, the scientific basis of the test no matter whether the substance is purely synthetic or can normally be found in the body fluids. One of the duties of the IAAF Doping Commission is to evaluate the reported positive finding and to recommend action. In this paper it is described on which scientific ground a testosterone positive finding can be judged.

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1 Introduction

Testosterone is a potent androgenic anabolic steroid of known abuse. Testosterone positive findings represent each year the highest number of anabolic agent cases reported by the IOC accredited laboratories. In 1992, the late Prof. Manfred Donike (1) stated that the athletes involved in doping practices were increasingly using endogenous steroids such as testosterone in place of the synthetic steroids, more easily detected since the end of the 80's. The use of short acting preparations to shorten the detection period of time is also widespread.

Other natural androgens such as dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA) and androstendione are now part of the "testosterone-enhancing" pharmaceutical arsenal. The last two steroids are direct biosynthetic precursors of testosterone and both can easily be obtained in the U.S.A.

Contrary to purely synthetic substances such as stanozolol, testosterone is naturally present in females and males and will be found in their urine samples. This implies that the proof required to conclude that testosterone was administered *is* more complex than the sole identification of the substance in the specimens and that there must be documented evidence of an increased T/E value and urinary testosterone concentration.

2 The T/E value: the probe indicating testosterone administration

In 1983, Donike (1, 2) proposed, after studying the effects caused by the administration of testosterone, that the measure of its ratio to epitestosterone, the 17α-epimer, be regarded as a diagnostic parameter in urine. Observations made on the athletic population (male and female) tested in Cologne and on samples collected during the Moscow and Lake Placid Games had shown that the mean and median T/E values are around 1-2 and that the vast majority of the ratios are found to be below 5.3. The IOC then

adopted the threshold of 6.

Although numerous attempts were made to underscore its usefulness, the T/E value is still the unchallenged criterion of testosterone administration. Fifteen years later, different groups witnessed its usefulness after studying several thousands of samples collected from male and female athletes of different ethnicity (4-7). Their statistical evaluation confirmed values reported in 1983 by DONIKE.

Table 1: Populations in relation to their T/E values

	T/E values	Testosterone concentration
Males		
Cologne (18) n = 5000	reference limit 97.5% : 5.2	< 130 ng/ml [1]
Montreal (7) n = 3700[3]	99% below 4.14	99% below 225 ng/ml [2]
Indianapolis (5) n = 22806	99% below 6.5-7.0	
	90% below 3-3.5	
UCLA (16) n = 3700	99% below 5.6	
Females		
Cologne (18) n = 1700	reference limit 97.5% : 6.3	< 60 ng/ml [1]
Montreal (19) n = 3,300 [4]	99% below 4.5	99% below 40 ng/ml [2]
	99.8% below 6	99.9% below 86 ng/ml [2]
UCLA (20) n = 8000	3 results higher than 6	
Males and Females		
Huddinge (12, 21)	Males: 26 results higher than 6	
n = 8946 (~ 30% of females)	Females: 2 results higher than 6	

[1] corrected (adjusted to 1.020)
[2] not corrected
[3] excluding positive cases
[4] including positive cases

On the other hand, considering only the values above 6 as positive creates false negative results. There are individuals for which normal T/E values are lower than 1 (7, 8). Studies have shown that some of these individuals receiving testosterone may still produce urine samples for which the threshold of 6 will not be reached (9, 10). This was clearly demonstrated in a group of Chinese volunteers for whom only the comparison of previous "basal-level" results would have enabled detection of the administration of testosterone (11).

It is known that the T/E values of a given male individual's urine samples are stable and naturally vary by less than 30% from the mean and that the T/E values of male athletes will not be affected by exercise, training, altitude (3-7). In females, due to the very low levels of both urinary testosterone and epitestosterone, the individual T/E values show greater variation sometimes twice those observed in males (4, 13-16). However, the use of anabolic steroids such as stanozolol or of enzyme inhibitors can have an influence on the endocrine system and disturb the urinary steroid profile, including the T/E values (1, 17).

Table 1 summarises the observations made on athletic populations in relation to their T/E values.

It has been known since 1984 that few individuals naturally produce urine samples in which elevated T/E values are systematically measured (3-7, 22-24). A low excretion of epitestosterone glucuronide is frequently the cause of the elevated ratio most of the time found between 6 and 10. This prompted the search of complementary markers such as the T/LH value (25, 26), the serum T/17α-hydroxyprogesterone value (27), the measurement of epitestosterone-sulphate (9, 28) and the use of ketoconazole to inhibit natural testosterone production (25), all of these however showing limitation. Other urinary parameters such as the A/T and the T/LH values are not indicative in every situation since they are not affected to the same extent as the T/E values and for an equally long period of time (7, 29). Moreover, following administration of oral DHEA and androstendione, the urinary concentration of androsterone and etiocholanolone increases noticeably rendering worthless the diagnostic value of the A/T (30, 31). Blood parameters can only be of some help if collection is made simultaneously to the urine specimen and this has not been the case.

Lately, three different groups introduced a promising way to complement the T/E value measurement proposing a procedure to discriminate between endogenous and exogenous testosterone using the difference observed in their respective content of ^{13}C (32-34). This method now needs to be validated and its sensitivity must be improved.

3 The definitions of a testosterone positive finding

When the IOC introduced testosterone on its list of banned substances, the sole measurement of a urinary T/E value above 6 was believed to be the result of the administration of testosterone or of the use of any other manipulation having a similar effect. Several years later in 1992, the definition was modified taking into account the natural conditions reported to have produced false positive results (35). A ratio higher than 6 was considered to reflect the administration of testosterone "unless there is evidence that this ratio is due to a physiological or pathological condition. For T/E values between 6 and 10, the IOC Medical Commission recommends that further tests be conducted before considering the

result as positive or negative. Such investigations may include: review of previous tests, endocrinological investigations, unannounced testing over several months".

For the IAAF, "the definition of a positive depends upon the following: the administration of testosterone or the use of any other manipulation having the result of increasing the ratio in urine of testosterone/epitestosterone".

Since 1994 the IOC definition abandons the brackets of 6 and 10, considers all results greater than 6 as an offence and defines examples of "pathological or physiological conditions such as a low epitestosterone excretion, androgene production of tumour, enzyme deficiencies". Details of the mandatory investigation to be conducted are also given as "a full written report will include a review of previous tests, subsequent tests and any results of endrocrine investigations" (38).

For the IAAF, the definition now reads as follows: "*a sample will be deemed to be positive for testosterone where either the ratio in urine of testosterone to epitestosterone, or the concentration of testosterone in urine, so exceeds the range of values normally found in humans as not to be consistent with normal endogenous production*" (39). This definition reflects accurately the observations and studies made by researchers, taking into account the individual natural condition and the effects of the different modes of administration of testosterone on the urinary steroids. It also makes possible the prevention of the false negatives caused by a very low T/E basal level (*vide supra*) if proof is established of an elevation higher than the individual's normal level, even though the threshold value of 6 is not reached.

4 The evaluation of the elevated T/E value result

4.1 Literature

There is no indication given by the IOC on how to conduct the evaluation of these cases, neither is the description made of the endocrine investigations to be obtained and therefore different interpretations coexist. For example, CATLIN et al. (16) propose the measurements of serum CBC, HDL and LDL cholesterol, free and total testosterone, SHBG, DHT, FSH, LH and a complete medical history and physical examination with emphasis on the hypothalamic-pituitary-testicular axis and the adrenal. For GEYER et al. (18), an endocrine study means establishing with urine samples collected during two days, the individual's reference range of T/E values and of the other steroid ratios related to testosterone administration (testosterone, epitestosterone, androsterone, etiocholanolone, LH). One blood sample is obtained during this period for the measure of the T/17-hydroxyprogesterone value. SEGURA proposed discriminatory tests in both urine and blood samples including research of the testosterone ester administered (40) and measure of the epitestosterone sulphate (12). Other groups advocate the application of the ketoconazolone test (41), although many pointed out its inapplicability in many countries and the potential adverse effects.

However, it is generally recognised that all these different forms of endocrine investigations are only needed when the analysis of the previous and subsequent test results and the comparison with the suspicious finding cannot provide a clear definitive answer.

4.2 The actual protocol followed

As stated above, the IAAF definition of a testosterone positive finding is either a urinary T/E ratio or a concentration of testosterone exceeding the range of values normally found in humans and not consistent with normal endogenous production. It is therefore necessary to analyse and compare the results of several samples given by the athlete.

In 1993, DONIKE et al. (6) gave a clear description of how to conduct the evaluation of a reported positive finding. The investigation is aimed at determining if the measured T/E value is higher than the normal of the reference population (population-based reference range) and of the given individual (subject-based reference range). In order to establish the individual's normal value, the mean, standard deviation and coefficient of variation of a minimum of three other T/E values of previous (if available) or subsequent tests are calculated excluding the suspicious sample. If necessary, the samples can be collected under quarantine-like conditions.

According to statistical definitions accepted in the field of clinical biochemistry, the individual's highest normal value expected can be calculated by adding to the mean value a defined number of times the standard deviation. This method constitutes the basis for the evaluation of reported adverse findings by numerous responsible authorities.

The variation of all the individual's T/E values gives, in most cases, a clear indication of a normal or abnormal situation. The administration of testosterone causes quite significant fluctuations in the T/E values and elevated variations of 67 to 130% are observed (7, 11 ,12, 29). The period of time during which the T/E values remain elevated is highly variable depending upon the mode of administration (16). For example, after the intake

of oral testosterone undecanoate, the T/E values return to normal within 24 hours and only exceed the threshold of 6 for few hours (29). In a natural condition, the normal T/E values will vary by less than around 30% in males and up to around 60% in females. There are however borderline cases reported for which some sport authorities required further investigations such as the ketoconazole test or endocrine studies.

According to the IAAF definition, another evidence of testosterone administration is a urinary concentration of testosterone exceeding the levels normally found in humans. As reported earlier, the naturally elevated T/E values are in most instances due to a systematic low excretion of epitestosterone glucuronide whilst the concentration of testosterone is normal. The abnormally elevated T/E value is due to an increased urinary testosterone concentration. Two groups in Montreal and Cologne described the testosterone concentrations measured in athletes' samples taking into account the specific gravity of the specimens. Most of the IOC accredited laboratories estimate the urinary concentration of testosterone and epitestosterone during screening procedures. Although not given by all with the same level of accuracy, the comparison of the testosterone concentration with the population and individual range has often provided highly valuable information, a sudden increase reflecting the administration of testosterone.

Each year, around 10,000 to 15,000 tests are done by IOC accredited laboratories in Athletics. During 1993-1996, although this may not represent accurately all the detected positive findings, records indicate that about thirty-four elevated T/E cases (26 males, 8 females) were reported to the IAAF by the National Sport Authorities or the IOC accredited laboratories in accordance with the IOC Medical Code. In fourteen of these, the IAAF Doping Commission attributed the results to a natural excretion of elevated T/E values whilst the others were given as positive. The opinion of the Doping Commission was formulated after reviewing the results according to the protocol developed by DONIKE (cf. box next column).

Protocol for elevated T/E values

Step 1. Collection of information and comments from the laboratory reporting the adverse finding. A form is suggested to the laboratory.

Step 2. Collection of available information on other previous or subsequent tests (minimum of three).

Step 3. Evaluation of the T/E values: calculation of the mean value, the standard deviation and the variation (%).

Step 4. Evaluation of the concentration of testosterone and epitestosterone taking into account the specific gravity of the specimens.

Systematic natural excretion of elevated T/E values.

- The T/E values do not exceed 10-12
- The variation of the values is normal e.g. below or around 30% in males and up to 60% in females
- The concentration of testosterone glucuronide is not elevated or suddenly increased and the epitestosterone glucuronide is low.
- Other available parameters of the steroid profile are normal

Abnormal result: indication of testosterone administration

- The T/E variation exceeds what is normally measured (*vide supra*)
- The suspicious result differs from the subject normal T/E values. The highest T/E value expected is given by adding 3 to 5 (for females) times the standard deviation to the mean basal value (excluding the positive finding).
- The concentration of testosterone is increased in the suspicious specimen taking into account the specific gravity.
- The concentration of testosterone is found in the upper range or above of the measurements made on the athletic population of the same gender.

Borderline result: benefit of the doubt is given to the athlete.

5 Potential loopholes and factors affecting the urinary T/E value

5.1 False negatives

The use of testosterone may remain undetected. For example, the use of short acting preparations will drastically reduce the detection period, results returning to normal within a few hours. Secondly, the use of epitestosterone can mask more or less successfully the otherwise elevated T/E value especially if concentrations are not considered. The third situation raises more concerns since it is directly derived from the incapacity of the test with its actual threshold of 6, to detect the use of testosterone in a group of the athletic population. As pointed out earlier,

individuals for which basal T/E values are very low may receive testosterone without ever producing specimens with T/E values exceeding 6. The only way to circumvent this problem at this time is the establishment of the individual's baseline values, an unrealistic approach with the existing resources.

5.2 Factors invoked to invalidate the test and its significance

Some of the most frequently raised arguments against the significance of the T/E value will now be discussed.

5.2.1 The bacterial contamination of the samples

The specimens are not collected under sterile conditions and therefore may be contaminated. Delays in delivering the samples to the laboratory and improper refrigeration during transportation and storage may offer good conditions for the microbial growth and affect the validity of the test as far as natural substances are concerned. In fact, nearly 95% of the samples received will be contaminated following an incubation of several days at 37°C. Not all contaminated samples will show signs of degradation invalidating the test results. The effects of the bacterial degradation on the urinary steroids are easily detectable and consist mainly in the hydrolysis of the steroid conjugates and in oxydoreductive reactions leading to the abnormal presence of steroids in the free form and the accumulation of the 5α- and 5β-androstan-3,17-dione (7, 42, 43). The presence of more than 5% of testosterone in the free fraction or of measurable amounts of androstandiones will invalidate a T/E positive finding. During a lecture, GEYER et al. (44) reported having observed the formation of testosterone in a female athlete's urine sample kept refrigerated for months. This bacterial formation and accumulation of testosterone in a urine sample was however never observed and reproduced during research experimentation (7, 42, 43).

5.2.2 The variation of the T/E values during the menstrual cycle

As stated earlier, the variation of the T/E values in females is greater than in males and this is obviously attributed to the very low levels of androgens in the specimens and in some protocols, to coeluting substances (13-15). The results of one study aimed at evaluating the stability of the T/E values during the menstrual cycle in four female volunteers was presented in 1994 (13) and showed a dramatic increase of the T/E values in two cases reaching values of 8 and 13 respectively. The daily variation of the individual T/E values was up to 79%. The authors, however, mentioned that the increased T/E value was directly linked to a decrease in the already very low epitestosterone concentration. Other groups observed in some volunteers an increase of the T/E values during the menstrual cycle (16, 19) but not to the extent described in the above study.

In the feminine athletic population, thousands of tests analysed in Europe and North America only gave a handful of T/E results exceeding the threshold of 6 (*vide supra*). This clearly demonstrates that the phenomenon described by MARECK-ENGELKE et al. is overestimated in terms of the frequency of the drastic increase (2 out of 4 females) and of its magnitude.

5.2.3 The variation of the T/E values caused by oral contraceptives

MARECK-ENGELKE et al. presented in a lecture in 1996 the results of a study indicating that the T/E values decreased during withdrawal of oral contraceptives in each of the four volunteers (15). This was attributed to an increase of the epitestosterone excretion. In all cases, the urinary concentrations were said to be very low, near the detection limit. The variation of the T/E values remained lower than 60%. The authors concluded that the application of oral contraceptives can lead to an increase of the T/E ratio due to the suppression of the epitestosterone excretion.

5.2.4 The effect of alcohol intake

As discussed by CATLIN et al. (16), although four studies tried to investigate the effects of alcohol on the T/E values, the results are contradictory. They concluded prudently that a high intake of inebriating alcohol may produce a significant increase of the T/E values in some females. In 1998, FALK et al. (45), reported a variable increase ranging from 30 to 90% of the T/E values in four volunteers following a high intake of alcohol (2g/kg of body weight) taken within four hours. None of the T/E values measured exceeded 2.4. Later other groups reported contradictory effects with similar doses (46-48). In some studies, T/E values of up to 8, 12 and even 22 were reported. One may question the interpretation given to these findings considering the very low levels of both epitestosterone and testosterone in female samples and the normal variation of their T/E values. For example one group described what they considered to be a noticeable effect on the T/E values caused by an intake of alcohol (1.4 to 2g/kg of body weight) (47). The detailed observation of the T/E values reported indicates that alcohol induced a variation of 18 to 72% from the individual's mean. However, such variations of the T/E values are normal in females as reported before on several occasions by the same group.

6 Conclusion

Some studies seem to cast serious doubts over the validity of the T/E value as a measure of the administration of testosterone in female athletes. Menstruation, intake of oral contraceptives or alcohol, nutrition, supplements and the bacterial contamination of the samples, very frequent and natural phenomena, were all reported or invoked to increase significantly the T/E value in female volunteers up to levels well-above the threshold of 6. On the other hand, thousands of female and male urine samples are tested each year during routine controls performed in competition or out-of-competition therefore in every possible normal and extreme conditions encountered during the course of an athletic *career*. As shown earlier, actual observations made on the female athletic population in Europe and North America, all indicate that the vast majority of the T/E values are found below 6 and that only a handful of test results exceeded the threshold of 6.

Testosterone is frequently used. This is most probably due to its alleged efficiency, the highly diversified forms and preparations available and certainly, as a consequence of the problems associated with its detection. Two direct biosynthetic precursors of testosterone, DHEA and androstendione, can be easily obtained in U.S.A. Both were shown in preliminary studies to affect testosterone, epitestosterone and other major steroid metabolites such as androsterone and etiocholanolone. Their increasing popularity should be kept in mind when reviewing an elevated T/E positive finding.

In the future, if the approach using the carbon isotope ratio to identify directly exogenous urinary testosterone is proven to be robust, flexible and sensitive enough, we may envisage lowering the threshold value of the "classical" T/E test. The confirmation of the exogenous nature of testosterone in the same specimen along with the evaluation of previous or subsequent test results may solve the major problem of testosterone detection: the number of false negative results.

REFERENCES

1 Donike, M.: Steroid profile in Cologne. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U.; Rauth, S. (eds.): Proceedings of the 10th Cologne workshop on dope analysis, 7th to 12th June 1992, Köln, 1993, p. 47

2 Donike, M.; Barwald, K.-R.; Klostermann, K.; Schänzer, W.; Zimmermann, J.: Nachweis von exogenem Testosteron. In: Heck, H.; Hollmann, W.; Liesen, H.; Rost, R. (eds.): Sport: Leistung und Gesundheit. Cologne, 1983, p. 293

3 Donike, M.; Rauth, S.; Wolansky, A.: Reference ranges of urinary endogenous steroids determined by GC/MS. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U.; Rauth, S. (eds.): Proceedings of the 10th Cologne workshop on dope analysis, 7th to 12th June 1992, Cologne, 1993, p. 69

4 Mareck-Engelke, U.; Geyer, H.; Donike, M.: Stability of steroid profiles. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U.; Rauth, S. (eds.): Proceedings of the 10th Cologne workshop on dope analysis, 7th to 12th June 1992, Cologne, 1993, p. 87

5 Baenziger, J.; Bowers, L.: Variability of T/E ratios in athletes. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U.; Rauth, S. (eds.): Proceedings of the 11th Cologne workshop on dope analysis, 7th to 12th March 1993, Cologne, 1994, p. 41

6 Donike, M.; Rauth, S.; Mareck-Engelke, U.; Geyer, H.; Nitschke, R.: Evaluation of longitudinal studies, the determination of subject based reference ranges of the T/E ratio. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U.; Rauth, S. (eds.): Proceedings of the 11th Cologne workshop on dope analysis, 7th to 12th March 1993, Cologne, 1994, p. 33

7 Ayotte, C.; Goudreault, D.; Charlebois, A.: Testing for natural and synthetic anabolic agents in human urine. In: J. Chromatogr. B. (1996), 687, p. 3

8 Catlin, D.H.; Hatton, C.K.; Straus, P.W.; Starcevic, B. In: Cowan, D.A.; Kicman, A.T. (eds): Control of doping with anabolic agents. Proceedings of the Scientific Meeting of the 4th Permanent World Conference on Anti-Doping in Sport, London, 1993, p. 2

9 Dehennin, L.; Masumoto, A.M.: Long-term administration of testosterone enanthate to normal men: alterations of the urinary profile of androgen metabolites potentially useful for detection of testosterone misuse in sport. In: J. Steroid Biochem. Mol. Biol. (1993), 44, p. 179

10 Palonek, E.; Garle, M.: Single injection of testosterone to 7 volunteers: Results from this study. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U.; Rauth, S. (eds.): Proceedings of the 10th Cologne workshop on dope analysis, 7th to 12th June 1992, Cologne, 1993, p. 131

11 Segura, J.: Testosterone doping detection and confirmation by means of T/E ratio and other parameters. In: Harmonisation of Doping Issues in the IAAF, IAF Seminar 1995, Paris, 10-11 October 1995

12 Garle, M.; Ocka, R.; Palonek, E.; Bjorkhem, I.: Increased urinary testosterone/epitestosterone ratios found in Swedish athletes in connection with a national control program. Evaluation of 28 cases. In: J. Chromatogr. B (1996), 687, p. 55

13 Mareck-Engelke, U.; Geyer, H.; Donike, M.: Stability of steroid profiles (4): The circadian rhythm of urinary ratios and excretion rates of endogenous steroids in female and its menstrual dependency. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (2). Proceedings of the 12th Cologne workshop on dope analysis, 10th to 15th April 1994, Cologne, 1995, p. 135

14 Mareck-Engelke, U.; Flenker, U.; Donike, M.: Stability of steroid profiles (5): The annual rhythm of urinary ratios and excretion rates of endogenous steroids in female and its menstrual dependency. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (3). Proceedings of the 13th Cologne Workshop on dope analysis, 12th to 17th March 1995, Cologne, 1996, p. 177

15 Mareck-Engelke, U.; Flenker, U.; Schänzer, W.: Stability of steroid profiles (6): The influence of oral contraceptives on steroid profiles. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (4). Proceedings of the 14th Cologne Workshop on dope analysis, 12th to 17th March 1996, Cologne, 1997, p. 139

16 Catlin, D.H.; Hatton, C.K.; Starcevic, S.H.: Issues in detecting abuse of xenobiotic anabolic steroids and testosterone by analysis of athlete's urine. Doping in Sport Symposium. In: Clin. Chem. 43 (1997), 7, p. 1280

17 Donike, M.; Geyer, H.; Kraft, M.; Rauth, S.: Long-term influence of anabolic steroid misuse on the steroid profile. In: Belotti, P.; Benzi, G.; Ljungqvist, A. (eds.): Doping in sport. Monte Carlo, 1989. Monte Carlo: International Athletic Foundation, 1990, p. 107

18 Geyer, H.; Mareck-Engelke, U.; Schänzer, W.; Donike, M.: The Cologne protocol to follow up high testosterone/epitestosterone ratios. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (4). Proceedings of the 14th Cologne Workshop on dope analysis, 12th to 17th March 1996, Cologne, 1997, p. 139

19 Ayotte, C.; Charlebois, A.: Unpublished results (1997)

20 Catlin, D.H.: Unpublished results (1997)

21 Garle, M.: Personal communication (1997)

22 Namba, O.; Miyachi, Y.; Irie, M.; Kuroda, Y.: Urinary testosterone and epitestosterone secretion in a doping positive subject. International Congress of Endocrinology, Kyoto, 1988, abstract 16-22-324

23 Raynaud, E.; Audran, M.; Brun, J.F.; Fedou, C.; Chanal, J.L.; Orsetti, A.: False-positive cases in detection of testosterone doping. In: Lancet (Letter) (1992), 340, p. 1468

24 Oftebro, H.. In: Lancet (1992), 339, p. 941

25 Cowan, D.A.; Kicman, A.T.; Walker, C.J.; Wheeler, M.J.: Effect of administration of human chorionic gonadotrophin on criteria used to assess testosterone administration in athletes. In: J. Endocrinol. (1991), 131, p. 147

26 Kicman, A.T.; Brooks, R.V.; Collyer, S.C.; Cowan, D.A.; Nanjee, M.N.; Southan, G.H.; Wheeler, M.J.: Criteria to indicate testosterone administration. In: Br. J. Sport Med. 24 (1991), p. 253

27 Carlstrom, K.; Palonek, E.; Garle, M.; Oftebro, H.; Stanghelle, J.; Bjorkhem, I.: Detection of testosterone administration by increased ratio between serum concentrations of testosterone and 17 alpha-hydroxyprogesterone. In: *Clin. Chem. 38 (1992), p. 1779*

28 Dehennin, L.. In: J. Steroid Biochem. Mol. Biol. 44 (1993), p. 171

29 Wright, F.; Lafarge, J.P.; Antréassian, J.; Lagoguey, M.; Pères, G. In: Hemmersbach, P.; Birkerland, K.I. (eds.): Long term study of steroid and peptidic hormones in the plasma of healthy young men under controlled testosterone undecanoate therapy. In: Blood sample in doping control. Second International Symposium on Drugs in Sports. Lillehammer, Norway, 1993, p. 65

30 Ayotte, C.; Charlebois, A.; Lévesque, J.F.: Unpublished results

31 Kazlauskas, R.: Unpublished results

32 Becchi, M.; Aguilera, R.; Farizon, Y.; Flament, M.M.; Casabianca, H.; James, P.: Gas chromatography/combustion/isotope-ratio mass spectrometry analysis of urinary steroids to detect misuse of testosterone in sport. In: Rapid Commun. Mass Spectrom. 8 (1994), p. 304

33 Aguilera, R.; Becchi, M.; Casabianca, H.; Hatton, C.K.; Catlin, D.H.; Starcevic, B.: Improved method of detection of testosterone abuse by gas chromatography /combustion/isotope ratio mass spectrometry analysis of urinary steroids. In: J. Mass Spectrom. 31 (1988), p. 169

34 Horning, S.; Geyer, H.; Machnik, M.; Schänzer, W.; Hilkert, A.; Oebelmann, J.: Detection of exogenous testosterone by 13C/12C analysis. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (4). Proceedings of the 14th Cologne Workshop on dope analysis, 12th to 17th March 1996, Cologne, 1997

35 IOC Medical Commission: List of banned substances and methods of doping – May 1992

36 IAAF Procedural Guidelines for Doping Control, Schedule 1, Prohibited substances, Part 1, 1992

37 IOC Medical Commission: List of banned substances and methods of doping – May 1992

38 IOC Medical Commission: List of banned substances and methods of doping – January 1997

39 IAAF Procedural Guidelines for Doping Control, Schedule 1, Prohibited substances, Part 1, March 1996

40 Torre, X. de la; Segura, J.; Polettini, A.; Montagna, M.: Detection of testosterone esters in human plasma. In: J. Mass Spectrom. 30 (1995), p. 1393

41 Kicman, A.T.; Oftebro, H.; Walker, C.; Norman, N.; Cowan, D.A.: Potential use of ketoconazole in a dynamic endocrine test to differentiate between biological outliers and testosterone use by athletes. In: Clin. Chem. 39 (1993), p. 1798

42 Hemmersbach, P.; Birkeland, K.I.; Norli, J.R.; Ringertz, S.H.: Urine storage conditions and steroid profile analysis. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (4). Proceedings of the 14th Cologne Workshop on dope analysis, 12th to 17th March 1996, Cologne, 1997, p. 99

43 Lapointe, S.: M.Sc. Thesis, INRS-Santé, Université du Québec, 1997

44 Geyer, H.; Schänzer, W.; Mareck-Engelke, U.; Donike, M.: Factors influencing the steroid profile. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (3). Proceedings of the 13th Cologne Workshop on dope analysis, 12th to 17th March 1995, Cologne, 1996, p. 95

45 Falk, O.; Palonek, E.; Bjorkhem, I.: Effect of ethanol on the ratio between testosterone and epitestosterone in urine. In: Clin. Chem. 34 (1988), 7, p. 1462

46 Karila, T.; Kosunen, V.; Leinonen, A.; Tahtela, R.; Seppala, T. In: J. Chromatogr. B. (1996), 687, p. 109

47 Mareck-Engelke, U.; Geyer, H.; Schindler, U.; Flenker, U.; Iffland, R.; Donike, M.: Influence of Ethanol on Steroid Profile Parameters. In: Donike, M.; Geyer, H.; Gotzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (3). Proceedings of the 13th Cologne Workshop on dope analysis, 12th to 17th March 1995, Cologne, 1996, p. 143

48 Seppenwoolde-Waasdorp, J.A.; Boer, D. de; Engelen, H.M.J. Van; Vrijdag, A.D.; Maes, R.A.A.: Evaluation of endogenous steroid profiles in urine (2) effects of ethanol intake reinvestigated. In: Donike, M.; Geyer, H.; Götzmann, A.; Mareck-Engelke, U. (eds.): Recent advances in doping analysis (3). Proceedings of the 13th Cologne Workshop on dope analysis, 12th to 17th March 1995, Cologne, 1996, p. 157