

Nutritional supplements and doping controls

by Christiane Ayotte

Numerous „nutritional“ supplements are now part of many athletes' diet: vitamins, minerals and electrolytes. With the exception of caffeine and ephedrines, none of these should contain banned substances or produce a positive finding according to the ingredients listed on the label. Since 1996, the Sport Nutritional Industry in the USA has offered an increasing number of natural or legal steroids that although regulated in other countries, are easily available around the world. This situation is even more disturbing since with an apparent lack of appropriate surveillance, members of this industry distribute supplements that are shown in some cases to contain banned substances that are either misidentified or not listed on the label. The author concludes that a worrying number of so-called „dietary“ supplements actually contain prohibited steroids that may or may not be listed on the label. Their intake will result in a positive finding.

Numerous „nutritional“ supplements are now part of many athletes' diet; vitamins (C, E, A, B complex, B6, B12), minerals and electrolytes (Ca, Fe, Mg, Zn, Se, K), chromium picolinate, carbohydrates, creatine, caffeine, ephedrines, amino acids, l-carnitine, glutamine, glucosamine, protein powders, antioxidants (CoQ10), fatty acids, hydroxymethylbutyrate (HMB), herbal preparations (Echinacea, Ginseng, St-John's Wort, Spiruline, Ginkgo biloba, Arnica, garlic) and shark cartilage to name just some of the most frequently mentioned on doping control forms.

With the exception of caffeine and ephedrines, none of these should contain banned substances or produce a positive finding according to the ingredients listed on the label. However, since 1996, the Sport Nutritional Industry in the USA has offered an increasing number of natural or legal steroids that although regulated in other countries, are easily available around the world. Oral preparations of androgens, pro-hormones or precursors such as DHEA (dehydroepiandrosterone), androstenedione, androstenediol, 19-norandrostenedione, 19-norandrostenediol are now commercially available as such or in countless combinations. These so-called supplements can also be obtained from the Internet.

The oral administration of DHEA, androstenedione and androstenediol was shown to markedly alter the urinary steroid profile and to increase the T/E urinary ratio of some individuals. Norandrostenedione, norandrostenediol and nandrolone (19-nortestosterone) are metabolised and excreted as norandrosterone and noretiocholanolone. The urine samples collected

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after the administration of these supplements will definitely test positive.

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Introduction

Testosterone and 19-nortestosterone (nandrolone) are potent androgenic anabolic steroids at the centre of known abuse in sport. Anabolic steroids have been banned in Olympic sports for more than 20 years and, since 1986, the exogenous steroids most frequently found in athletes' urine samples have been testosterone and nandrolone metabolites. Now that synthetic anabolic agents can be efficiently detected with sensitive methods, athletes wishing to use doping agents are turning to natural steroids. This development was highlighted by the late Prof. Manfred Donike in 1992 (1). The use of short acting preparations to shorten the detection period of time is also suspected.

It was reported (2) that in the 1980's, knowing that a test would soon be adopted to detect the use of testosterone, scientists in East Germany tried to circumvent it by developing short acting testosterone preparations and nasal sprays containing a testosterone biosynthetic precursor, androstenedione.

One of the first hormonal supplements that became commercially available in the USA was DHEA around 1996. The list steadily expanded and now includes testosterone precursors, DHEA, androst-4-en-3,17-dione, androst-4-en-3,17-diol, androst-5-en-3,17-diol as well as 19-nortestosterone precursors, 19-norandrost-4-en-3,17-dione and 19-norandrost-4-en-3,17-diol. These come in various combinations or "stacks" with other substances touted to possess very specific performance-enhancing properties and are presented as being supported by scientific research. The following extract from an Internet advertisement sets the tone (3):

Stacking recommendations : it is probably most effective to stack Nor-Androstenedione with Androdiol, Saw palmetto, and Chrysin. Saw palmetto is suggested to help side-effects associated with DHT from androgens. Chrysin is thought to have anti-aromatase properties. To aid the enzymatic conversion process, the following supplements may also help : Zinc, Pyruvate, and Phosphates...

Warning to everyone : we are not aware of any studies conducted on the effects (good or bad) of Nor-Androstenedione supplementation for muscle growth or strength increases. This is definitely new territory. Use at your own risk!

Special warning to tested competitors : Nor-Androstenedione may cause a positive showing on a steroid test for Nandrolone. Just as is the case with Androdiol and Androstenedione, it increases the epitestosterone/testosterone ratio which is frequently used to indicate steroid use.

The use of these steroids has always been prohibited in sport as they are related to testosterone and 19-nortestosterone. Since they are legally available in USA, the IOC and the Sport Authorities listed them by name as banned anabolic agents (4).

Despite repeated warnings from many sport authorities, a growing number of diversified dietary supplements are reported by athletes on doping control forms filled during sample collections.

Doping controls

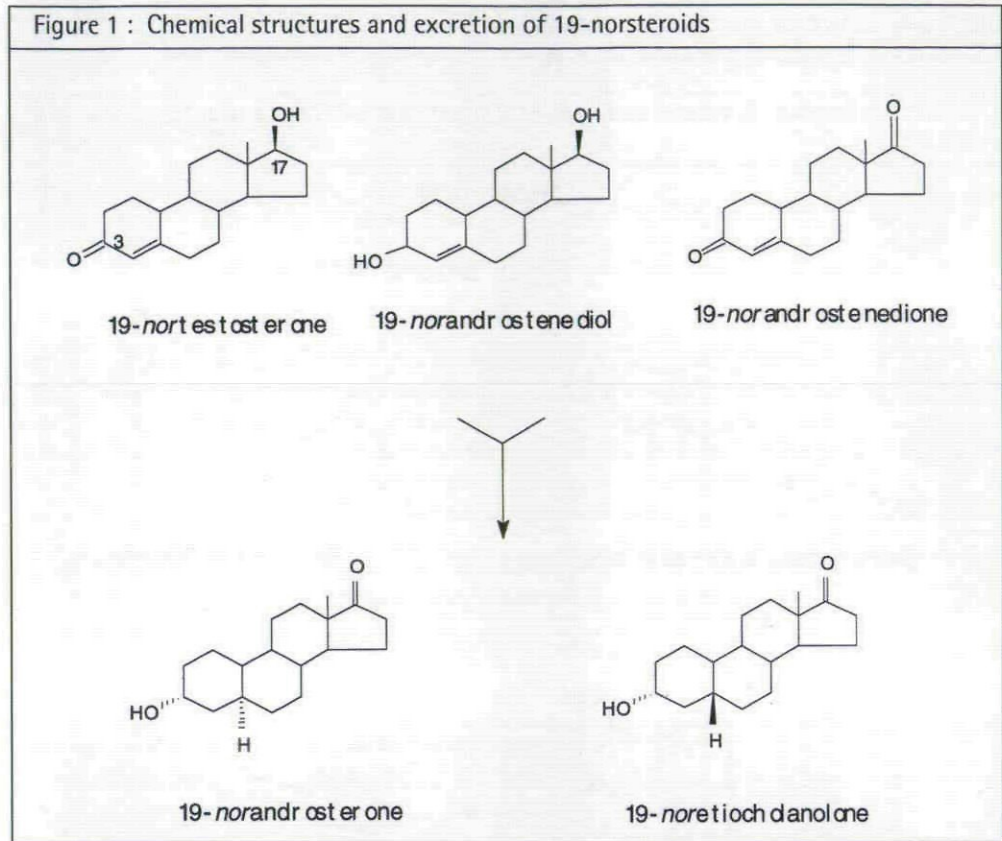
Five years ago, the administration of oral precursors of testosterone and 19-nortestosterone was not a real issue in athletic drug testing because there was no such preparation easily available. Intensive work has had to be done in recent years to characterise the urinary metabolites and to study the effects of their administration on the urinary steroid profile.

Detection of 19-nortestosterone, 19-norandrostenedione and 19-norandrostenediol administration

These steroids are chemically related as

shown in figure 1, possessing functionality of different oxidation level (ketone or hydroxyl groups) in position C-3 and C-17.

The oral administration of the nutritional supplements of 19-norandrostenedione and 19-norandrostenediol also results in the pres-



The intra-muscular administration of pharmaceutical oil-based preparations of 19-nortestosterone long-chain esters has been known for more than forty years and has been shown by several groups to be excreted mainly as 19-norandrosterone and also as 19-noretiocholanolone and 19-norepiandrosterone. These metabolites are found in the human urine as the glucuro- and/or sulfoconjugates. With 19-norepiandrosterone being sulfoconjugated, 19-norandrosterone and 19-noretiocholanolone glucuronides are the metabolites detected and identified by GC/MS in doping control analysis (5). Following the administration of 19-nortestosterone, 19-norandrosterone glucuronide has been found in higher amounts than 19-noretiocholanolone(6).

ence of 19-norandrosterone and 19-noretiocholanolone in the urine samples (7-8).

Detection of androst-4-en-3,17-dione and DHEA oral administration

Androstenedione and DHEA are biosynthetic precursors of testosterone (9). The chemical structures of the three steroids that are shown in figure 2 are similar, varying by the oxidation level of the groups in position C-3, C-17 or by the position of the double bond in C-4 or C-5. Androstenedione in itself is considered as a weak androgen and DHEA is marketed by the pharmaceutical industry as an androgen (Prasterone).

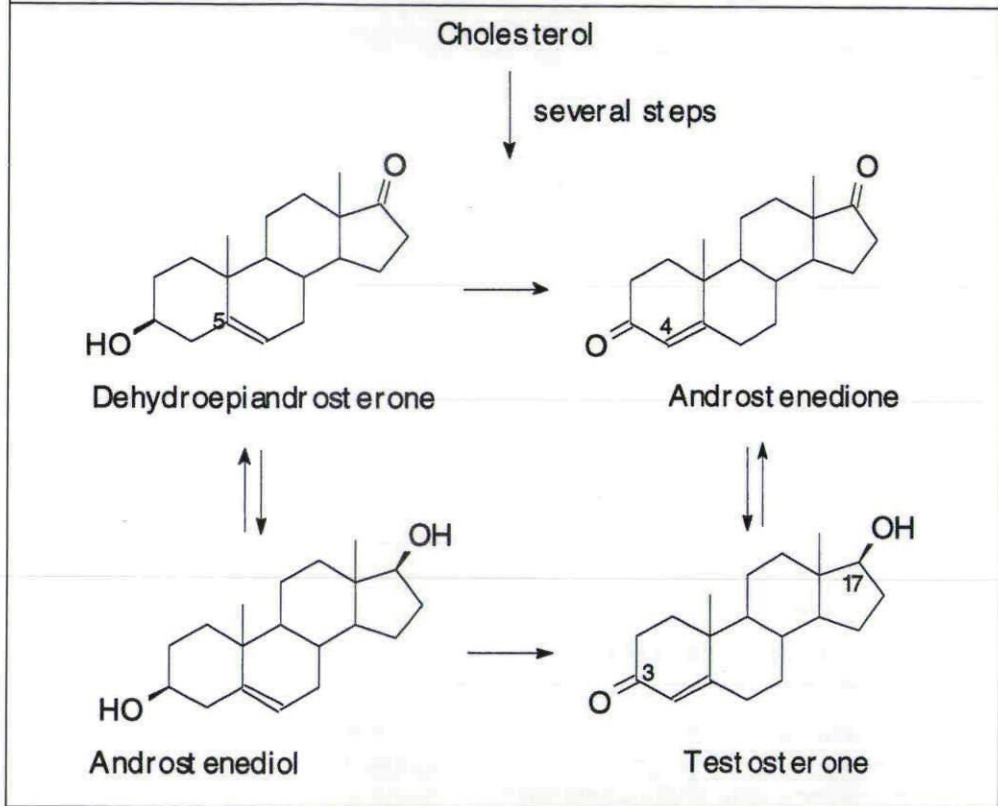
The administration of testosterone is first detected in human urine by the GC/MS measurement of a testosterone/epitestosterone

(T/E) value higher than 6 that is caused by the relative increase of excreted testosterone glucuronide (10).

The oral intake of androstenedione and DHEA was shown to increase the excreted T/E values in females and in some male vol-

unteers they are purchasing do not have to pass the stringent quality controls imposed on approved medications. Their claimed actions, efficiency or potency have not been thoroughly investigated by controlled clinical studies and remain for the most part

Figure 2: Chemical structure and biosynthesis of testosterone, androstenedione and DHEA



unteers (8,11). Other alterations of the urinary steroid profile, such as the presence of characteristic hydroxylated metabolites and of abnormally high concentrations of androsterone and etiocholanolone, are also used to prove the administration of these precursors. The GC/C/IRMS is also a very powerful complementary technique to demonstrate the synthetic or natural nature of the urinary androgens.

Analysis of the content of some "nutritional" supplements

The customers of hormonal "nutritional" supplements should be made aware that the

anecdotal. The potentially serious adverse effects from the self-administration of nutritional and steroids supplements are not fully known (12).

It was pointed out in a study published in 1998 by a group of American scientists that the hormonal so-called dietary supplements available for self-administration are not evaluated by the USA Food and Drug Administration (FDA) for their safety and efficacy (13). Their production and manufacturing do not have to be made in compliance with the regulations of the FDA, neither do they have to meet the standards of quality.

The authors reported the results of the analysis they made of sixteen different DHEA preparations in different dosages. They found that the actual content of this steroid ranged from 0% to 150% of the dosage indicated on the label.

This is however not the only concern that one should have about so-called dietary supplements. It was shown from different IOC Accredited Laboratories that the labelling of some preparations may not reflect their actual content (14). In Sweden, some supplements supposed to contain androstenediol, alone or in combination with androstenedione, that were seized at customs were shown instead to contain testosterone, a regulated substance. In Montreal, we have purchased with the authorisation of Health Canada some hormonal supplements advertised on the Internet and have analysed their content. In two preparations labelled as androstenedione and androstenedione + DHEA "complex" we found the presence of minor amounts of testosterone. The preparation of androstenediol was instead composed principally of testosterone with other steroids such as androstandiol, androstenolone and even epitestosterone present in lower amounts. Two supplements of 19-norandrostenedione and 19-norandrostenediol were also supposed to contain progesterone but it was found in only one of the two supplements.

In 1999, the Cologne IOC Accredited Laboratory reported to the IOC Medical Commission and to the IAAF Anti-Doping Commission that two sport dietary supplements manufactured in USA contained steroids not listed on the label and that the intake of these contaminated preparations would have caused a positive finding to anabolic agents. These non-hormonal preparations of Chrysin and Tribulus touted to possess respectively anti-aromatase activity and stimulating effects on testosterone secretion should not have produced positive results after a doping control test. They were found to contain prohibited substances not labelled such as androstene-

dione, androstenediol, 19-norandrostenedione and 19-norandrostenediol. The administration of these contaminated products was shown to produce positive findings of norandrosterone and noretiocholanolone, both metabolites of the 19-norsteroids, increased T/E values and altered steroid profiles.

Conclusion

Athletes are targeted by the Sport Nutritional Industry. It is often believed that preparations that are sold legally as supplements cannot be harmful or contain banned substances. Athletes were warned years ago about the presence of stimulants such as the ephedrines in herbal preparations, particularly those containing Ma Huang. A worrying number of so-called "dietary" supplements actually contain prohibited steroids that may or may not be listed on the label. Their intake will result in a positive finding. This should not however be our only concern. The self-administration of hormonal "supplements" that have not been submitted to stringent clinical trials may have detrimental health effects and their performance-enhancing properties may be over estimated. The heads of the IOC accredited laboratories stated their concern in October 1999 in a public statement:

"In spite of athletes' strict liability, governmental authorities are strongly advised to take measures to prohibit the sale, manufacture, and importation of unlicensed preparations of nutritional supplements containing prohibited substances whether labelled or not."

References

M. Donike, Steroid Profile in Cologne, Proceedings of the 10th Cologne Workshop on Dope Analysis, 7th to 12th June 1992, M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth eds, Sport und Buch Strausse Edition Sport, Koln (1993) p.47

W. W. Franke and B. Berendonk, Hormonal doping and androgenization of athletes : a secret program of the German Democratic Republic government, Doping in Sports symposium, Clinical Chemistry, 43:7, 1262-1279 (1997)

Internet advertisement : , p.3, 29 October 1998

IOC Medical Commission, List of banned substances and methods of doping - January 1999

L. L. Engel, J. Alexander and M. Wheeler, Urinary metabolites of administered 19-nortestosterone, J. Biol. Chem., 231, 159 (1958); D.F. Dimick, M. Heron, E.E. Baulieu and M.F. Jayle, Comparative study of the metabolic fate of testosterone, 17-methyltestosterone, 19-nortestosterone, 17-methyl-19-nortestosterone and 17-methyl-estr-5(10)-ene-17-ol-3-one in normal males, Clin. Chimica Acta, 6, 63 (1961); R.J. Ward, C.H.L. Shackleton and A.M. Lawson, Gas chromatographic-mass spectrometric methods for the detection and identification of anabolic steroid drugs, Br. J. Sports Med., 9(2), 93 (1975); C. Ayotte, D. Goudreau and A. Charlebois, Testing for natural and synthetic anabolic agents in human urine, J. Chromatogr., 687, 3 (1996)

W. Schanzer, Metabolism of anabolic androgenic steroids, Clin. Chem., 42 (7), 1001 (1996)

P. Kintz, V. Cirimele et B. Ludes, Norandrosténone et norétiocanolone... les métabolites révélateurs, Acta Clin. Belg. Suppl., 1, 68 (1999)

V.P. Uralets and P.A. Gillette, Over-the-Counter anabolic steroids 4-androsten-3,17-dione; 4-androsten-3,17-diol and 19-nor-4-androsten-3,17-dione: excretion studies in men, Recent advances in doping analysis (6), Proceedings of the Manfred Donike Workshop, 16th Cologne Workshop on Dope Analysis, March 1998, p. 147, idem, Communication 17th Cologne Workshop, March 1999;

W.J.J. Leunissen, Quantitative aspects of the determination of steroid profiles from urine by capillary gas chromatography, Ph.D. Thesis, Eindhoven, Netherlands (1979)

M. Donike, K.-R. Barwald, K. Klostermann, W. Schanzer and J. Zimmermann, Nachweis von exogenem Testosteron in Sport : Leistung und Gesundheit, H.

Heck, W. Hollmann, H. Liesen, R. Rost eds, Deutscher Ärzte Verlag Koln, (1983) 293; C. Ayotte, Evaluation of elevated testosterone/epitestosterone values in athletes' urine samples, IAF New Studies in Athletics, Vol. 12 (2-3) September 1997 (and references cited).

J.F. Lévesque and C. Ayotte, Proposed criteria for the detection of androstenedione oral administration, lecture, 17th Köln Workshop on Dope Analysis, Institut für Biochemie, Köln, Germany, March 1999; J.F. Lévesque, D. Goudreau and C. Ayotte, The administration of oral DHEA : the efficiency of steroid profiling, lecture, 17th Köln Workshop on Dope Analysis, Institut für Biochemie, Köln, Germany, March 1999; L.D. Bowers, Oral Dehydroepiandrosterone supplementation can increase the testosterone /epitestosterone ratio, Clin. Chem., 45 (2), 295 (1999); T.Z. Bosy, K.A. Moore and A. Poklis, The effect of oral dehydroepiandrosterone (DHEA) on the urine testosterone/epitestosterone (T/E) ratio in human male volunteers, J. Anal. Toxicol., 22 (6), 455 (1998); P. Van Eenoo, F. R. Delbeke, N. Desmet and P. De Backer, Excretion studies with 4-androstene-3,17-dione, Recent advances in doping analysis (6), Proceedings of the Manfred Donike Workshop, 16th Cologne Workshop on Dope Analysis, March 1998, p. 171; M. Garle and E. Palonek, Androstenedione: excretion studies from single and multiple dose experiments, Recent advances in doping analysis (6), Proceedings of the Manfred Donike Workshop, 16th Cologne Workshop on Dope Analysis, March 1998, p. 181

D.S. King, R.L. Sharp, M.D. Ubkovich, G.A. Brown, T.A. Reifenrath, N.L. Uhl and K.A. Parsons, Effects of oral androstenedione on serum testosterone and adaptations to resistance training in young men, A randomized controlled trial, J. Amer. Med. Ass., 281 (21), 2020 (1999); S. M. Sirrs and R. A. Bebb, DHEA: panacea or snake oil?, Can. Fam. Physician, 45, 1723 (1999)

M. Parasrampur, K. Schwartz and R. Petesch, Quality Control of Dehydroepiandrosterone Dietary Supplement Products, Letter, J. Amer. Med. Ass., 280 (8), 1565 (1998)

M. Garle, Huddinge IOC Accredited Laboratory, Huddinge University Hospital, personal communication to IOC Accredited Laboratories and to the IOC Medical Commission (1997); C. Ayotte and J. F. Lévesque, Montreal IOC Accredited Laboratory, INRS-Institut Armand-Frappier, personal communication to IOC Accredited Laboratories and to the IOC Medical Commission (1999); W. Schanzer, Cologne IOC Accredited Laboratory, Institut für Biochemie, Deutsche Sporthochschule Koln, report to the IAAF and the IOC, 1999