

Nutritional supplements - risk for inadvertent doping

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Abstract

Food additives are defined as substances that contain concentrated nutrients or other elements with a nutritional or physiological effect, alone or in combination, distributed in certain dosage forms designed to enrich diet. Food additive market is growing all over the World. Consumption of these products is growing in the last 2 decades because they claim many benefits for human health. In professional sport about 100% of athletes take nutritional supplements to enhance their performance, for quick recovery after training, to avoid fatigue or dehydration. Are food additives safe? This is a really current issue for sport medicine. In the last 17 years different studies have demonstrated that nutritional supplements could be a source of positive doping cases because some supplements contain prohibited non-labeled substances. If the food additives are not safe enough for athletes are they safe for the other customers all over the world? In this review we have discuss some non labeled substances found in nutritional supplements via different analytical techniques.

Key words: doping, sport, food additives, nutritional supplements

Introduction

Sports success is dependent primarily on genetic endowment in athletes with morphologic, psychologic, physiologic and metabolic traits specific to performance characteristics vital to their sport. Such genetically-endowed athletes must also receive optimal training to increase physical power, enhance mental strength, and provide a mechanical advantage. However, athletes often attempt to go beyond training and use substances and techniques, often referred to as ergogenics, in attempts to gain a competitive advantage. Pharmacological agents, such as anabolic steroids and amphetamines, have been used in the past, but such practices by athletes have led to the establishment of anti-doping legislation and effective testing protocols to help deter their use. Thus, many athletes have turned to various dietary strategies, including the use of various dietary supplements (sports supplements), which they presume to be effective, safe and legal [1].

In professional sports about 100% of athletes take nutritional supplements. Sport supplementation is not only used by professional sportsman but also by many physically-active individuals in different social categories. Many recent studies show that most people use nutritional supplements without any reflection of the risk/benefit relation. According to the European Community Directive of 2002 (Directive 2002/46/EC) and according to Bulgarian legislation, issued by the Ministry of Health 01.08.2005, food additives are defined as substances that contain concentrated nutrients or other elements with a nutritional or physiological effect, alone or in combination, distributed in certain dosage forms designed to enrich diet [2]. Dietary supplements contains a number of biological-

ly active substances, including protein products [3] and amino acids [2,3], vitamins [4-12], minerals, omega fatty acids, enzymes and many other substances, which has plant or animal origin. The fact that one food additive has a plant or animal origin, does not make it safe. [2] The use of nutritional supplements is not always safe. Even that the food additives market is growing the control of these products is minor or very often absent, the registration mode is extremely liberal all over the World. Nutritional supplements could be purchased via: pharmacies/drugstores, supermarkets, internet, club stores and also health-food stores. Many recent studies showed that some food supplements could contain impurities and others do not to contain ingredients listed on the label. World Anti Doping Agency (WADA) announced that some food supplements contain doping agents that are not declared on the label. If a professional athlete use contaminated nutritional supplements there is risk of inadvertent doping and also of many other negative side effects.

WADA

The World Anti-Doping Agency (WADA) was established in 1999. WADA is an international independent agency composed and funded equally by the sport movement and governments of the world. Its key activities include scientific research, education, development of anti-doping capacities, and monitoring of the World Anti Doping Code (Code) – the document harmonising anti-doping policies in all sports and all countries [13]. WADA could be described as guardian of the values and sport spirit inherent in the Code. WADA is based on the highest ethical standards and has developed many policies, procedures and practices that reflect justice, equity and integrity in sport. WADA supports many global researches to identify and detect doping substances and methods; exploring new models for enhanced detection. Every year WADA develops and maintains the annual List of Prohibited Substances and Methods. The use of doping substances not only violate the rules in sport but also is very harmful for humans health. The intake of prohibited substances could be associated with a wide range of adverse side effects as acne, gynecomastia, heart attacks and many other disorders. Most of people have only a crude knowledge about the serious side effects of doping substances. For this reason WADA has created many effective anti-doping education programmes for doping preventing for professional athletes and young people from different social categories and different professions.

Prohibited List

The Prohibited List (List) was first published in 1963 under the leadership of the International Olympic Committee. Since 2004, as mandated by the World Anti-Doping Code (Code), WADA is responsible for the preparation and publication of the List [14]. Every substance in the list is considered as doping substance. Every year WADA published an annual List. The use of a prohibited substance by professional athlete is possible only for medical reasons.

International convention against doping in sport

The development and entry into force of the Convention is a significant step in the fight against doping in sport. It represents the first time that governments around the world have collectively decided to focus their considerable powers and resources on tackling the doping problem. The Convention was needed to complement the actions taken by the sporting movement under the Code and to address particular limitations that have impeded progress. A series of measures for governments to avert or eliminate doping in sport and to foster cooperation are outlined. Specific actions include restricting the availability of prohibited substances and methods, targeting those that facilitate doping, funding doping controls, addressing problems associated with nutritional supplements and

promoting education as the central tool in prevention. All of these provisions, and those engaged in their implementation across the globe, share a single purpose - that future generations are able to enjoy and excel in doping-free sport [15]. On 1 February 2007, the International Convention against Doping in Sport entered into force. This landmark occasion signified the most successful international convention in the history of the United Nations Educational, Scientific and Cultural Organization (UNESCO) in terms of the speed of its development and entry into force. Important as this achievement might be, the enactment of the Convention is of greater significance to the future of sport. Never before have global anti-doping efforts been stronger and more focused on providing an honest and equitable playing environment for athletes. The Convention provides the hitherto absent legal framework with which all governments can address the growing prevalence and increasingly insidious use of performance-enhancing substances and methods in sport [15]. Anti-doping programmes, therefore, seek to preserve the essence of sport characterised by values such as honesty, fairness, respect, courage, commitment and solidarity [15].

Non labeled substances in nutritional supplements

Since 1999 several groups have analysed nutritional supplements with mass spectrometric methods (GC/MS, LC/MS/MS) for contaminations and adulterations with doping substances. These investigations showed that nutritional supplements contained prohibited stimulants as ephedrine, caffeine, methylenedioxymetamphetamine and sibutramine, which were not declared on the labels. An international study performed in 2001 and 2002 on 634 nutritional supplements that were purchased in 13 different countries showed that about 15% of the nonhormonal nutritional supplements were contaminated with anabolic-androgenic steroids (mainly prohormones). Since 2002, also products intentionally faked with high amounts of 'classic' anabolic steroids such as metandienone, stanozolol, boldenone, dehydrochloromethyl-testosterone, oxandrolone etc. have been detected on the nutritional supplement market. These anabolic steroids were not declared on the labels either. The sources of these anabolic steroids are probably Chinese pharmaceutical companies, which sell bulk material of anabolic steroids. In 2005 vitamin C, multivitamin and magnesium tablets were confiscated, which contained cross-contaminations of stanozolol and metandienone. Since 2002 new 'designer' steroids such as prostanazol, methasterone, androstatrienedione etc. have been offered on the nutritional supplement market. In the near future also cross-contaminations with these steroids are expected. Recently a nutritional supplement for weight loss was found to contain the beta2-agonist clenbuterol. The application of such nutritional supplements is connected with a high risk of inadvertent doping cases and a health risk. For the detection of new 'designer' steroids in nutritional supplements, mass spectrometric strategies (GC/MS, LC/MS/MS) are very appropriate [16].

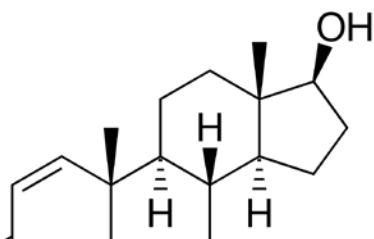
N. Baume, N. Mahler, M. Kamber, P. Mangin, M. Saugy made one of the biggest study for determination the real compositions of 103 dietary supplements. They have screened the supplements for contaminations with major anabolic steroid parent compounds, stimulants and traces of testosterone, nandrolone and their precursors. The analysed products were ordered at the end of 2002 on different web sites. These products were classified into the following four categories: 37 supplements in the prohormones category, 42 creatine, 12 "mental enhancers" and 12 branched chain amino acids (BCAAs). It has to be noted that prohormones are banned by the International Olympic Council (IOC)/WADA, either directly by name or under the umbrella of being a substance related to anabolic-androgenic steroids. The MS detector used was a 5971 coupled to a 5890 GC and a 7873 injector acquired from Agilent. All analyses were performed with a DB-XLB column (15 m, 0.25 mm, 0.25 μ m, J&W Scientific, Folsom, CA, USA). The injection port was maintained at 280°C and oven conditions started at 150°C with an initial time of 1 min. The ramp consisted of 10°C/min to 270°C. All injections were splitless. The transfer line was maintained at 310°C. The

analyses were performed in a single ion-monitoring mode. The number of mislabeled supplements represents 18% of the 103 products analyzed. Fourteen of these supplements were in the prohormones, three in the “mental enhancers” and one in the creatine category. The “mental enhancer 8” product was in this category because it was mainly composed of ephedrine, caffeine and synephrin but it also contained androstenediol. The most frequent contaminant was the testosterone parent molecule and the amounts were smaller in the creatine and “mental enhancers” than in the prohormone products [17,18]. The results of this study show that 20% of analysed food supplements were contaminated. Martello et al. have investigated 64 nutritional supplements for contamination. They have performed LC-MS/MS analyses in selected reaction monitoring (SRM) on an ion-trap equipped with an atmospheric pressure chemical ionization (APCI) probe operating in positive-ion mode. A total of 12.5% of the nutritional supplements analysed contained banned substances not declared on the label (anabolic steroids and ephedrine) [19]. The use of dietary supplements is widespread not only in sport but among many people from different ages, cultures, social categories. One of the most popular category dietary supplements is for weight loss. Obesity and overweight are considered as a high-income problem [20]. That is why nutritional supplements for treatment of overweight are very popular. In pharmacies and drug stores are available many food supplements that are recommended for a healthy lifestyle-enhancing tone the body and reduction of body fat [21]. People who purchase these products not always are well informed about the potential risks. Many researchers have announced for contaminated food supplements with sildenafil, tadalafil, vardenafil [22], sibutramine (12), ephedrine, clenbuterol [16].

Several precursors of testosterone and nandrolone introduced on the nutritional supplement market as performance enhancing drugs are banned in sports. Until now they are legally sold without a prescription in the US. Results of excretion studies with related compounds including 7-keto-DHEA and 1-androstenediol are presented. The main metabolites of 7-keto-DHEA are 7-hydroxylated compounds. The commercial 1-androstenediol preparation was contaminated with several other anabolic steroids. Oxidation of 1-androstenediol to 1-androstenedione seems to be the major renal metabolic pathway. Additionally contaminated nutritional supplements containing banned substances not indicated on the label were administered. The results of the excretion studies indicate that after the intake of amounts substantially lower than the recommended dose athletes can fail a doping test for periods up to 120 h [24].

New analogs of androgens that had never been available as approved drugs are marketed as “dietary supplement”. They are mainly advertised to promote muscle mass and are considered by the governmental authorities in various countries, as well as by the World Anti-doping Agency for sport, as being pharmacologically and/or chemically related to anabolic steroids [15]. Recently so-called prohormones of 1-testosterone (17 β -hydroxy-5 α -androst-1-en-3-one), namely 1-androstenedione (5 α -androst-1-ene-3,17-dione, 1-AD) and 1-androstenediol (5 α -androst-1-ene-3 β ,17 β -diol) are advertised as food supplements.(5) M.K. Parr and W. Schanzer (German Sport University, Cologne, Germany) have made an important research about the urinary metabolism of 1-androstenedione. The main metabolites were monitored following administration of a single oral dose of 50 mg to six male volunteers. 1-Testosterone, 3 α -hydroxy-5 α -androst-1-en-17-one (1-DHA), 3 β -hydroxy-5 α -androst-1-en-17-one (1-DHEA), 5 α -androst-1-ene-3 α ,17 β - and -3 β ,17 β -diol were detected. Additionally, common urinary steroid profile ratios were altered after the administration. Especially the ratios of androsterone/etiocholanolone and 5 α -/5 β -androstane- 3 α ,17 β -diol and the excretion rate of androsterone were increased for about 2 days post administration [15]. The authors of the research conclude that 1-testosterone (fig 1) is an active hormone. The inclusion of this substance in food supplements hides many risks for customers health.

Fig 1. 1-testosterone



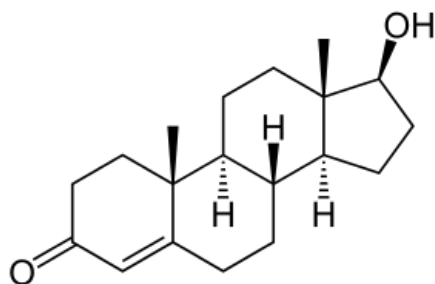


Figure 2. Testosterone

Conclusion

Via different analytical techniques like LC-MS/MS, GC/MS numerous studies have demonstrated that high percent nutritional supplements could contain pro hormones, anabolic steroids, ephedrine or other substances not declared on the label. For the world of professional sport this is a serious problem because it expose athletes of risk for inadvertent doping. For other customers this hides many potential side effects and health disorders. The quality control of food additives must be raised to assure the safety of these products.

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