



## Use of nutritional supplements contaminated with banned doping substances by recreational adolescent athletes in Athens, Greece



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### ABSTRACT

Although the use of nutritional supplements by adult athletes has been extensively studied, information on supplements consumption by adolescent athletes is still limited. The present study reports on the use of nutritional supplements contaminated with banned doping substances among 170 recreational adolescent athletes from eleven, randomly selected, gym centres, in Athens, Greece. Nutritional supplements consumption was reported by almost 60% of the study population, with proteins/amino acids and vitamins being the most popular. Nine per cent of the users were found to consume nutritional supplements contaminated with anabolic steroids, prohormones, selective androgen receptor modulators (SARMs) and aromatase inhibitors, all pharmacological substances with endocrine modulating properties not stated on the label. None of these individuals had previously consulted a physician or a nutritionist. A representative sample (ca 15%) of the protein/aminoacids and creatine preparations used by the study population were also tested and found free from doping substances. The majority (63%) of adolescents purchased products from the internet. In conclusion, exercising adolescents can have easy access to contaminated nutritional supplements and “black market” products, which could constitute a risk for public health. Low level of awareness and low involvement of medical care professionals among recreational adolescent athletes is also observed.

### 1. Introduction

Nutritional supplements are freely marketed to teenagers through the internet, without any legal restrictions and more importantly without warnings for possible risks for their health (Herriman et al., 2017).

In Greece, as elsewhere, the intake of supplements among people exercising in gyms is usually self-prescribed (Goston and Correia, 2010) and free of legal restrictions. Relevant information and recommendations are commonly obtained from fellow athletes, coaches, friends, or family (Froiland et al., 2004; Tian et al., 2009), a fact that is extremely risky, especially for young people. For every new brand of nutritional supplement available in the Greek market a notification file has to be

deposited to the National Organization for Medicines with appropriate labeling on the product in the Greek language (Directive, 2000/13/EC/20–03–00). No additional laboratory analysis of the declared composition and no preventive sampling controls are required.

Adulteration of nutritional supplements with hindered banned substances has been identified and reported (Mathews, 2018; Martínez-Sanz et al., 2017; Kioukia-Fougia et al., 2017; Watson et al., 2009). Such contaminated nutritional supplements could be proven problematic both to professional athletes and the general public; professional athletes could be accused for doping violation (Abbate et al., 2015; Geyer et al., 2000, 2004, 2008), and the general population could be non-intentionally exposed to substances with pharmacological properties. It is important to note that the use and possession of doping

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substances without medical prescription is prohibited for the general population in Greece under Hellenic Law 2725/1999 as amended and in force.

The present study reports on the use of nutritional supplements contaminated with banned doping substances by recreational adolescent athletes in Athens, Greece. At the same time data on the level of consumption of nutritional supplements in a sample of Greek teenagers who worked out regularly in Athens gyms is provided.

## 2. Methods

Nutritional supplements surveyed in this study are defined according to the Directive 2002/46/EC and in agreement with previous reports (Petroczi et al., 2007), as concentrated sources of nutrients or other substances with a nutritional or physiological effect, indicated to supplement the normal diet. They are marketed in dose form and do not bear health or medicinal claims.

Among 1100 individuals, regularly exercising in 11 gym centres randomly selected in Athens, Greece, 170 adolescent athletes replied to a validated, self-administered questionnaire regarding the use of nutritional supplements (J. Cohen, 1988; Tsitsimpikou et al., 2011). The term “nutritional supplement” was not explicitly defined for the responders, but they were asked to answer if they used supplements in addition to what they considered their diet. The questionnaire was accompanied with an explanatory opening page, where it was clearly stated that the results of the survey were to be published. The return of the completed questionnaire was considered as signing a written consent by the study population. The use of nutritional supplements including carbohydrates, proteins/amino acids, vitamins, minerals, creatine, carnitine, caffeine, herbal preparations, enzymes/coenzymes, homeopathic preparations and anabolic agents was sought in one multiple-choice question. The use of isotonic drinks/electrolytes was addressed in a different question.

Preparations declared as anabolic agents by the users were submitted to analysis for anabolic steroids prohibited for doping control. Responders using anabolic agents were requested in writing to provide the commercial preparations that they were consuming and leave them at the reception desk of their gym, in order to have them analysed for their content. In addition, they were requested to provide the protein/amino acids and creatine preparations they were using to be submitted also to the same laboratory analysis, as these products are more prone to be contaminated with anabolic agents due to their mode of action. All users complied with both requests. Both concentrated methanolic extracts of the preparations and the derivatized material after addition to the dry methanolic extracts of 80 µl of MSTFA and incubating at 80 °C for 45min, were screened for the presence of endogenous/synthetic anabolic steroids. Both liquid and gas chromatography – tandem mass spectrometry were used as previously described in order to screen for substances prohibited in doping control with the exception of peptide hormones. (Tsitsimpikou et al., 2011; Krug et al., 2014). The identification was then based on comparison of the obtained full scan spectra with spectra in the NIST library (National Institute of Science and Technology, Gaithersburg, MD, USA), retention time and literature data. At the same time, a positive control sample was used containing stanozolol, nandrolone, oral turinabol, methandienone, ostarine, LGD-4033 prepared from the respective pharmaceutical preparations extracted with methanol at a concentration around 1 ppm.

The Research Committee of the Department of Medical Laboratories, Technological Educational Institute of Athens, provided approval for the conduct of the study and distribution of the questionnaire, as part of the dissertation thesis of the student N. Christostomou (AM 03/164).

Statistical analysis was performed using the Statistical Package for Social Science version 22.0 (SPSS Inc, Chicago, IL, USA). Descriptive data were calculated as frequencies and percentages. Chi-square ( $\chi^2$ ) tests were computed to reveal meaningful associations between

**Table 1**

Prevalence of the type of nutritional supplements used by study participants.

Type of supplement	Nutritional Supplements Users (N = 100) <sup>a</sup>
Proteins/Aminoacids	84
Vitamins	67
Minerals/Trace elements	45
Carnitine	22
Carbohydrates	35
Creatine	78
Herbal preparations	14
Other	17
Anabolic agents	12
Homoeopathic preparations	3
Enzymes/coenzymes	2
Isotonic drinks/electrolytes	80 (47.1) <sup>b</sup>
Combined Use	75
<b>TOTAL</b>	<b>100 (58.8)</b>

<sup>a</sup> Numbers in parentheses represent percentages.

<sup>b</sup> Percentage is expressed on the whole study population (n = 170) (see Methods).

supplements use and the categorical study variables (gender, level of education etc) and Pearson correlation was performed for continuous variables (i.e. age, exercise years etc). Significance was set at  $p \leq 0.05$ .

## 3. Results

The study population consisted of 110 males ( $16.2 \pm 1.5$  years old) and 60 females ( $17.8 \pm 0.11$  years old), who were regularly exercising at private gym centers (81.2% 2 times per week) for almost 1 h per training session (70.6%).

Almost 60% of the adolescent recreational athletes that participated in this study reported use of supplements, with proteins/amino acids and vitamins being the most popular (Table 1). Isotonic drinks were consumed by almost half of the responders, with no statistical significance for the type of exercise (aerobic, anaerobic etc) practised. Among users, 42% declared use of more than one type of supplement and 85% used them more than 3 times per week. The frequency of supplement use was increased in those who trained for professional sport clubs (66%) and combined aerobic and resistance exercise (52%). Sex ( $\chi^2 = 17.1$ ,  $df = 1$ ,  $p < 0.001$ ), type of exercise ( $\chi^2 = 14.7$ ,  $df = 3$ ,  $p = 0.01$ ) and training frequency ( $\chi^2 = 9.3$ ,  $df = 2$ ,  $p = 0.024$ ) were associated with the adolescents' decision to consume nutritional supplements.

The majority of users (63%) purchased products from the internet, 15% of them from a pharmacy store and 16% asked their supervisors at the gym to provide them with nutritional supplements. Only 8% of them had consulted a physician or a nutritionist. Other sources of supplements recommendations included their personal trainer (34%), other co-athletes and friends (30%), while 25% of the users had decided on their own. Only 2 responders had sought advice from their family.

Among the 170 adolescent recreational athletes that responded to the present survey, no one declared being aware of the relevant legislation, while the vast majority of them (85.6%) did not check the labelling of the chosen product. Half of the responders wouldn't know where to refer to in order to have the consumed products analysed.

Twelve male adolescents,  $17.0 \pm 0.8$  years-old, declared supplementation with anabolic agents. From them, 9 were practising a combination of anaerobic training and martial arts and 3 were training also for professional sport clubs and combined aerobic and resistance exercise. All of them were training 3–4 times a week for 1½-2 h per training session. All these products were subjected to laboratory analysis and the results are summarised in Table 2. From these anabolic supplements, only 3 were preparations not prohibited under doping control legislation, namely the plant extract *Tribulus terrestris* (TT),

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