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

Konstantinos Tarouhas\textsuperscript{a}, Nasia Kioukia–Fougia\textsuperscript{b}, Petros Papalexis\textsuperscript{c}, Aristidis Tsatsakis\textsuperscript{d}, Dimitrios Kouretas\textsuperscript{e}, Flora Bacopoulou\textsuperscript{f}, Christina Tsitsimpikou\textsuperscript{g,*}

\textsuperscript{a} Department of Cardiology, University Hospital of Larissa, Messourlo, Larissa 41110, Greece
\textsuperscript{b} Doping Control Laboratory of Athens, OAKA “Sp.Louis”, Kifissias 37, Maroussi 15223, Greece
\textsuperscript{c} Spiliopoulio General Hospital “Agia Eleni”, 21st Dim. Soutsou Str., Ampelokipi, Athens 11521, Greece
\textsuperscript{d} Department of Forensic Sciences and Toxicology, Faculty of Medicine, University of Crete, Heraklion 71003, Greece
\textsuperscript{e} Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece
\textsuperscript{f} Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care, First Department of Pediatrics, National and Kapodistrian University of Athens, Aghia Sophia Children’s Hospital, Athens 11527, Greece
\textsuperscript{g} General Chemical State Laboratory of Greece, 16, An. Tsocha Str., Athens 11521, Greece

A R T I C L E   I N F O

Keywords:
Minors
Supplementation
Exercise
Doping substances
Contamination

A B S T R A C T

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 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; Martinez-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
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 (Petrozzi 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 45 min, 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 stanazolol, 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. Chrisostomou (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 nutritional 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 ± 1.5 years old) and 60 females (17.8 ± 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 ($x^2 = 17.1, df = 1, p < 0.001$), type of exercise ($x^2 = 14.7, df = 3, p = 0.01$) and training frequency ($x^2 = 9.3, df = 2, p = 0.024$) were associated with the adolescents’ decision to consume nutritional supplements.

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 ($x^2 = 17.1, df = 1, p < 0.001$), type of exercise ($x^2 = 14.7, df = 3, p = 0.01$) and training frequency ($x^2 = 9.3, df = 2, p = 0.024$) 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 ± 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),

### Table 1

<table>
<thead>
<tr>
<th>Type of supplement</th>
<th>Nutritional Supplements Users (N = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins/Aminoacids</td>
<td>84</td>
</tr>
<tr>
<td>Vitamins</td>
<td>67</td>
</tr>
<tr>
<td>Minerals/Trace elements</td>
<td>45</td>
</tr>
<tr>
<td>Carnitine</td>
<td>22</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>55</td>
</tr>
<tr>
<td>Creatine</td>
<td>78</td>
</tr>
<tr>
<td>Herbal preparations</td>
<td>14</td>
</tr>
<tr>
<td>Other</td>
<td>17</td>
</tr>
<tr>
<td>Anabolic agents</td>
<td>12</td>
</tr>
<tr>
<td>Homoeopathic preparations</td>
<td>3</td>
</tr>
<tr>
<td>Enzymes/Coenzymes</td>
<td>2</td>
</tr>
<tr>
<td>Isotonic drinks/electrolytes</td>
<td>80 (47.1)</td>
</tr>
</tbody>
</table>

| Combined Use                       | 75                                     |

**TOTAL** 100 (58.8)

* Numbers in parentheses represent percentages.

b Percentage is expressed on the whole study population (n = 170) (see Methods).
which were found free of any banned contamination. Another plant extract (Butea superba) actually contained the prohormone dihydrotestosterone (DHT) and carried a misleading label (doping free). One other preparation was actually creatine containing the selective androgen receptor modulator (SARM) LGD-4033, purchased from the internet. Four individuals provided us with small food bags containing pink (stanozolol) and yellow-white powders (nandrolone) declaring them as natural testosterone boosters. Laboratory analysis revealed the presence of synthetic anabolic steroids (i.e. stanozolol and nandrolone) in a matrix full of amino acids. The protein/aminoacids (14% of the total preparations used by the study population) and creatine preparations (15% of the total preparations used by the study population) tested, showed no adverse analytical findings regarding the presence of banned substances.

In total, 9% of the adolescent recreational athletes of the study population were exposed through the use of nutritional supplements to anabolic steroids, prohormones, SARMs and aromatase inhibitors, all pharmaceuticals, and other dangerous chemicals. Health side-effects of anabolic steroids are well-documented both in humans and in animal models (Darke et al., 2014; Kanayama et al., 2008; Germanakis et al., 2013; Vasilaki et al., 2016; Tsitsimpikou et al., 2016; Kara et al., 2017). In addition, various pharmaceutical preparations used for doping purposes are reported to be contaminated or of poor quality, such as GH-labelled injection vials without any pharmaceutically active content, unpurified long-R(3) -IGF-1 and unlabelled ampoules containing the SARM Andarine (S-4) (Kohler et al., 2010). Energy drinks and adulterated weight-loss products have been associated with high risk in adolescents (Smolinske, 2017). Several incidents reported in the literature involve cross-contamination of nutritional supplements with doping substances, even the growth hormone releasing peptide-2 (GHRP-2), which were unintentionally provided to professional athletes (Abbate et al., 2015; Geyer et al., 2000, 2008; Watson et al., 2009; Kohler et al., 2010). Creatine products have also been reported contaminated with prohormones not declared on the label (Baume et al., 2006), while T3 extracts are reported to contain androgenic anabolic steroids (4-androstene-3,17-dion, 4-androstene-3,17β-diol, 5-androstene-3,17β-diol, 19-nor-4-androstene-3,17-dion and 19-nor-4-androstene-3,17-diol) not listed on the label (Geyer et al., 2000). Previous studies have indicated the presence of banned substances in nutritional supplements due to impurities carried over from pharmaceutical bulk production (Gabriels and Lambert, 2013). Similarly, the level of contamination detected in five of the anabolic agents screened in the present study is calculated approximately around several mg per kg of the analyzed nutritional supplement. The rates of contamination of nutritional supplements commercially available range between 12 and 58% (Martínez-Sanz et al., 2017).

Nowadays athletes and exercising individuals in general, are prone to doping due to various psychological factors (Nicholls et al., 2017; Mattila et al., 2010; Dwyer et al., 2013). In the present study, 9% of the adolescent recreational athletes were using supplements containing doping substances. The users were consciously using anabolic agents, to increase protein synthesis and muscle building (Whitehouse and Lawlis, 2017), but in most cases the products they were using were advertised as natural boosters. Both professional and recreational athletes are using ‘black market’ products to enhance their performance (Kohler et al., 2010). This was true in our study, too, regarding the detected synthetic anabolic steroids, used in bulk preparations.

4. Discussion

The extensive use of nutritional supplements among adolescents and other recreational athletes derives from their intrinsic need to improve their body image or to face various medical conditions in an alternative way to classic medicine, without considering possible risks to their general health (Austin et al., 2017; Biggs et al., 2017; O’Dea, 2003; Alves and Lima, 2009). Use of supplements by children is not usually recommended by a health care professional and their contribution to health promotion is often questionable (Bailey et al., 2013).

In our study almost 60% of adolescents regularly exercising in gyms used nutritional supplements. The number is rather high compared to Italian and Australian teenagers and the results from the NHANES 1999–2000 study, who targeted adolescents in general without any special involvement with sports (Briefel and Johnson, 2004; del Balzo et al., 2014; Gallagher et al., 2014; Dorsch and Bell, 2005). Increased intake of nutritional supplements may disturb body homeostasis (Troppmann et al., 2002). It is suggested that uncontrolled use of nutritional supplements by youngsters could end up in abuse with unpredictable effects (Biggs et al., 2017).

Nutritional supplements sold for various reasons may have unexpected consequences for public health (Harris et al., 2017). Nutritional supplements used for weight loss, muscle building and even sexual function enhancement are not medically recommended and have been shown to be ineffective in many cases and pose serious health risks to consumers due to adulteration with banned substances, prescription pharmaceuticals, and other dangerous chemicals. Health side-effects of anabolic steroids are well-documented both in humans and in animal models (Darke et al., 2014; Kanayama et al., 2008; Germanakis et al., 2013; Vasilaki et al., 2016; Tsitsimpikou et al., 2016; Kara et al., 2017). In addition, various pharmaceutical preparations used for doping purposes are reported to be contaminated or of poor quality, such as GH-labelled injection vials without any pharmaceutically active content, unpurified long-R(3) -IGF-1 and unlabelled ampoules containing the SARM Andarine (S-4) (Kohler et al., 2010). Energy drinks and adulterated weight-loss products have been associated with high risk in adolescents (Smolinske, 2017). Several incidents reported in the literature involve cross-contamination of nutritional supplements with doping substances, even the growth hormone releasing peptide-2 (GHRP-2), which were unintentionally provided to professional athletes (Abbate et al., 2015; Geyer et al., 2000, 2008; Watson et al., 2009; Kohler et al., 2010). Creatine products have also been reported contaminated with prohormones not declared on the label (Baume et al., 2006), while T3 extracts are reported to contain androgenic anabolic steroids (4-androstene-3,17-dion, 4-androstene-3,17β-diol, 5-androstene-3,17β-diol, 19-nor-4-androstene-3,17-dion and 19-nor-4-androstene-3,17-diol) not listed on the label (Geyer et al., 2000). Previous studies have indicated the presence of banned substances in nutritional supplements due to impurities carried over from pharmaceutical bulk production (Gabriels and Lambert, 2013). Similarly, the level of contamination detected in five of the anabolic agents screened in the present study is calculated approximately around several mg per kg of the analyzed nutritional supplement. The rates of contamination of nutritional supplements commercially available range between 12 and 58% (Martínez-Sanz et al., 2017).

Nowadays athletes and exercising individuals in general, are prone to doping due to various psychological factors (Nicholls et al., 2017; Mattila et al., 2010; Dwyer et al., 2013). In the present study, 9% of the adolescent recreational athletes were using supplements containing doping substances. The users were consciously using anabolic agents, to increase protein synthesis and muscle building (Whitehouse and Lawlis, 2017), but in most cases the products they were using were advertised as natural boosters. Both professional and recreational athletes are using ‘black market’ products to enhance their performance (Kohler et al., 2010). This was true in our study, too, regarding the detected synthetic anabolic steroids, used in bulk preparations.

5. Conclusions

Contaminated nutritional supplements and “black market” products that adolescents can easily be supplied with, could constitute a risk for public health (Ianosi et al., 2016; Vari et al., 2017). Despite withdrawals of nutritional supplements when found contaminated after relevant market alarms (Cohen et al., 2014a; P. A. Cohen et al., 2014b),
our results point to loose controls and market surveillance enforced by the authorities. Special provision should be taken for retailers providing contaminated nutritional supplements to minor consumers (Herriman et al., 2017). Nutritionists should take into account non-intentional doping through the use of nutritional supplements, when establishing a supplementation program. In addition, awareness of new products on the black market and in nutritional supplements is of utmost importance for laboratories to develop detection methods accordingly and screen for new substances as early as possible.

Authors’ contribution

The study was designed by CT, KT and FB; data were collected and analyzed by NF-K, PP, AT, and KT; data interpretation and manuscript preparation were undertaken by KT, NF-K, CT and FB. All authors approved the final version of the paper.

Conflicts of interest

The authors declare no competing interests and no funding has been received for this study.

Acknowledgments

Special acknowledgments to Dr Fotini Vasiliaki for the laboratory analysis of the nutritional supplements at the Laboratory of Forensic Sciences and Toxicology in the University of Crete.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2018.03.043.

References