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RECENT ADVANCES
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Some Good News from the Field of Nutritional Supplements
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Some good news from the field of nutritional supplements

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Abstract

Since 1999 it is known that nutritional supplements may contain anabolic androgenic steroids, which are not declared on the label. The consumption of such nutritional supplements may lead to positive doping results. To seek for low risk alternatives for athletes, products of the “Rote Liste”, the index of therapeutics of the German pharmaceutical industry, were analysed. 201 therapeutics were selected containing similar ingredients as nutritional supplements like vitamins, minerals, herbal products etc. All products were tested with GC/MS for 9 different prohormones and testosterone, nandrolone and metandienone.

Results: In the 201 products no anabolic androgenic steroids were detected. These results show that the probability of a contamination with anabolic androgenic steroids in products from the German pharmaceutical industry is very low. Products of the “Rote Liste” may be used by athletes as low risk alternatives to nutritional supplements.

Introduction

Several recent studies have shown that nutritional supplements may contain anabolic androgenic steroids which are not declared on the label [1-6]. The consumption of such supplements may lead to positive doping results. To seek for low risk alternatives for athletes products of the index of therapeutics of the German pharmaceutical industry, called “Rote Liste”, should be tested for different prohormones and testosterone, nandrolone and metandienone as described before [7]. Special focus is driven to products which are recommended by medical attendants and sports physicians, used by athletes or mentioned in the list of permitted substances of the German NADA [8].

Experimental

Questionnaire

During the doping control procedure athletes were asked for medicaments applied during the last hours. For the entire investigation the athletes from soccer, athletics, swimming, cycling and weightlifting were accounted (tab. 1). The declarations were analysed for the use of analogues of nutritional supplements listed in the index of therapeutics of the German pharmaceutical industry called “Rote Liste”.

Additionally the medical attendants and sports physicians of German premier league soccer clubs were asked for their recommended preparations of the “Rote Liste”.

The list of permitted substances of the German NADA [8] was also evaluated for analogues of nutritional supplements from the “Rote Liste”.

tab. 1: Number of Doping control forms from athletes of different sports which were evaluated for the declaration of used medicaments and nutritional supplements (routine doping control 2002, total number 5321)

sport	number	sport	number
soccer national	415	soccer international	2229
athletics national	753	athletics international	287
swimming national	309	swimming international	203
cycling national	499	cycling international	201
weightlifting national	52	weightlifting international	373
total	2028		3293

Supplements

187 therapeutics containing similar ingredients as nutritional supplements like vitamins, minerals, herbal products etc. were selected from the “Rote Liste”. Thereby products from the list of permitted substances of the German NADA [8] as well as products brought up in the questionnaire were favoured. Further 14 nutritional supplements from pharmaceutical companies were included in the investigation.

The products were purchased from February until July 2003 in a German pharmacy and analysed for their prohormone content.

tab. 2: Pharmaceutical formulation of the analysed products

formulation	number	formulation	number
tablets	64	fizzy tablets	16
capsules	40	lozenges	10
injection solutions	29	powders and granulate material	9
syrops, juices and drops	24	infusion solutions	8

tab. 3: Contents of the analysed products

contents	number	contents	number
minerals	93	carbohydrates	6
vitamins	84	enzymes	4
herbal products	29	carnitine	3
amino acids	9	miscellaneous	13

Chemicals

DHEA, Testosterone, 5α -Androstane- $3\beta,17\beta$ -diol and 19-Nor-4-androstene-3,17-dione were purchased from Sigma (St. Louis, USA), 5-Androstene- $3\beta,17\beta$ -diol and 19-Nortestosterone from Serva (Heidelberg, Germany). 4-Androstene- $3\beta,17\beta$ -diol, 19-Nor-5-androstene- $3\beta,17\beta$ -diol, Androstadienedione and d_3 -19-Noretiocholanolone were obtained from Steraloids (Wilton, USA) and 4-Androstene-3,17-dion from Schering (Berlin, Germany). 19-Nor-4-androsten- $3\beta,17\beta$ -diol the internal standards d_3 -Testosteron and d_3 -Nortestosteron and the 1-N,N-diisopropylamino-n-alkanes were synthesised in our laboratory. N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) was purchased from Chem. Fabrik Karl Bucher (Waldstetten, Germany). Other reagents and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany).

Sample preparation

The samples were analysed as described earlier [7]. In brief, the samples (1 g) were extracted with methanol after the addition of a mixture of deuterated internal standards (d_3 -Nortestosterone, d_3 -Testosterone and d_3 -Noretiocholanolone). The supernatant was evaporated to dryness. The residue is re-dissolved in KOH (0.1 M, pH >12) and extracted with n-pentane. The n-pentane layer is re-extracted with MeOH (95% with H₂O). The

methanolic layer is evaporated to dryness after the addition of a mixture of 1-N,N-diisopropylamino-n-alkanes (DIPA 14-23, 1 µg each) and the residue derivatised with TMIS reagent (MSTFA/ ammonium iodide/ ethanethiol, 1000:2:3, v:w:v).

Aberrant, for oily samples an aliquot of 500 ml of the supernatant after the first methanol extraction was further treated as described above. Deviant the final methanolic extract is washed with n-pentane prior to evaporation.

For the analysis of liquid samples for infusion 100 ml were spiked with the above mentioned internal standards and the pH was adjusted to >12. The mixture was extracted twice with 30 ml of n-pentane. The n-pentane extracts were washed with 30 ml of water each and evaporated to dryness after combining and addition of the DIPA solution. The residue was derivatised with 100 µl of TMIS reagent.

Instrumentation

For the analyses of the samples the GC-MS was operated with the following parameters:

GC-MS:	GC: Hewlett Packard (HP) 6890, MSD: HP 5973
Injection param.:	Volume: 2 µl, Temp.: 300°C
Column:	HP 5 MS; 16.5 m; 0.25 mm i.d.; 0.25 µm film thickness
Carrier gas:	Helium, splitless, head pressure 13 psi
Oven temp.:	100°C with 40°C/min to 190°C, with 5°C/min to 240°C, with 40°C/min to 320°C, 3 min hold
Ionisation:	70 eV, electron impact (EI)
Data aqu.:	SIM

Results and Discussion

Questionnaire

The declarations of athletes on routine doping control forms were evaluated for the use of analogues of nutritional supplements listed in the index of therapeutics of the German pharmaceutical industry called “Rote Liste”. The products mentioned are listed in tab. 4 sorted by their contents.

The preparations recommended by the medical attendants and sports physicians of German premier league soccer clubs are shown in tab. 5.

In addition the frequencies of use of analogues of nutritional supplements from the „Rote Liste“ are illustrated in fig. 1.

The list of permitted substances of the German NADA [8] contains several analogues of nutritional supplements listed especially in section 21 – vitamins, minerals and trace elements. These products are listed in tab. 6.

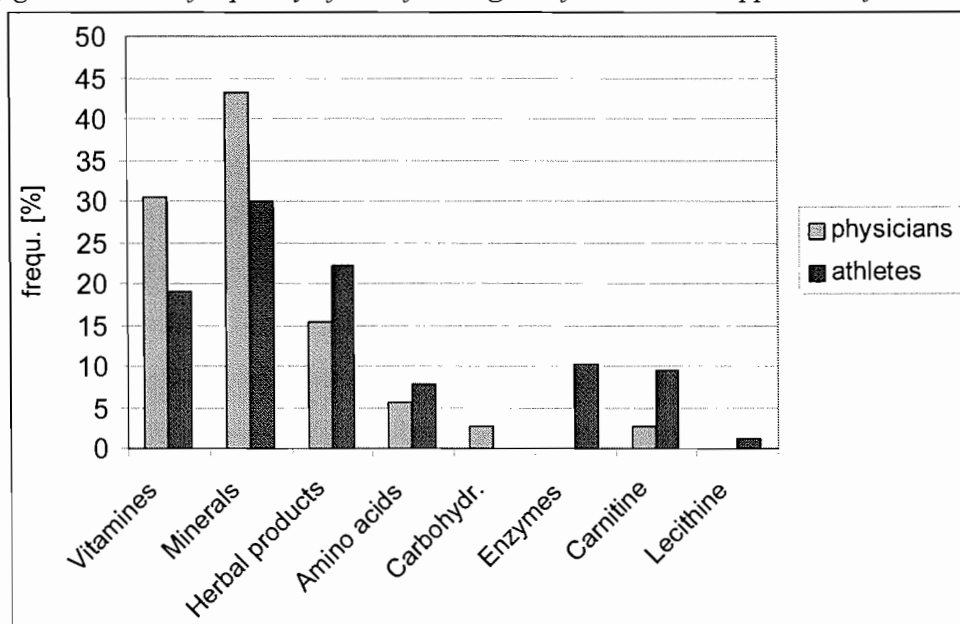
tab. 4: Products of the „Rote Liste“ declared by athletes on doping control forms

contents	number		contents	number
minerals	128		lecithine	5
vitamins	81		enzymes	44
herbal products	95		carnitine	40
amino acids	33			

tab. 5: Products of the „Rote Liste“ recommended by sports physicians from premier league soccer teams, olympic summer and winter teams and olympic centers

contents	number		contents	number
minerals	31		amino acids	4
vitamins	22		carbohydrate solutions	2
herbal products	11		carnitine	2

fig. 1: Relative frequency of use of analogues of nutritional supplements from the „Rote Liste“



tab. 6: List of permitted substances (NADA Germany 2004 [8])
Vitamins, minerals and trace elements

21. Vitamin-, Mineralstoff- und Spurenelementpräparate		
anabol-loges	frubiase calcium T	Neuro-Effekton B
Aquo-Cytobion 500	Hexobion	Phosetamin
Betabion	Inzelloval	Polybion
Biomagnesium	Inzolen	Rekawan
BVK Roche	Kaliner	selenase
Calcium	Kendural	Spondyvit
Cebion	Löscalcon	Tridin Rp
Cetebe	Lösferron	Tromcardin
Curazink	Macalvit	Trophicard
Cytobion	magnerot	Unizink
Elotrans	Magnesiocard	Vigantol
Eryfer	Magnesium-Diasporal	Vigantolekten
Eunova	Magnesium Verla	Vitamin A+E-Hevert
E-Vitamin-ratiopharm	Magnetrans	Vitamin B1-ratiopharm
Ferrelecit	Medivitan N	Vitamin B6-ratiopharm
ferro sanol	Mg 5 - Longoral	Vitamin B12-ratiopharm
Folgamma	Multibionta	Vitasprint B12
Folsan	Multivitamin	Zentramin Bastian N
frubiase calcium forte	Natabec	Zinkorotat

Supplement analyses

In the 201 products no anabolic androgenic steroids were detected. These results show that the probability of a contamination with anabolic androgenic steroids in products from the German pharmaceutical industry is very low. Products of the "Rote Liste" may be used by athletes as low risk alternatives to nutritional supplements.

Summary

Analogues to nutritional supplements of the German "Rote Liste" are "low risk" supplements. Another source of „low risk“ supplements are products of the database of the Olympic Centre Cologne (<http://www.osp-koeln.de>). This database consists of list of companies, which perform a quality check for prohormones and/or which can guarantee that they have no contact with prohormones in the production and transportation processes.

Athletes are strongly advised to take only „low risk“ supplements to minimize unintentional doping cases.

Acknowledgements

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