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## **The Analysis of “Non-Hormonal” Nutritional Supplements for Prohormones**

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### **Introduction**

Since 1996 so called prohormones are available on the US sports nutrition market. According to the doping rules of the IOC, these substances belong to the prohibited class of anabolic agents (1).

Recent studies have shown, that “non-hormonal” supplements as Tribulus Terrestris-, guarana- and chrysin-products contained prohormones, which were not declared on the label. These “contaminations” led to positive results in doping control for the nandrolone metabolite norandrosterone (2, 3). With this knowledge, we continued to analyse further nutritional supplements for prohormones.

### **Experimental**

#### Analysis of nutritional supplements

153 “non-hormonal” nutritional supplements were analysed with GC/MS for prohormones. Most products were from follow up studies of positive doping cases, i.e. nutritional supplements of the possession of positive athletes were analysed. If prohormones were detected, original packed containers of these products were bought on the supplement market and also analysed for prohormones. 12 “non-hormonal” products were bought randomly from companies, which sell also prohormones.

The nutritional supplements were analysed for the following prohormones:

prohormones of testosterone: 4-androstene-3,17-dione (4-Adion), dehydroepiandrosterone (DHEA), 5-androstene-3 $\beta$ ,17 $\beta$ -diol (5-Adiol), 4-androstene-3 $\beta$ ,17 $\beta$ -diol (4-Adiol) and testosterone (Test)

prohormones of nandrolone (19-nortestosterone): 19-nor-4-androstene-3,17-dione (4-Nordion), 19-nor-4-androstene-3 $\beta$ ,17 $\beta$ -diol (4-Nordiol), 19-nor-5-androstene-3 $\beta$ ,17 $\beta$ -diol (5-Nordiol) and 19-nortestosterone.

The analyses were performed according to the procedure described by Geyer et al. (2, 3). The flow scheme of the sample preparation is presented in figure 1.

#### Excretion studies with the nutritional supplements

Male volunteers administered contaminated nutritional supplements. Before the application and several hours after the application of the supplements, urine samples were collected and analysed by gas-chromatography/mass-spectrometry for the glucuronides of norandrosterone according to the screening procedure for anabolic steroids (4, 5)

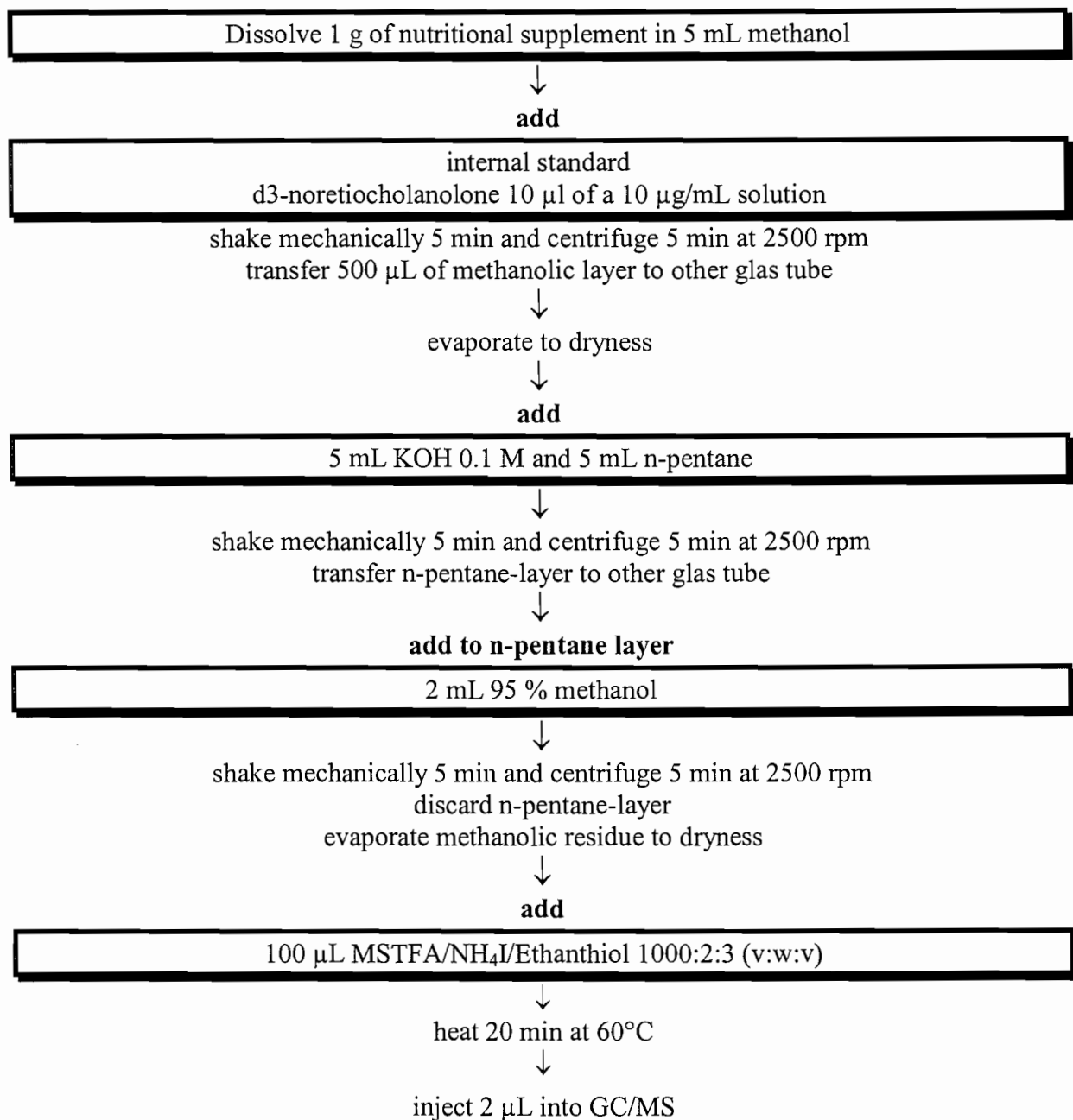


Fig. 1: Flow scheme for the sample preparation for the analysis of nutritional supplements for anabolic-androgenic steroids.

## Results

In 18 different nutritional supplements from 12 different companies prohormones could be detected, which were not declared on the label. 15 of the 18 supplements contained prohormones of nandrolone. The positive supplements have been bought in USA, UK, Germany, Belgium, Sweden, Israel and Austria. In most cases the location of the manufacturing company was not mentioned. The prohormones occurred in tablets, capsules, powders, drinking ampouls and oily solutions. The declared main-ingredients of the positive supplements were carnitine, creatine, vitamins and minerals, Tribulus Terrestris, ribose, branched chain amino acids (BCAA), ornithine keto-glutarate (OKG), zinc, pyruvate, chrysin, guarana, conjugated linolic acid (CLA), herbal extracts and glutamine. The most frequently detected prohormones were 19-nor-4-androstene-3,17-dione and 4-androstene-3,17-dione. These substances were detected in 14 and 11 supplements respectively (see fig. 2).

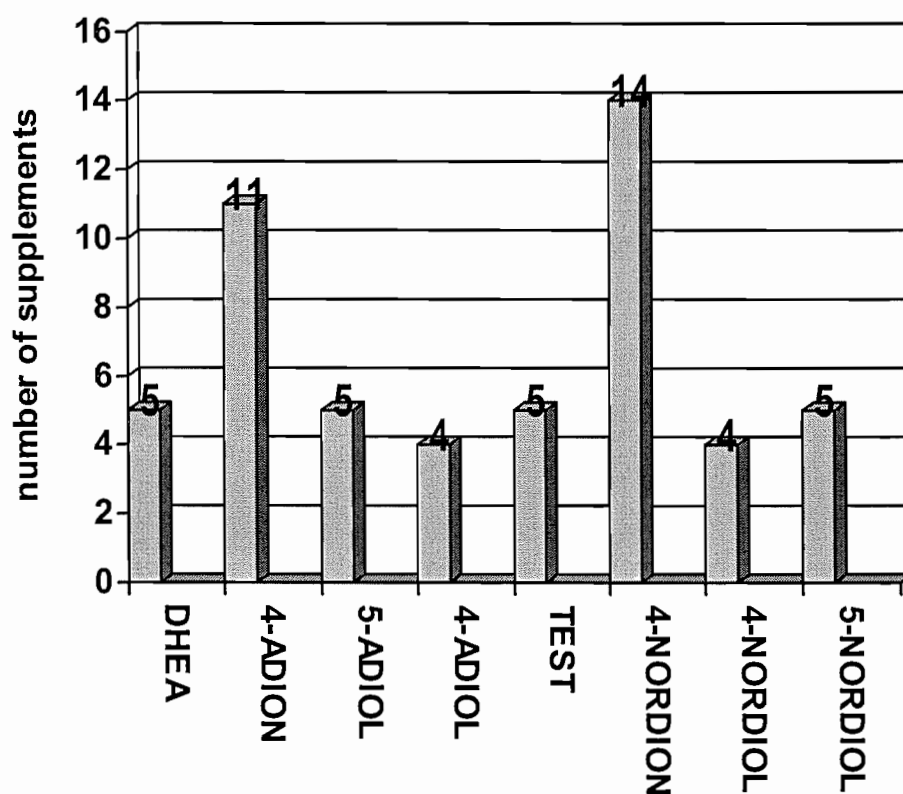


Fig. 2: Frequency of prohormones in the 18 “non-hormonal” nutritional supplements. The most frequently detected prohormone was 19-nor-4-androstene-3,17-dione (4-Nordion). 4-Nordion was detected in 14 supplements.

Most supplements contained several prohormones. In figure 3 is shown a chromatogram of a creatine product with 7 prohormones and testosterone. The distribution of the detected concentrations of the prohormones are presented in figure 4. About 50 % of the analysed supplements showed concentrations of prohormones higher than 10 µg per capsule or tablet. The maximum concentration of prohormones, detected in a guarana capsule, was 5140 µg (sum of 4 prohormones).

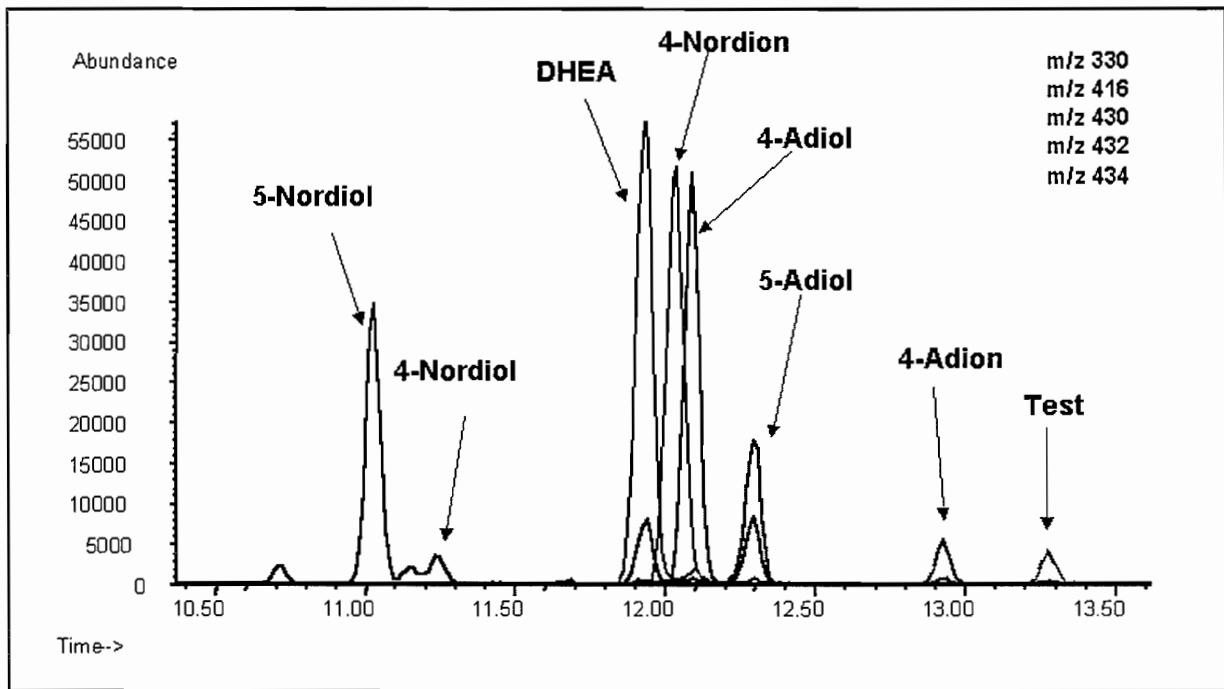


Fig. 3: Chromatogram of a creatine product with 7 prohormones and testosterone.

Concerning the prohormones of nandrolone about 40% of the positive supplements showed concentrations above 10 µg per capsule or tablet (fig. 5) The total amount of prohormones and the profile of the prohormones varied from charge to charge and from capsule to capsule within one charge. The results of a supplement with Tribulus Terrestris are presented in table 1. The total amount of steroids varied between 0,3 µg and 79,8 µg per capsule.

Excretion studies with supplements which contained 4-norandrostendione in amounts of 0,8 µg, 3,6 µg and 5,6 µg resulted in maximum urinary norandrosterone concentrations of 0,5 ng/ml, 11,6 ng/ml and 12,6 ng/ml respectively (fig. 6).

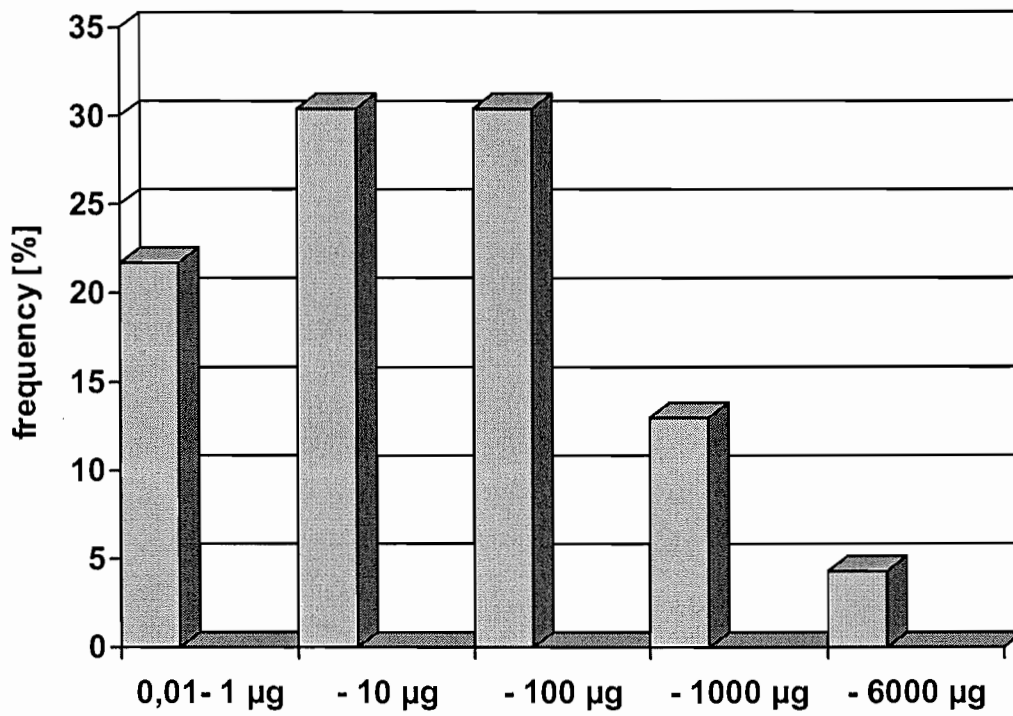


Fig. 4: Concentrations of prohormones (per capsule/per tablet) in contaminated nutritional supplements (n=46)

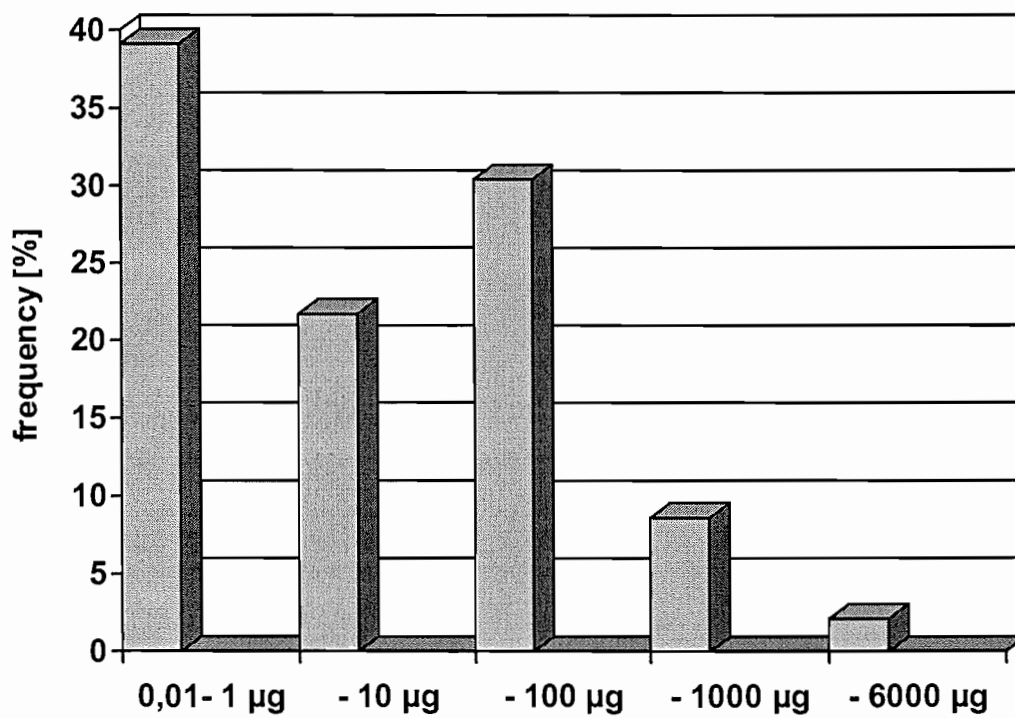


Fig. 5: Concentrations of prohormones of nandrolone (per capsule/per tablet) in contaminated nutritional supplements (n=46)

Tab. 1: Amount of not-declared prohormones (in microgramm per capsule) in capsules (Kap) of three different charges of a Tribulus Terrestris product.

TRIBULUS TERRESTRIS	Charge 1			Charge 2			Charge 3		
	Kap1	Kap2	Kap3	Kap1	Kap2	Kap3	Kap1	Kap2	Kap3
4-Adion	0,3	1,3	76,3	0,6	2,6	0,9	1,3	1,4	0,8
5-Adiol	-	-	-	-	-	-	16,6	30,1	5,6
4-Adiol	-	0,7	1,5	0,3	1,4	0,8	1,8	3,8	0,8
4-Nordion	0,04	0,13	0,64	0,08	0,12	0,17	0,48	0,51	0,22
4-Nordiol	-	0,74	1,36	-	1,02	0,51	3,7	15,1	1,6
<b>total amount of prohormones</b>	0,3	2,9	79,8	1,0	5,1	2,4	23,9	50,9	9,0
<b>amount of nandrolone prohormones</b>	0,04	0,9	2,0	0,08	1,1	0,7	4,2	15,6	1,8

Within the 153 analysed supplements were also 12 “non-hormonal” products, which randomly were bought from companies in the United States, which also sell prohormones. From this randomized study 4 products contained prohormones, which were not declared on the label (tab. 2)

Tab. 2: 4 of 12 nutritional supplements, randomly bought from prohormone selling companies, contained prohormones not declared on the label (NEM...= laboratory code for nutritional supplements)

	NEM 130 [µg/capsule]	NEM 131 [µg/capsule]	NEM 132 [µg/capsule]	NEM 152 [µg/capsule]
4-Nordion	-	0,4	2,8	3,7
4-Adion	2,6	3,8	1,2	-
Test	0,3	-	-	-
5-Adiol	0,6	1,1	5,0	-
4-Adiol	2,4	-	-	-

## Discussion

The results show, that more than 10 % of the analysed supplements contained prohormones, not declared on the label. This number is probably not representative for the whole nutritional supplement market, because most supplements were not bought randomly but in connection

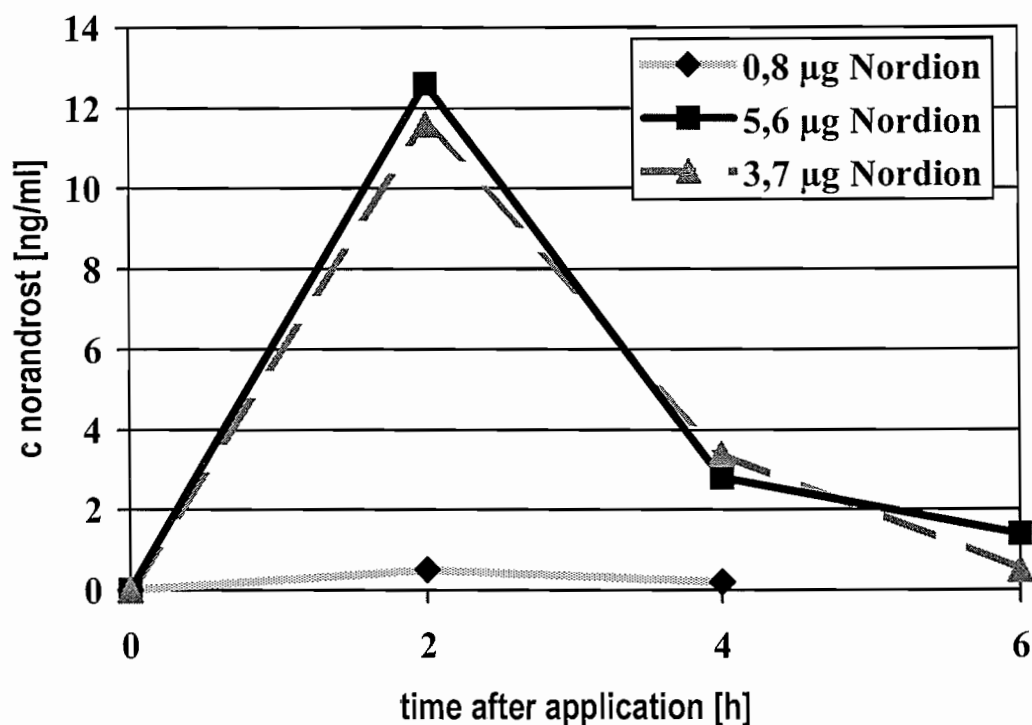


Fig. 6: Urinary concentration of norandrosterone after application of nutritional supplements contaminated with different amounts of norandrostendione.

with positive doping control cases. The probability, that a supplement contains non-declared prohormones is much higher in products from companies which also sell prohormones. 33 % of randomly bought nutritional supplements (4 of 12) of such companies contained not-declared prohormones (tab. 2).

The amounts of the prohormones in the analysed supplements are much lower (see fig. 4 and 5) than the amounts in the lowest concentrated commercially available prohormone supplements, which contain 25 000 µg of prohormones. Additionally the concentrations show strong variations from charge to charge and capsule to capsule and tablet to tablet respectively



(tab. 1). Both facts, low concentrations and varying concentrations are indicators for so called cross-contaminations and not for intentional admixtures. We suppose, that the contaminations occur in companies, which process and transport prohormones. If the same machines and vessels are used in the processing of other nutritional supplements as vitamins, minerals etc., contaminations of these “non-hormonal” supplements are the consequence of an insufficient cleaning of the processing tools and vessels. These cross-contaminations are possible, because the nutritional supplement industry has not to fulfill GMP (Good Manufacturing Practices) conditions as the pharmaceutical industry.

According to studies with androstendione (6-9) physiological effects or adverse effects after the oral application of such low amount of steroids, as detected in the contaminated supplements, are improbably but cannot be excluded.

The excretion studies with the prohormones contaminated with low amounts of norandrostendione showed positive results for urinary norandrosterone ( $> 2$  ng/ml) for about 4 hours (fig. 6). From these results can be concluded, that the application of contaminated nutritional supplements with maximal amounts of 19-norsteroids of more than 1000  $\mu$ g/capsule (fig. 5), can lead to positive results for norandrosterone for more than 24 hours, especially if the recommended dose –several capsules per day- is taken. It is difficult to predict the expected maximum concentrations of norandrosterone, because of the interindividual differences in metabolism and the strong variation of the concentrations of the steroids in the capsules. Urinary values of norandrosterone of more than 500 ng/ml are possible with the strongest contaminated supplements (2, 3).

Regarding the amount of prohormones of testosterone in the contaminated supplements, positive results in dope control, e.g. increase of the ratio testosterone/epitestosterone should not occur. Nevertheless, such an increase cannot be excluded especially in females with natural low concentrations of testosterone and epitestosterone (2, 3)

## **Conclusion**

The sports community should be aware of the danger of nutritional supplements contaminated with prohormones. Such contaminated nutritional supplements can lead to positive results in

doping control, especially for the nandrolone metabolite norandrosterone. To minimize the risk of contaminations, athletes should buy only nutritional supplements from companies, which perform a quality control for prohormones and/or which do not sell prohormones.

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