



Unintentional doping through the use of contaminated nutritional supplements

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Objective. To determine whether the intake of contaminated nutritional supplements could cause an athlete to fail a dope test.

Design. A contaminated nutritional supplement was used, identified in an ongoing study screening over-the-counter nutritional supplements. One capsule of the supplement, containing small amounts of 19-nor-4-androstenedione and 4-androsten-3,17-dione, not listed on the label, was administered to 5 healthy male volunteers. Fractional urine collection was done at prescribed intervals.

Outcome measures. The samples were analysed using gas chromatography/mass spectrometry (GC/MS). Samples containing 19-norandrosterone, the main metabolite of 19-nor-

4-androstenedione, were quantified using GC/MS.

Results. All the volunteers had urinary concentrations of 19-norandrosterone above the World Anti-Doping Agency threshold of 2 ng/ml from 2 hours post administration. In 2 volunteers 19-norandrosterone above the threshold value could still be detected beyond 36 hours post administration. The highest concentration of 19-norandrosterone found in a sample was 54.6 ng/ml at 8 hours post administration.

Conclusion. The results of this study showed that the intake of microgram amounts of a prohibited substance in a nutritional supplement could cause an athlete to fail a dope test.

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Inadvertent doping through the use of nutritional supplements is a potentially important cause of the increase in positive doping cases among high-profile Olympic athletes. There are a wide variety of nutritional substances on the market specifically designed for athletes. These supplements are promoted among athletes for their performance-enhancing properties, and for faster recovery during training, manipulation of body composition and reduction in illness and infection risks. Athletes assume that these supplements do not contain prohibited substances because they are readily available without prescription and are sold legally as supplements.

In many countries the manufacture of nutritional supplements is not regulated appropriately. This means that the ingredients of the supplement may not match those listed on the label of the container. Other problems associated with this lack of regulation are batch composition differences and possible contamination with prohormones, which are prohibited substances in sport. Recent studies¹ have shown that non-hormonal nutritional supplements such as vitamins, minerals and amino acids may contain undeclared substances not listed on the label. In some cases the undeclared substances found in the supplement can include those prohibited by the Anti-Doping Code of the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA). Results from an IOC study² on nutritional supplements (13

countries excluding South Africa) showed that 94 of 634 samples (14.8%) contained prohormones not listed on the label. Both Catlin et al.³ and Kamber et al.⁴ analysed nutritional supplements and found hormone contaminants not listed on the labels. Results from our previous study on nutritional supplements available on the South African market showed that approximately 7% of supplements tested could be mislabelled or contaminated with prohibited substances.⁵ The results of the effect of contaminated nutritional supplement ingestion may be useful to clinicians working in this field.

The aim of this study was to determine if the intake of a nutritional supplement containing microgram amounts of prohibited substances could cause an athlete to fail a dope test.

Materials and methods

Contaminated supplements were identified in an ongoing study that screened over-the-counter nutritional supplements. Five healthy male volunteers (age 24 - 55 years, weight 77 - 99.4 kg) received 1 capsule of a nutritional supplement that was contaminated with 19-nor-4-androstenedione and 4-androstenedione (previously determined as 8.4 - 31.8 µg/capsule for 19-nor-4-androstenedione and 0.1 - 0.4 µg/capsule for 4-androstenedione).⁵ Urine samples were collected before administration and at 2, 4, 8, 12, 24, 30, 36 and 48 hours post administration. Urine samples were refrigerated after collection pending analysis. The samples were analysed using gas chromatography/mass spectrometry (GC/MS) for the glucuronides of 19-norandrosterone, testosterone and epitestosterone according to the screening procedure for anabolic steroids.⁶ Samples containing 19-norandrosterone

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were quantified by GC/MS using a calibration curve. The samples were analysed in duplicate.

The study was approved by the Ethics Committee for Medical Research of the University of the Free State. The volunteers were informed of the low amounts of prohormones in the product, and written consent was obtained.

Results

Fig. 1 shows the urinary concentrations of 19-norandrosterone (the main metabolite detected in urine after the intake of 19-nor-4-androstenedione) in the volunteers after the administration of the contaminated supplement. The highest concentration of 19-norandrosterone found in the samples was 54.6 ng/ml. The peak concentrations of 19-norandrosterone varied between 11 ng/ml and 55 ng/ml. The urinary concentration of 19-norandrosterone in 1 volunteer dropped below the threshold level between 8 and 12 hours post administration. In 2 volunteers, 19-norandrosterone above the threshold level could still be detected for longer than 36 hours post administration.

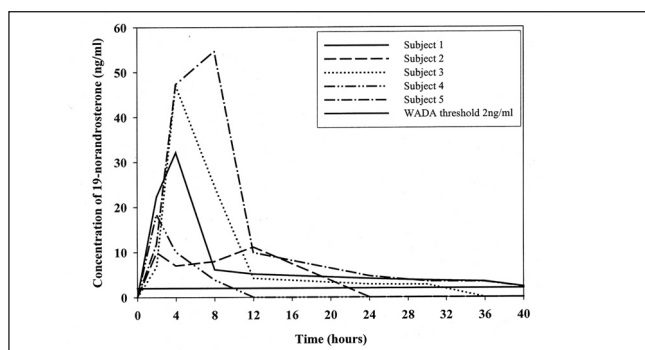


Fig. 1. Urinary concentrations of 19-norandrosterone in 5 male volunteers after administration of a contaminated nutritional supplement.

Discussion

Several studies have shown that nutritional supplements can be contaminated with prohormones, and the supplements available in South Africa are no exception.⁵ The quantity of prohormone in the supplement was lower than the amount found in commercially available prohormone supplements. The contaminated nutritional supplement was administered to 5 male volunteers to determine if the ingestion of these small amounts of 19-nor-4-androstenedione (0.02 - 0.06% of the usual dose of 50 mg) would lead to a positive dope test. All the volunteers showed concentrations above the WADA threshold of 2 ng/ml from 2 hours post administration.

The exact dosage of 19-nor-4-androstenedione administered to the volunteers was unknown because of the variation in

concentration of 19-nor-4-androstenedione in the capsules.⁵ These results were obtained after the ingestion of only 1 capsule. However, the recommended dosage according to the manufacturer is 4 capsules 3 times a day, which can result in even higher concentrations of 19-norandrosterone and for longer time periods. The results are similar to those obtained by Geyer *et al.*² where the administration of nutritional supplements containing different amounts of 19-nor-4-androstenedione (0.8 - 24.6 µg/capsule) also led to positive doping tests 2 hours post administration. According to Geyer *et al.*² it can be estimated that nutritional supplements containing nandrolone prohormones in concentrations higher than 1 µg may result in a positive dope test. However, supplements with smaller concentrations of nandrolone prohormones could still lead to a positive test depending on the recommended dosage of the manufacturer.

The use of testosterone and its prohormones, like DHEA and 4-androstenedione, lead to an increase in the urinary testosterone/epitestosterone ratio. No increase in the testosterone/epitestosterone ratio was observed in any of the urine samples in this study and therefore none of the volunteers tested positive for 4-androstenedione. This could be because of the low concentration of 4-androstenedione in the capsules, which ranged from 0.1 to 0.4 µg/capsule.⁵

Conclusion

The intake of microgram amounts of prohibited substances in contaminated supplements can cause an athlete to fail a dope test. Our results are of particular interest to team physicians and other clinicians prescribing supplements to competitors in sport. Athletes taking nutritional supplements should take them with the full knowledge and acceptance of WADA's strict liability rule, which states that an athlete is ultimately responsible for substances found in his/her body fluids irrespective of their origin. There is no guarantee that taking a nutritional supplement will not result in a positive dope case, as shown in this study.

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