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A crossover study of 19-norandrostenedione contamination in sports supplements: preliminary findings

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Introduction

In recent years there has been a huge increase in the manufacture and sale of nutritional supplements, mainly targeting the human sport and lifestyle markets. Pro-hormones or precursors such as 19-norandrostenedione, androstenedione and dehydroepiandrosterone (DHEA) have also been detected as contaminants in many non-hormonal products (Geyer *et al.* 2004). Human athletes who fail a drug test frequently cite contamination of dietary supplements as a defence.

19-norandrostenedione is closely related in chemical structure to nandrolone. Oral administration of this substance to human volunteers has led to detection in the urine of the nandrolone metabolites, 19-nor-androsterone (19-NA) and 19-nor-etiocholanolone (19-NE) (Catlin *et al.* 2000, Geyer *et al.* 2004). The levels of 19-NA detected have exceeded 2 ng/ml and therefore would constitute a doping violation under World Anti-Doping Agency (WADA) regulations.

This current study seeks to determine the urinary profiles of the major metabolites of nandrolone, 19-NA and 19-NE, following repeated administration of a creatine supplement contaminated with a known level of 19-norandrostenedione (10 μ g) and to determine the variability in the detection periods for a number of volunteers (n=18).

Experimental

The spiked creatine supplement was prepared by dissolving pure creatine (5 g) in warm bottled drink water (500 ml). A 100 μ g/ml standard of 19-nor-androstenedione (Sigma-Aldrich, St Louis MO, USA) in ethanol was prepared and added to the creatine-water mix to

give a supplement spiked with 10 μ g of steroid. In addition, a placebo supplement was prepared by dissolving 5 g creatine in warm bottled water (500 ml).

Subjects (n = 18) were randomised into the single blind study and received either the steroidcontaining supplement or the placebo. The study was approved by Loughborough University Ethical Advisory Committee (Ref R05-P39). The supplement was administered once a day (morning) for a 5 day period. On day 6, no supplement was administered. All urine samples were collected for Days 1, 5 and 6. On Days 2, 3 and 4 only the initial pre-dose urine samples were collected. After a rest period of a minimum of 48 hours the subjects were crossed over and were administered either spiked supplement or placebo for a further 5 day period.

All urine samples were analysed quantitatively for the metabolites 19-NA and 19-NE, relative to D_4 -19-norandrosterone internal marker. The specific gravity and pH of all samples was recorded prior to analysis. Samples were analysed using a validated method developed for the quantitative analysis of 19-NA in line with WADA guidelines (solid phase extraction, to yield an eluant which was treated to form the enol-trimethylsilyl (TMS) derivative). Each sample batch was extracted/ analysed alongside two sets of control samples (urine spiked with 19-NA and 19-NE at 2 and 8 ng/ml) and a set of calibration samples (urine spiked with 19-NA and 19-NE at 0, 1, 2, 5, 7, 10 ng/ml).

GCMS analysis was carried on a Shimadzu QP2010 instrument, with a Valcobond VB-5 column. All samples were quantitated for both metabolites using XCALIBUR LCQUAN

In addition, the pure bottled water, placebo creatine supplement and spiked creatine supplements were analysed using HFL's Gold nutritional supplement screening method (visit www.SupplementAware.com for details of this screen).

Results

The presence of 19-NA in the spiked creatine supplement mix was confirmed using the HFL Gold supplement screen. No steroid was detected in the placebo creatine supplement samples.

All urine samples from subjects taking spiked creatine and a selection of urine samples from subjects taking placebo creatine were analysed quantitatively against calibration curves for 19-NA and 19-NE. All points along the curve in all assays were well within the acceptance

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criteria established for the quantitative method. In addition, the low (2 ng/ml) and high (8 ng/ml) control samples were within the acceptance criteria in all assays.

All urine samples from subjects taking spiked creatine showed significant levels of the major metabolite, 19-NA, with levels post first dose on Day 1 ranging from 2.9 - 13.8 ng/ml and levels post dose on Day 5 ranging from 2.3 - 19.3 ng/ml. (N.B. concentrations of 19-NA and 19-NE were corrected for specific gravity where necessary). Typical profiles obtained for 19-NA can be seen in Figure 1. These levels are well above the current threshold of 2 ng/ml for a positive doping finding under WADA rules.



Figure 1. Typical profile for 19-NA levels following initial dose of creatine spiked with 10 μ g 19-norandrostenedione on Day 1 (note: on Days 2, 3 and 4 only 1 **pre-dose** urine sample was collected; on Days 1, 5 and 6 **all** urine samples passed during the day were collected).

The mean highest value of 19-NA in Day 1 post first dose samples was 6.5 ng/ml (standard deviation 3.1): almost 4 times higher than the mean 19-NE value (mean 1.7, standard deviation 1.0). The mean highest value of 19-NA in Day 5 post dose samples was 6.8 ng/ml (standard deviation 5.2) compared to mean value for 19-NE of 2.1 (standard deviation 2.5).

Day 1 post first dose urine samples were analysed for all subjects taking placebo supplement. All of the calculated levels for both 19-NA and 19-NE fell well below the lowest limit of quantification of the assay, as did all other placebo samples analysed.

Preliminary analysis of the urinary clearance of the major metabolite, 19-NA showed that it fell to below the 2 ng/ml threshold approximately 0.5 - 10 hours post first dose on Day 1.

Conclusions and Further Work

The presence of the nandrolone metabolites, 19-NA and 19-NE in the urine was directly related to the consumption of supplement containing the parent steroid, 19-nor-androstenedione. These metabolites are not detected in placebo urine samples.

Contamination of 19-nor-androstenedione in supplements as low as 10 μ g/500ml (20 ng/ml) was shown to give rise to a positive drugs test in all cases. In general, levels of the major metabolite, 19-NA, were still detectable in urine up to 10 hours post initial dose. Further work is now underway to determine the effect of lower amounts of contamination and analysis is currently being carried out on the data obtained from this study to establish the variation between male/female subjects, body mass, etc. These findings will be reported in the near future.

References

Geyer, H., Parr, M.K., Mareck, U., Reinhart, U., Schrader, Y., and Schanzer, W. (2004) Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroids – results of an international study. *Int. J. Sports Med.* 25, 124-129.

Catlin, D.H., Leder, B.Z., Ahrens, B., Starcevic, B., Hatton, C.K., Green, G.A. and Finkelstein, J.S. (2000) Trace contamination of over-the-counter androstenedione and positive urine test results for a nandrolone metabolite. *J. Am. Med. Assoc.* 284, 2618-2621.