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One Year of Nandrolone at the Antidoping Laboratory of Rome

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One year of nandrolone at the antidoping laboratory of Rome*

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INTRODUCTION

The number of positivity cases due to metabolites of 19-nortestosterone and/or of its congeners increased in Italy in the last year, and several interpretations to explain the causes of such a growth have been proposed, the most credible among them being:

1. Intentional doping, with either nandrolone or its precursors
2. Far earlier therapeutic use of nandrolone-based drugs (i.e. after a severe injury)
3. Ingestion of contaminated nutritional supplements
4. Ingestion of meat illicitly contaminated by nandrolone
5. Ingestion of high doses of non-contaminated nutritional supplements
6. Endogenous production, increased by peculiar physiological conditions (i.e. following heavy physical exercise)
7. Conditions 5 and 6 occurring contemporarily

This study originates from the observation that, at present, roughly 1% of all samples analyzed by the antidoping laboratory of Rome are suspected to contain norandrosterone (NA) and/or noretiocholoanolone (NE), in concentrations levels ranging from sub ng/mL to over 40 ng/mL. The present survey considers samples analyzed by the laboratory of Rome in the first year

* It was not possible to evaluate in their entirety the experimental results of this study since most of the scientific documents are presently under investigation by the "Procura della Repubblica di Torino". Further studies on the "below the threshold" samples could not be performed since the samples themselves have been confiscated by the Procura della Repubblica di Torino as well.

following full reaccreditation, and additional control samples obtained from other sources, comprising non-athletes volunteers. Data on the percent of positivity cases of nandrolone on meat samples analyzed in Italy and in other EU countries are also considered.

EXPERIMENTAL

a) Samples

The study refers to 5453 samples, received by the antidoping laboratory of Rome in the period March 2000 – February 2001.

b) Methods, instrumental apparatus and reagents

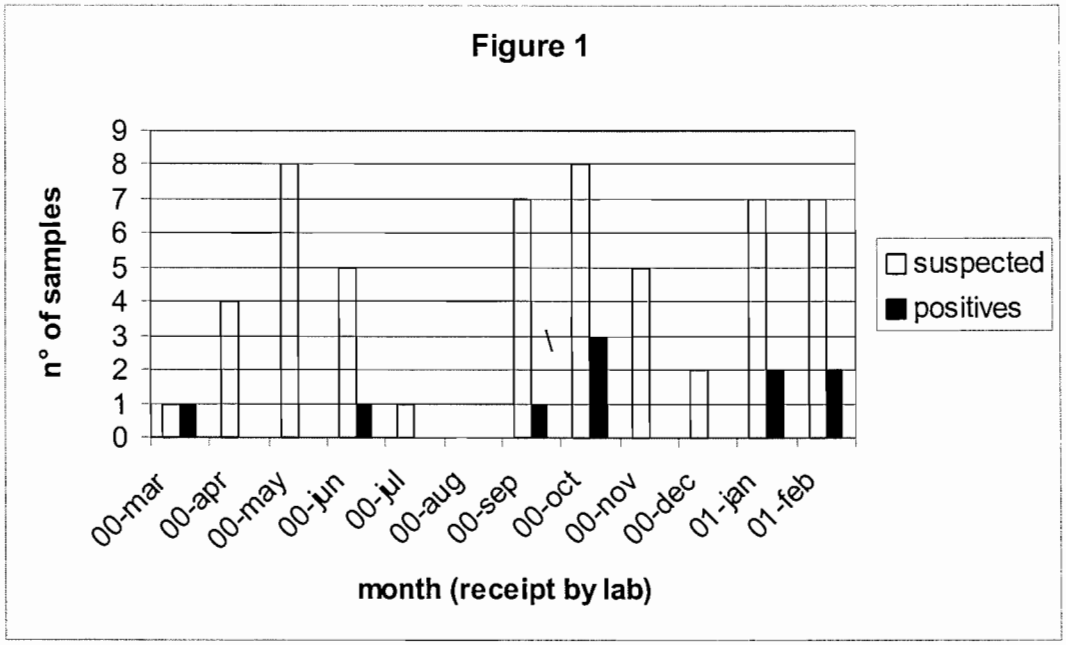
Screening: carried out on all samples; TMS-derivatives; GC-MS-EI (HP 6890-5973), SIM (m/z 405 and 420 for both NA and NE).

Quantitation: performed according to the IOC-MC guidelines, with respect to the IOC thresholds of 2 and 5 ng/mL for male (M) and female (F) athletes respectively; carried out on all samples not excluded by the screening; TMS-derivatives; GC-HRMS (Fisons Autospec TOF) (12K res at 10% valley), SIM (m/z 315.2144, 405.2645 and 420.2880); comparison with ISTD (NA–d4 and NE–d4) with correction for the response factor evaluated in the positive reference urine.

Final confirmation (qualitative): carried out only on samples with [NA] >2ng/mL (M) and >5 ng/mL (F); TMS-derivatives, GC-HRMS (Fisons Autospec TOF) (12K res at 10% valley), SIM (m/z 315.2144, 405.2645 and 420.2880) and/or GC-HRMS-MS (TOF) full scan (3K res at 10% valley on MS1).

c) Experimental evidence

The presence of NA and/or NE was suspected after the screening analysis in 55 out of 5449 samples; the presence of NA was also suspected in other 10 samples, not being further investigated (concentration < 0.1 ng/mL); 10 samples were reported as positive after the confirmation analysis. Concentration of estradiol (not included in the screening of samples collected before July 2000) was always in the normal range. The distribution of suspect samples along the year is given in Figure 1.



d) Studies on non-athletes population

The urines of non athletes volunteers (17 males and 17 females, age 15-93) were assayed as a part of a parallel study aimed to verify the periodicity of urinary steroid concentration as a function of age and of various physiological conditions and/or pharmacological stimulation/inhibition. NE was never detected. NA was detected only in the urine of 3 subjects, all female, 2 of them pregnant.

e) Meat samples

Data referring to the period 1998-1999, during which almost 90000 samples of bovine meat samples were analyzed by the EU authorized laboratories, show that no positive cases for nandrolone (not searched for in all samples) were reported; while several cases of contamination by beta-agonists (including clenbuterol) were detected.

CONCLUSIONS

1. Positivity due to the ingestion of contaminated meat is highly unlikely, at least according to the Italian situation.

2. Basal endogenous production of NA-NE and/or their analogues/precursors is not confirmed in males by the analysis of urines from non-athletes volunteers. The solidity of the endogenous production hypothesis is further reduced by the low percent of females among athletes whose urine was not negative after the screening analysis (50 males vs. 5 females, only one with NA>5 ng/mL).
3. The effect of physical exercise and of the possible role of (non contaminated) nutritional supplements should be investigated in more details, even if they do not seem to play a primary role on the basis of the results obtained on athletes, that, with roughly 99% of them testing negative for both NA and NE (LOD 0.5 and 0.8 ng/mL respectively), could be considered a reliable "reference population".
4. The identification, and possibly quantitation, of NE, and consequently the urinary concentration ratio NA/NE, can supply additional evidence in most of the positive cases.
5. A further insight into the relevance of the third metabolite is also needed.
6. Criteria for positivity should possibly be the same in all sport disciplines.
7. Periodicity of analytically positive cases, with reference to the major seasonal events, has to be monitored.
8. The real contribution of contaminated nutritional supplements to the increasing number of cases (independently from the IOC threshold) has to be further ascertained.