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# Retrospective Investigation of Illicit Drug Use in Amateur Athletes

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## Abstract

***Background:** The rate of intake of illicit drugs by athletes is not limited to the elite athletes, and subjects not practising elite sport use several doping substances. We evaluated the rate of intake of illicit drugs in competitive, but not elite, and recreational Italian athletes.*

***Materials:** In the period of 3 years, a total of anonymised 2787 urinary samples from competitive, but not elite, and recreational Italian athletes was screened for cannabis, cocaine, opioids and amphetamines.*

***Results:** A total of 7.5% urine samples were positive for illicit substances. The metabolites of cannabis were discovered in 148 samples, and cocaine metabolites in 28. Amphetamines were present in 6 samples, and ephedrine in 1. The metabolites of nandrolone were identified in 3 samples. In a further 24 samples, the ratio of testosterone/epitestosterone level was inversed, but no illicit substance or metabolites were identified.*

***Conclusions:** In non-elite athletes, the prevalence of illicit drugs use is comparable to that described in elite athletes.*

## Introduction

Regular physical activity prevents several cardio vascular, dysmetabolic and neoplastic conditions. In addition, physical exercise can promote social contact and reduce the risks of drug addiction [1,2]. A relationship has been demonstrated between malaise and sporting activity, prevalently in the young. Life style investigations have demonstrated that most young subjects involved in rehabilitation and recovery programmes after a period of drug addiction regularly practiced competitive sport; also, many students who currently undertake regular competitive sport habitually take illicit substances [3].

We were not aware of studies investigating the rate of illicit drug use in non-elite athletes in our region, and we therefore studied a large sample of such athletes involved in a wide variety of sports.

## Materials and Methods

All the procedures described in the present study were approved by our local Ethics Committee. All subjects gave their informed written consent to participate in the study.

A group of 2787 subjects, including 361 women, were tested. Their mean age was 25 y (range 14 to 42), and all were competitive non-elite sport. A urine sample was later obtained from each subject. All samples were taken at the end of the training session or after a competition, or during the examination to obtain eligibility for participation in sports at the Sports Medical Center of Tuscany, a central region of Italy. The athletes came from 11 different sports including soccer, basket, swimming, cycling, athletics and baseball. They trained an average of two hours a day, 6 days a week, for at least 10 months of the year.

We analysed a total 361 (13%) of women's urine samples and 2571 of men's urine samples. In 5 cases,

mislabelling of the sample made it impossible to identify the sex of the athlete. We did not include these samples in the present investigation.

## Laboratory Urine Sample Analysis

Sample analysis included a screening test and a confirmatory test. The immunochemical method (KIMS: Kinetic interaction of micro particles in solution) was used for preliminary screening to detect the presence of illicit substances such as cannabinoids, cocaine and opioids in the urine samples. Detection of stimulant substances, anabolic agents and banned diuretics was performed (screening and confirmatory tests) with gas or liquid chromatographic-mass spectrometric (GC-MS or LC-$MS^n$) techniques.

## Determination of Stimulants

### *Screening*

The urine samples, alkalinized and treated with the addition of $Na_2SO_4$, were processed using a liquid-liquid extraction with tert-butylmethyl ether (MTBE). After centrifugation, the supernatant was reduced to 200 µl under nitrogen stream at 40°C, and injected into the GC-MS system [4,5]

### *Confirmation*

To confirm the presence of amphetamine and analogous substances (methyl amphetamine, methylendioxyamphetamine (MDA), methylendioxymethyl amphetamine (MDMA), methylendioxyethylamphetamine (MDEA), ***N***- methylendioxyphenylbutanamine (MBDB)) the urine samples, alkalinized and treated with the addition of $Na_2SO_4$, underwent liquid-liquid extraction with MTBE. After centrifugation, the supernatant was evaporated to dryness. 100 µl of N-methyl-trimethylsilyltrifluoroacetamide (MSTFA) were added to the dried residue and kept at 60°C for 5 min; after cooling at room temperature, 20 µl of N-methyl-bis-trifluoroacetamide (MBTFA) were added, and the mixture was mixed and incubated for 10 min at 60°. The sample was then injected into GC-MS [4].

To confirm the presence of ephedrine [4], the urine samples, alkalinized and treated with the addition of $Na_2SO_4$, underwent liquid-liquid extraction with MTBE. After centrifugation, the supernatant was evaporated to dryness. 100 µl of PFPA/Acetone 80/20 (v/v) were added to the dried residue, and kept at 60°C for 15 min. The sample was evaporated to dryness under nitrogen stream at 50°C; 200 µl of pentane were added, and the sample was injected into the mass spectrometer.

## Determination of Cannabinoids

Confirmation of 11-nor-carboxy-$\Delta^9$-tetrahydrocanna binolic acid (THC-COOH) was based on solid-phase extraction of the urine sample, hydrolysis, trimethylsilyl derivatization and GC-MS analysis [6].

## Determination of Cocaine and its Metabolites

The urinary presence of cocaine and its metabolites was determined using solid-phase extraction of the urine sample, hydrolysis, trimethylsilyl derivatization and GC-MS analysis [7].

## Determination of Anabolic Substances

Screening and confirmation of synthetic and natural anabolic agents was based on solid-phase extraction of the urine sample and enzymatic hydrolysis of the glucuroconjugates, followed by liquid-liquid extraction at basic pH, trimethylsilyl derivatization and GC-MS analysis [8].

Regarding the testosterone/ epitestosterone ratio, only samples with a range higher than 4 have to be submitted to further evaluation using Isotope Ratio Mass Spectrometry (IRMS), but this instrumentation is not yet available in the laboratories, thus the results obtained must be considered incomplete.

## Determination of Diuretics

All the analytical procedure were performed according to the protocols described .The urine samples were filtered and diluted with purified water, centrifuged at 4000 rpm for 5 min. The supernatant was placed in a glass vial and injected into the LC-MS/MS system [9].

All the instruments used in the present study were calibrated according to the manufacturer's instructions at weekly intervals.

The Instrumentation included*:* Immunochemical test: Cobas Integra 800- Roche® for the Determination of stimulants, cocaine and its metabolites, cannabinoids and narcotics

GC-MS mod. Saturn 2000 Varian, a Capillary column: 95% dimethyl-5% diphenyl polysiloxane, 30m x 0.25µm, ø 0.25mm by Scan acquisition, mass range: 70-450 amu was used. Oven temperature: 100°C, 1min; 20°C/min@250°C; 5°C/min@280; 30°C/min@300°C, 3min

The determination of anabolic agents was possible by using GC-MS mod. 5973 inert Agilent Technologies. The sample was analyzed by a Capillary column: 100%

dimethyl polysiloxane 30m x 0.25µm, ø 0.25mm. Single Ion Monitoring acquisition, mass range: 200-500 amu. Oven temperature: 100°C, 1min; 20°C/min@250°C; 5°C/min@280; 30°C/min@300°C, 3min. The determination of diuretics substances was made by using

HPLC mod. Prostrar 210 Varian; Mass spectrometry Ion Trap mod.500-MS Varian

Column: Synergi 2u-Polar, 50x2.00 mm 2 micron. Mobile phase A: formic acid 0.1%

Mobile phase B: Acetonitrile+0,1% formic acid

Gradient: from 0 to 0.30 min 90% of A, in 8.00 min at 25% of A, isocratic at 25 % at 10.30 min, in 1 min at 90% of A and re-equilibration for 8 min al 90% of A.

Flow: 200 µl/min

ESI source; MS parameters: Collision voltage: 58 V e 55 V

Excitation amplitude: 1.35 v e 0.60 V

Nebulizer gas pressure 60 psi

Drying gas pressure 25 psi

Drying gas temperature 300 °C

Needle voltage 4600V /-4600 V

## Results

A total of 210 samples, i.e. 7.5% of the global population studied, were positive for illicit or anabolic substances. Among the different kinds of sports activity, only 11 kinds were involved in a positive presence of illicit substances.

There is however a category including samples of subjects in whom the type of sport is not recognizable for the incomplete information.

Positivity was found as follows: in 148 samples for 11-nor-carboxy-Δ -tetrahydrocannabinolic acid; in 28 samples for cocaine metabolites (benzoylecgonine); in 6 samples for amphetamine and 1 ephedrine. In 3 samples the 19-nortestosterone metabolites, and in 24 samples the testosterone/epitestosterone ratio (>4/1), were found to be altered.

## Discussion

The literature reports that assumption of illicit substances is a widespread problem in the young athlete population, not limited to the elite category. The results obtained in the anti-doping laboratories are a confirmation of this, and they also suggest the importance of carefully monitoring the progressive diffusion of this behavior.

Several and similar motivations normally lead to the assumption of illicit substances in both athletes and the general population. First of all we must consider that illicit substance abuse is included in the concept of drug addiction, and that the athlete population, especially if it is young, must be treated as a risk category.

Commonly both normal subjects regularly practising sport as well as athletes show several types of distress, which are often the expression of a tendency to anxiety or depression and secondary to their genetic pattern (also dependent on genetic pattern), or else due to the problems specifically related to the sporting environment [10]

When sport is regularly practiced it can have a positive impact and therapeutic effects on the young. Abuse of illicit substances determines the development of pharmacological addiction, and the level of fitness achieved is often perceived by athletes to be higher than that actually acquired with the sporting activity alone.

In addition to this, we should bear in mind that athletes, especially young ones, are widely exposed to the assumption of illicit substances. They often have the habit of self-prescribing illicit drugs, particularly during competitions. In the case of prolonged exercise this custom can lead to the regular assumption of illicit substances, with progressive enhancement of the dose and the effects. This is commonly a consequence of the need to compensate for states of depression and anxiety [11].

Several French authors have conducted studies demonstrating the strong relationship between endurance sports, doping and abuse of illicit substances [12]

In conclusion the large diffusion of illicit substance assumption in athletes has brought to light a new social reality, in which athletes and drug addicts are considered in two different ways. While for the population at large drug addiction is synonymous of weakness and dishonesty, athletes that habitually take illicit substances continue to be perceived positively even in this condition.

For this reason the role of the physician in Sport Medicine may be crucial in avoiding the risk that sport becomes a cover for this particular, deviant behaviour [13,14].

Coaches and the physicians who are previously informed of the existence of these problems can work together in order to promptly detect the symptoms of illicit substance abuse and consequently reduce the damages. This can be done by carrying out programmes for the prevention and recovery of symptomatic subjects.

Local health authorities must be responsible for providing specialist assistance and also for following up the event.

Thus Tuscany too can offer an important contribution to epidemiologic knowledge of the diffusion of illicit substance abuse in the sporting world, even if the investigation is particularly focussed on recreational sport.

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