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Interpretation of urinary concentrations of pseudoephedrine and its metabolite cathine in relation to doping control

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Until the end of 2003 a urinary concentration of pseudoephedrine exceeding 25 μg/mL was regarded as a doping violation by the World Anti-Doping Agency. Since its removal from the prohibited list in 2004 the number of urine samples in which pseudoephedrine was detected in our laboratory increased substantially. Analysis of 116 in-competition samples containing pseudoephedrine in 2007 and 2008, revealed that 66% of these samples had a concentration of pseudoephedrine above 25 μg/mL. This corresponded to 1.4% of all tested in competition samples in that period. In the period 2001–2003 only 0.18% of all analysed in competition samples contained more than 25 μg/mL. Statistical comparison of the two periods showed that after the removal of pseudoephedrine from the list its use increased significantly. Of the individual sports compared between the two periods, only cycling is shown to yield a significant increase.

Analysis of excretion urine samples after administration of a therapeutic daily dose (240 mg pseudoephedrine) in one administration showed that the threshold of 25 μg/mL can be exceeded. The same samples were also analysed for cathine, which has currently a threshold of 5 μg/mL on the prohibited list. The maximum urinary concentration of cathine also exceeded the threshold for some volunteers. Comparison of the measured cathine and pseudoephedrine concentrations only indicated a poor correlation between them. Hence, cathine is not a good indicator to control pseudopehedrine intake. To control the (ab)use of ephedrines in sports it is recommended that WADA reintroduce a threshold for pseudoephedrine. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: doping; urine; pseudoephedrine; cathine; sports; ephedrine; amphetamine

Introduction

Stimulants are synthetic derivates of adrenaline and have similar pharmacological effects on mental function and behaviour, producing excitement and euphoria and increased motor activity. The ephedrines are one of the oldest groups of therapeutically applied stimulants. These substances can be found naturally in ephedra or ma huang (*Ephedra sinica*). All extracts of these plants are still used in nutritional supplements.[1]

Ephedrine-type stimulants are applied therapeutically for decongesting the respiratory tract. For example, pseudoephedrine (PEPH) is frequently used for allergic rhinitis while preparations containing ephedrine (EPH) are used in the treatment of coughs.

There has been much discussion concerning the effect of ephedrines in sports,[2–10] with conflicting findings concerning the ergogenity of PEPH. To control the use of ephedrines, the International Olympic Committee (IOC) and the World Anti Doping Agency (WADA) put them on the list of prohibited substances.[11,12] Because of the frequent therapeutic application and the debated ergogenic potency, the regulations are complicated: for cathine a threshold of 5 μg/mL and for ephedrine and methylephedrine a threshold of 10 μg/mL is applied. Until December 2003, PEPH and NEPH were also prohibited at concentrations higher than 25 and 10 μg/mL, respectively. Hence, the actual situation is that PEPH has been removed but its metabolite, cathine, remains on the prohibited list.

The aim of this study was to investigate urinary concentrations of PEPH and cathine after administration of different preparations and to compare the incidence of elevated PEPH-concentrations before and after its removal from the prohibited list. The results of the administration study were then compared with routine samples containing PEPH.

Experimental

Excretion study

The study was performed with six healthy volunteers (one female and five male) aged 23–39. The study protocol was reviewed and approved by the ethical committee of the Ghent University Hospital (UZ Gent, Project EC UZG 2006-404). Each volunteer signed an informed consent agreement and was given two tablets of Cirrus (UCB, Brussels, Belgium) containing 120 mg PEPH.HCl and 5 mg cetirizine.HCl each. Two weeks later, the same volunteers were given one tablet of Clarinase® (Schering-Plough, Brussels) containing 240 mg PEPH.sulfate and 10 mg loratidine

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Table 1. Routine samples with concentrations of PEPH above 25 µg/mL in the period 2001–2003 in our laboratory

Sport	No. of samples analysed	No of samples with PEPH >25 µg/mL	Percentage of samples with PEPH >25 µg/mL
Athletics	954	1	0.1
Cycling	3531	6	0.2
Tennis	134	1	0.7
Swimming	309	1	0.3
Ice hockey	190	2	1.1
Football	1070	3	0.3
Basketball	388	3	0.8
Tabletennis	132	1	0.8
Volleyball	441	2	0.5
Other sports	4216	0	0.0
Total	11 365	20	0.2

Table 2. Routine samples with concentrations of PEPH above 25 µg/mL in the period October 2007–December 2008 in our laboratory

Sport	No. of samples analysed	No. of samples with PEPH >25 µg/mL	Percentage of samples with PEPH >25 µg/mL
Athletics	447	2	0.4
Cycling	1548	60	3.9
Field hockey	116	2	1.7
Handball	153	1	0.7
Ice hockey	74	3	4.1
Indoor football	177	1	0.6
Martial arts	73	1	1.4
Judo	158	1	0.6
Rugby	29	1	3.4
Volleyball	295	5	1.7
Other sports	2878	0	0
Total	5948	77	1.3

each. Another two weeks later the same volunteers were given four tablets of Vasocedine Pseudoephedrine® (Qualiphar, Bornem, Belgium) containing 60 mg PEPH.HCl each. In all cases PEPH was administered in one administration of the maximal therapeutic dose over a 24-h period.

Urine samples were collected before (0h) and quantitatively at 1, 2, 3, 6, 9, 12 hours after intake of the tablets. Additional samples were collected after 24, 36, 48, 72, 96 and 120 h. All urine samples were stored at -20 °C awaiting analysis. Volume and density were measured and all samples were analysed in duplicate. Samples were analysed using a previously described LC-MS/MS method for the quantitation of ephedrines.[13]

Routine samples

To investigate the use of PEPH in sports, all in-competition routine urine samples containing PEPH (LOD 1 µg/mL) were collected from the fourth quarter of 2007 until the end of 2008. The concentration of PEPH and its metabolite cathine were determined using a previously described GC-NPD method[14] and compared with concentrations of PEPH in samples from the period 2001–2003 (before removal of PEPH from the list).

First, the association between sports type and PEPH concentration (above and below 25 µg/mL) in both periods (Table 1 and Table 2) was determined. Fisher's exact test was used as the conditions for performing a chi-square test were not fulfilled due to small cell values. Given the dimensions of both tables, a large number of Monte Carlo simulations (5×10^8) was used to compute the *p* values. Second, the common sports between both periods (athletics, cycling, ice hockey and volleyball) were compared. Since sufficient samples, for both PEPH concentrations above and below 25 µg/mL, are present for cycling in both periods, a chi-square test was used to test the null hypothesis that the number of samples with a PEPH concentration higher than 25 µg/mL is equal in both periods. Because of small cell values, Fisher's exact test (using the hypergeometric distribution) was used for the three other sports. Finally, a chi-square test was used to test whether the proportion of PEPH concentrations exceeding 25 µg/mL is equal in both periods for all sports combined. CRAN R version 2.8.1 was used to perform the statistical analyses.

Results and Discussion

Excretion study

Large individual differences were found in the concentrations of PEPH and cathine. A similar variation has also been observed in a previous study measuring urinary PEPH concentrations.[15] This variation can be assigned to the urinary pH and the urinary flow.[16,17]

After Clarinase intake, PEPH could already be detected one hour after intake (Figure 1). T_{max} varied between 4 h and 24 h. Maximum concentrations ranged between 65 and 233 µg/mL. The total amount of unchanged drug excreted during the first 12 hours varied between 21% and 29% of the administered dose.

For Vasocedine maximum concentrations of PEPH were reached between 4 and 12 h after administration. Maximum concentrations varied between 95 and 229 µg/mL. The total amount of unchanged drug excreted during the first 12 hours was between 27% and 50% of the administered dose. After intake of Cirrus, maximum urinary concentrations were between 98 and 163 µg/mL and the total excreted amount of unchanged drug varied between 19% and 41%.

The result of this excretion study show that urinary PEPH concentrations in an individual, taking the maximal therapeutic daily dose (24 h period), can greatly exceed the previously used threshold of 25 µg/mL. These observations were supported by previous studies. Tseng *et al*.[18] found maximum urinary concentrations of 114 µg/mL after administration of a single dose of 30 mg of PEPH.HCl. Chester *et al*. administered 360 mg of PEPH.HCl over a 36 h period.[15] The maximum urinary concentration of PEPH was 215 µg/mL. In another study[19] a single dose of 120 mg PEPH.HCl was administered and the maximum urinary concentration of PEPH was 91 µg/mL.

Cathine was also detected in the urine samples for all preparations (Figure 1). Maximum urinary concentrations varied between 3.66 and 10.39 µg/mL for Clarinase, 2.3 and 7.43 µg/mL for Cirrus and between 3.3 and 12 µg/mL for Vasocedine, respectively. Excreted amounts were between 0.4% and 2.6% of the dose. This low percentage is in accordance with previous work stating that cathine is only a minor metabolite of PEPH.[16,19,20] Nevertheless, the results in this work also show that exceeding the threshold of 5 µg/mL (Figure 1) is possible after administration

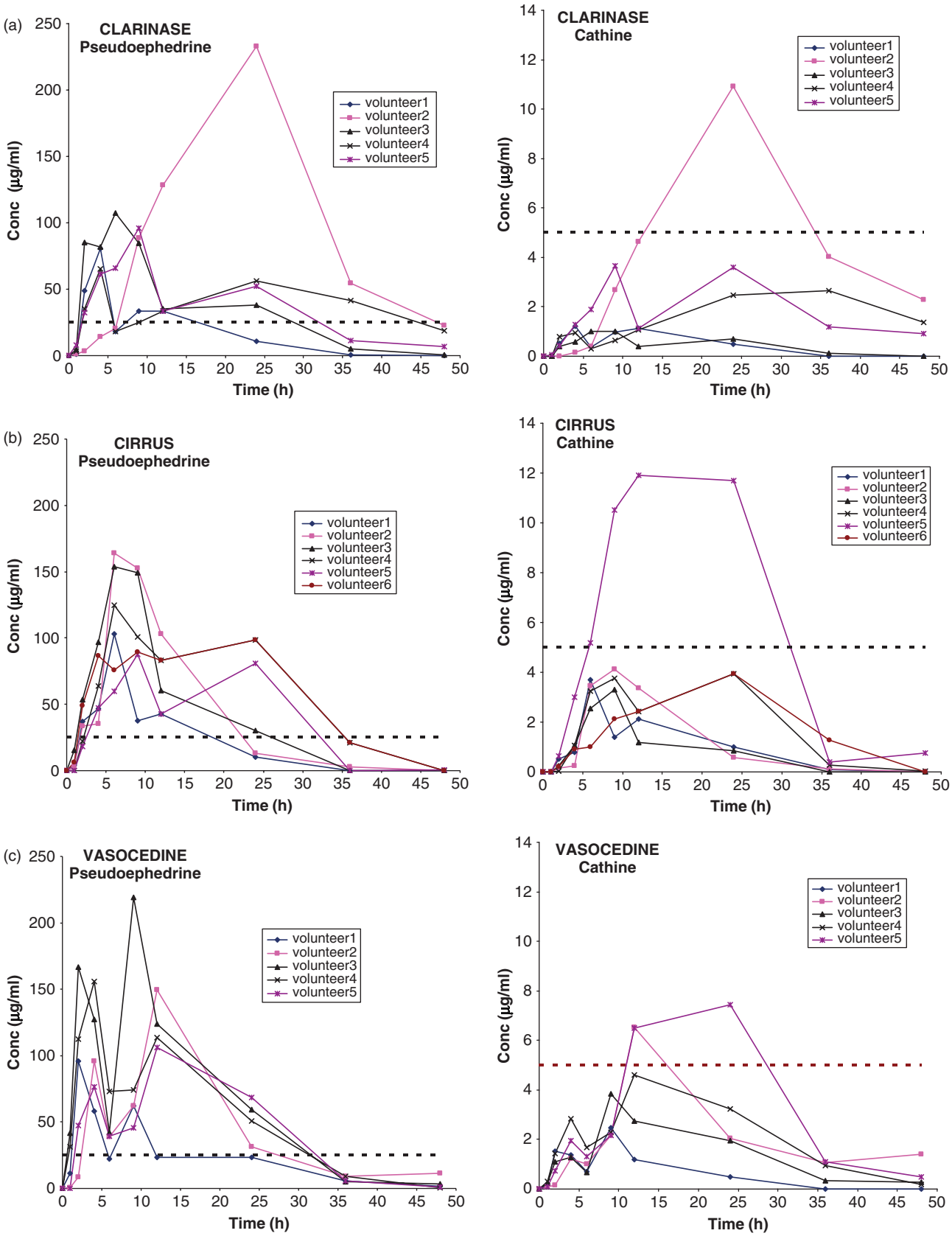

Figure 1. Urinary excretion curves for pseudoephedrine and cathine after administration of (A) Clarinase (240 mg pseudoephedrine sulphate), (B) Cirrus (2 × 120 mg pseudoephedrine.HCl) and (C) Vascosedine (4 × 60 mg pseudoephedrine.HCl). Dotted line: threshold for cathine (5 µg/ml), former threshold for pseudoephedrine (25 µg/mL).

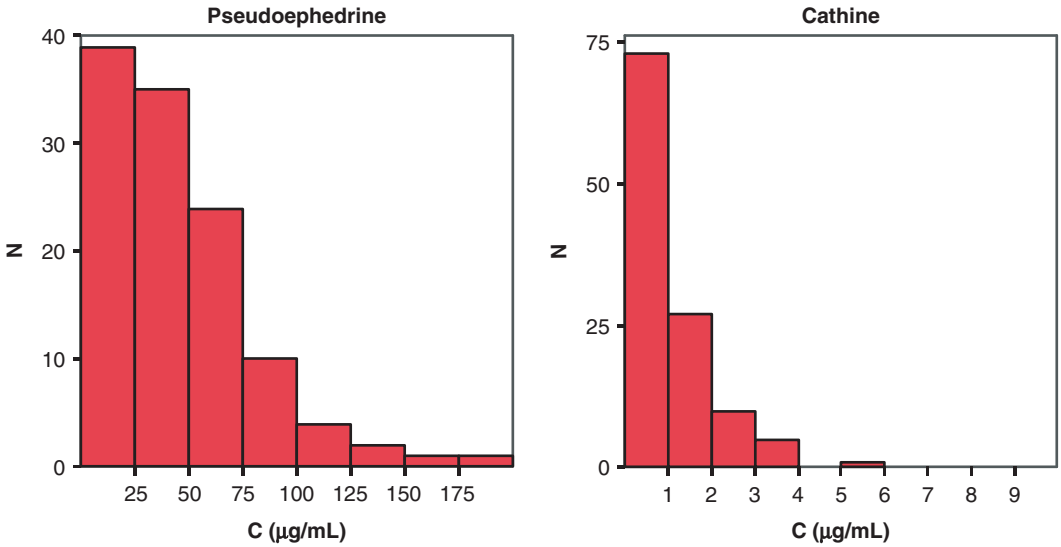

Figure 2. Distribution of PEPH and cathine levels in routine samples of 116 athletes tested for doping control purposes in the period from October 2007 to December 2008.

of high therapeutic doses of PEPH. This is in contrast with the only published scientific paper describing quantitative data on the excretion of cathine after PEPH intake so far, which indicates that after 120 mg PEPH.HCl the maximum concentration never exceeded 1 µg/mL.[19]

Routine samples

Before the removal of PEPH from the WADA-list, only few samples were declared positive for PEPH in the year 2001–2004 in our laboratory. Only 20 samples out of 11 365 (0.18%) (Table 1) contained more than 25 µg/mL of PEPH. These positive findings were always in combination with cathine concentrations below 7 µg/mL. Since the fourth quarter of 2007, 116 samples containing PEPH were collected. The distribution of PEPH concentrations in these samples is presented in Figure 2. Concentrations of PEPH in these samples varied between 5.2 and 197 µg/mL. 66% of these samples contained a concentration of PEPH above the previously used threshold of 25 µg/mL (Table 2). Taking into account the previously applied threshold, the highest percentages of adverse analytical findings (AAFs) would be observed in cycling, rugby and ice hockey. A total of 1.4% of the in competition-samples analysed (5948) would result in an AAF.

Testing the association between PEPH concentration and type of sports yielded a p value of 2×10^{-9} for the period from October 2007 until December 2008, thereby causing the rejection of the null hypothesis that PEPH concentration and type of sports are independent from one another. For the period from January 2001 until December 2003, a p value of 2.6×10^{-5} was obtained, yielding the same conclusion. In other words, the percentage of samples containing a PEPH concentration higher than 25 µg/mL is shown to differ significantly between sports types in both periods. In the period January 2001 until December 2003 ice hockey had the highest contribution to this p value and in the period from October 2007 until December 2008 cycling yielded the highest contribution to the p value. When the four common sports were compared between the two periods, the chi-square test yielded a p value of 3.0×10^{-26} for cycling, clearly leading to the rejection of the null hypothesis. Using the Fischer's test, the following p values were obtained for the other sports: 0.24 for athletics, 0.14 for ice hockey and 0.12 for volleyball, resulting in acceptance of the null hypothesis for each of these sports. Finally, comparing the two periods for all sports combined, a p value of 2.1×10^{-20} was obtained, thereby causing the rejection of this null hypothesis. Hence a significant difference was observed between the two periods for all sports combined and it can thus be concluded that the use of PEPH has increased since its removal from the prohibited list.

These results are remarkable when compared with a study performed after caffeine was removed from the list, which showed no increase in its use.[21]

Comparison of excretion study data and data from routine samples

To further understand the kinetics of PEPH and its metabolite, the relationship between PEPH and cathine was investigated with particular regard to the question of whether high concentrations of PEPH could yield high concentrations of cathine. Concentrations of PEPH and cathine of the excretion study were plotted against each other (Figure 3a). Only a weak positive correlation could be found ($R^2 = 0.38$) making it difficult to associate concentrations of PEPH with cathine. A similar weak correlation ($R^2 = 0.33$) was found when PEPH and cathine concentrations in routine samples were compared.

Conclusions

The administration experiments in this work show that concentrations of cathine and PEPH are difficult to correlate. Hence, cathine is not a good indicator for the control of the (ab)use of PEPH in sports. The excretion study also demonstrated that therapeutic use of PEPH can lead to positive testing, taking into account the threshold of 25 µg/mL previously used by WADA.

Comparison of urinary PEPH concentrations in samples, collected before and after removal of PEPH from the prohibited list, shows that the use of PEPH has increased significantly. Hence, the reintroduction of a threshold for PEPH, to control its (ab)use in sports, is highly recommended.

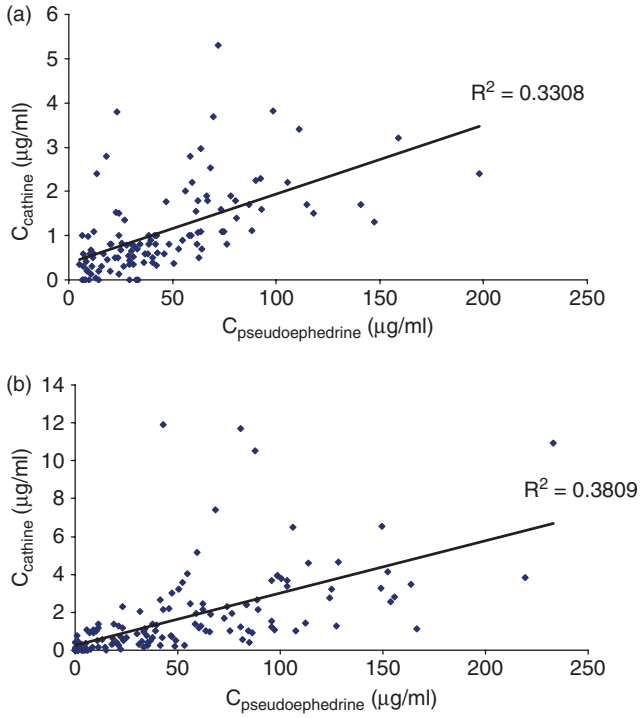

Figure 3. Correlation between PEPH and cathine in the routine samples (a) and excretion urine samples (b).

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