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Urinary concentrations of inhaled and orally administered salbutamol

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Introduction

Asthma is a well know respiratory illness with a low mortality rate and can be triggered by various factors such as viruses, food, smoke, dust, pollen, chemicals, emotional needs, temperature changes and exercise. The symptoms include breathlessness, tiredness, wheezing and chronic coughing. Chronic coughing during exercise is referred to as exercise induced asthma. Exercise induced asthma is very common during moderate to heavy exercise. The prevalence of exercise induced asthma is estimated to be between 15-20% of the population. Approximately 80% of asthma sufferers however experience exercised induced asthma. The symptoms of asthma normally present within 5 – 8 minutes after the commencing of exercise and can increase in severity 3 – 5 minutes after the exercise have been stopped.

β_2-adregeneric agonists such as salbutamol (albuterol) are the treatment of choice in asthma patients. Salbutamol can be administered through inhalation, orally or by intravenous injection. The adult inhalation dosage is 1-2 puffs 3-4 times a day. In the case of exercised induced asthma 2 puffs is used before commencing with exercise. Salbutamol is not extensively metabolised in the lungs. The concentration of salbutamol after inhalation depends on the percentage of the dose that is swallowed after impaction in the mouth and throat as well as absorbance from the gastrointestinal tract[1]. The oral dosage is 2-4 mg 3-4 times a day while the intravenous dosage is 4 μg/kg four hourly.

Athletes not only use salbutamol for exercised induced asthma but also for its anabolic and stimulating side effects[2]. The use of β_2-agonists is therefore prohibited by WADA.

Salbutamol, salmeterol, formoterol and terbutaline when administered by inhalation require a Therapeutic Use Exemption (TUE).

This study was conducted in 2006. According to the 2006 WADA list[3] a salbutamol concentration (free plus glucuronide) between 100 and 1000 ng/ml had to be reported as a β_2-agonist. WADA changed the 100 ng/ml to 500 ng/ml as from 1 January 2007[4]. In both the 2006 and 2007 list a concentration greater than 1000 ng/ml will be considered an adverse analytical finding for steroids despite the granting of a TUE unless the athlete proves that the abnormal result was the consequence of the therapeutic use of inhaled salbutamol.

The aim of this study was to determine the dosage of inhaled salbutamol which will result in a urinary concentration of more than a 100 ng/ml and to compare the urinary concentrations obtained after inhaled salbutamol with an oral therapeutic dosage.

Experimental

Chemicals and reagents

All reagents and solvents were of analytical grade. Aqueous solutions and buffers were prepared with 18 MΩ.cm water (Milli-Q, Millipore). β-glucuronidase from *E.Coli* was obtained from Roche Diagnostics and salbutamol and terbutaline reference standards were purchased from Sigma-Aldrich.

Excretion study

The clinical study was approved by the Ethics Committee of the University of the Free State and informed consent was obtained from the volunteers. It was an open single dose study conducted in 4 phases on 6 healthy male volunteers over a period of 23 days. Salbutamol (Venteze®) was administered by inhalation in different doses during the first three parts (2, 4 and 6 puffs respectively) of the study. Finally, an oral therapeutic dosage of 2 mg salbutamol (Venteze®) was administered. Fractional urine samples were collected at 2, 4, 8, 12 and 24 hours post administration for each of the different phases of the study. The urines samples were stored at -20^{o}C awaiting analysis.

Sample preparation

1 ml phosphate buffer (pH = 7), 50 µl Terbutaline (8.170 µg/ml, ISTD) and 25 µl β-glucuronidase were added to 1 ml urine. Hydrolysis was performed at 50 ^{o}C for 2 hours. Approximately 2 g Na_2SO_4 was added to the sample. The samples were then made alkaline by the addition of 200 µl K_2CO_3/$NaHCO_3$ buffer (pH = 9.6). Acetonitrile (0.5 ml) was added and the mixture extracted with 3 ml diethyl ether. After centrifugation the organic phase was separated and evaporated at 60^{o}C under high purity nitrogen. The dried residue was dissolved in 100 µl of mobile phase. The samples together with calibration standards, urine blanks and control samples were analysed by LC-MS/MS.

Instrumental analysis

Instrument:	Agilent 1100 HPLC with API 2000 Mass Spectrometer		
Column:	Phenomenex Luna C18 (150 mm × 2.00 mm) 5µ		
MS Parameters:	Scan type:	MRM	
	Transitions:	m/z 240 → 147.9	salbutamol
		m/z 226.1 → 151.9	terbutaline
Ionisation mode:	Positive		
Ion source:	Turbo Spray		
Dwell time:	300 ms (for each analyte)		
Time:	10 min		
LC Parameters:	Flow rate:	200 µl/min	
	Injection volume:	1 µl	
Mobile phase:	%A (1mM Ammonium acetate in 0.1% Acetic acid):		90
	%B (Acetonitrile):		10

Results and Discussion

On day one, 2 puffs equivalent to approximately 200 µg of salbutamol were administered through inhalation. 4 out of the 6 volunteers in the study showed salbutamol concentrations above 100 ng/ml 2 – 6 hours post administration (Figure 1).

In the second part of the study, on day 8 the volunteers were administered 4 puffs of salbutamol. The urinary salbutamol concentrations of all the volunteers except subject 6 were above 100 ng/ml 2 – 8 hours post administration (Figure 2). Four of the volunteers showed

salbutamol concentrations above 200 ng/ml. The highest salbutamol concentration measured was 304 ng/ml.

Six puffs of salbutamol or 600 μg of salbutamol were administered to the volunteers on day 15. Compared to 4 puffs some inconsistent results were obtained. Two volunteers showed salbutamol concentrations above 700 ng/ml and a further two volunteers showed concentrations above 300 ng/ml. The last two volunteers showed salbutamol concentrations of less than 200 ng/ml (Figure 3) but all 6 volunteers had at least one concentration above 100 ng/ml.

During the last phase of the study on day 22 a tablet containing 2 mg of salbutamol was administered to. Urinary salbutamol concentrations in excess of 1000 ng/ml were found in 4 of the volunteers. The peak salbutamol concentrations for these volunteers varied between 1215 and 2787 ng/ml. The peak urinary salbutamol concentration of subject 1 was just below 1000 ng/ml and that of subject 6 was 401 ng/ml (Figure 4).

Conclusion

Our results show that the intake of only 2 puffs (200 μg) of salbutamol by inhalation can give urinary salbutamol concentrations above 100 ng/ml.

Two puffs or 200 μg salbutamol is the recommended dosage for exercised induced asthma. According to the 2006 list all salbutamol concentrations above 100 ng/ml had to be reported. This resulted in a lot of salbutamol quantifications for laboratories as in most of these cases salbutamol was declared on the doping collection forms and corresponding TUE's for the condition existed. Generally the dosage of salbutamol declared was either 1 to 2 puffs or 2 to 4 puffs.

The oral administration of salbutamol led to higher urinary salbutamol concentrations than administration by inhalation. These results is in agreement with the results obtained by Ventura *et al*[3,4].

Since 1 January 2007 WADA has changed the reporting level for salbutamol as a β2-agonist from 100 ng/ml to 500 ng/ml. If this rule is applied to our results only two cases were found

after the administration of 6 puffs. Our results therefore support the decision of WADA to change the level from 100 to 500 ng/ml.

These results are in support of the 1000 ng/ml threshold level as the administration of one 2 mg tablet may lead to urinary salbutamol concentrations above a 1000 ng/ml.

References

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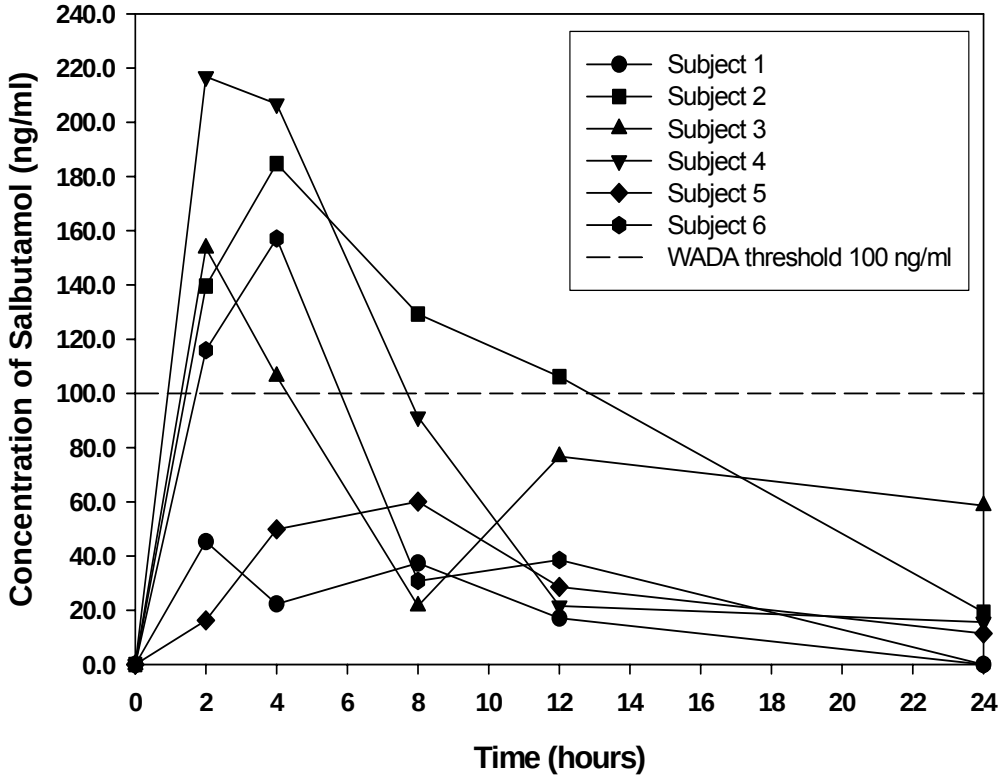

Figure 1: Urinary salbutamol concentrations in 6 male volunteers after the administration of 2 puffs of salbutamol.

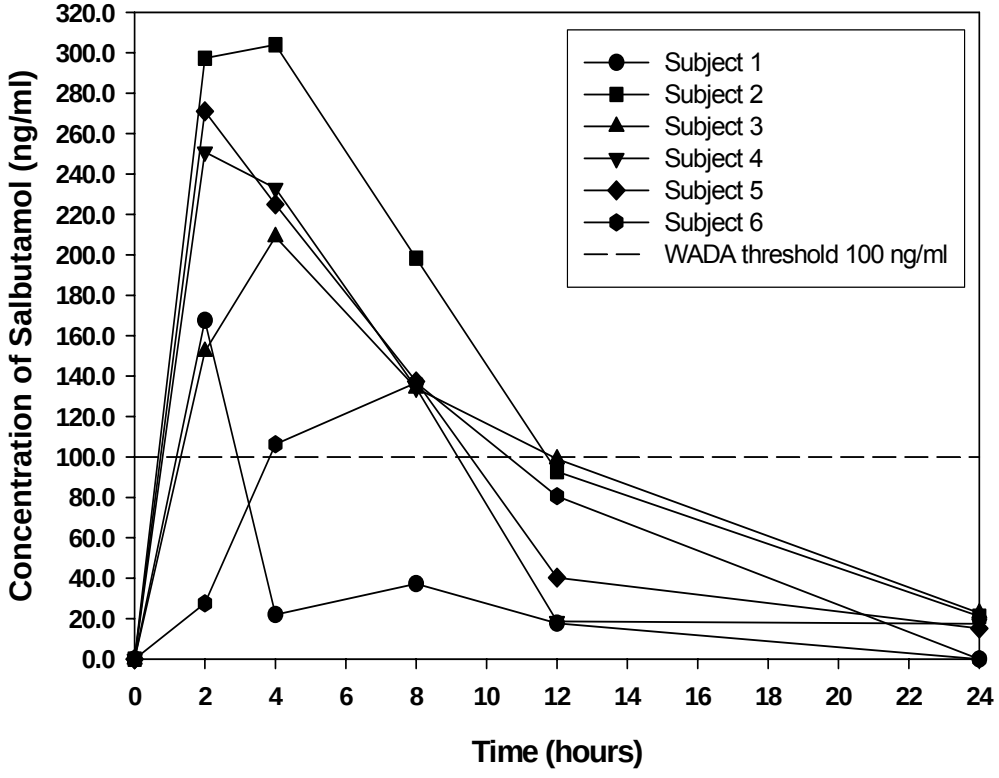

Figure 2: Urinary salbutamol concentrations in 6 male volunteers after the administration of 4 puffs of salbutamol.

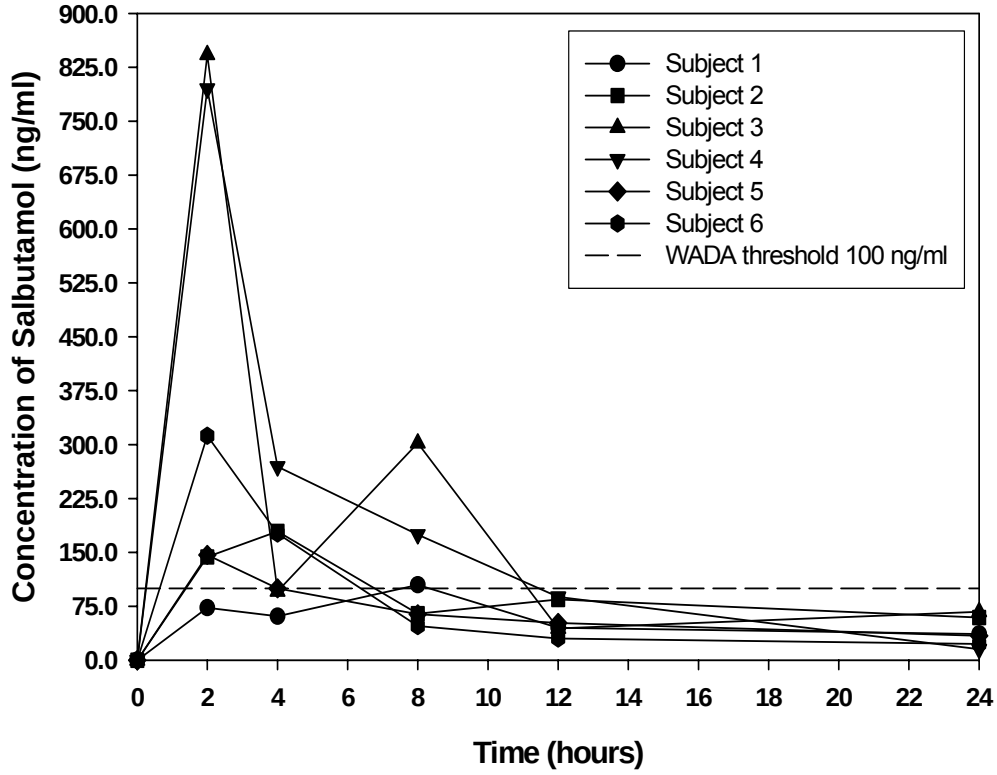

Figure 3: Urinary salbutamol concentrations in 6 male volunteers after the administration of 6 puffs salbutamol.

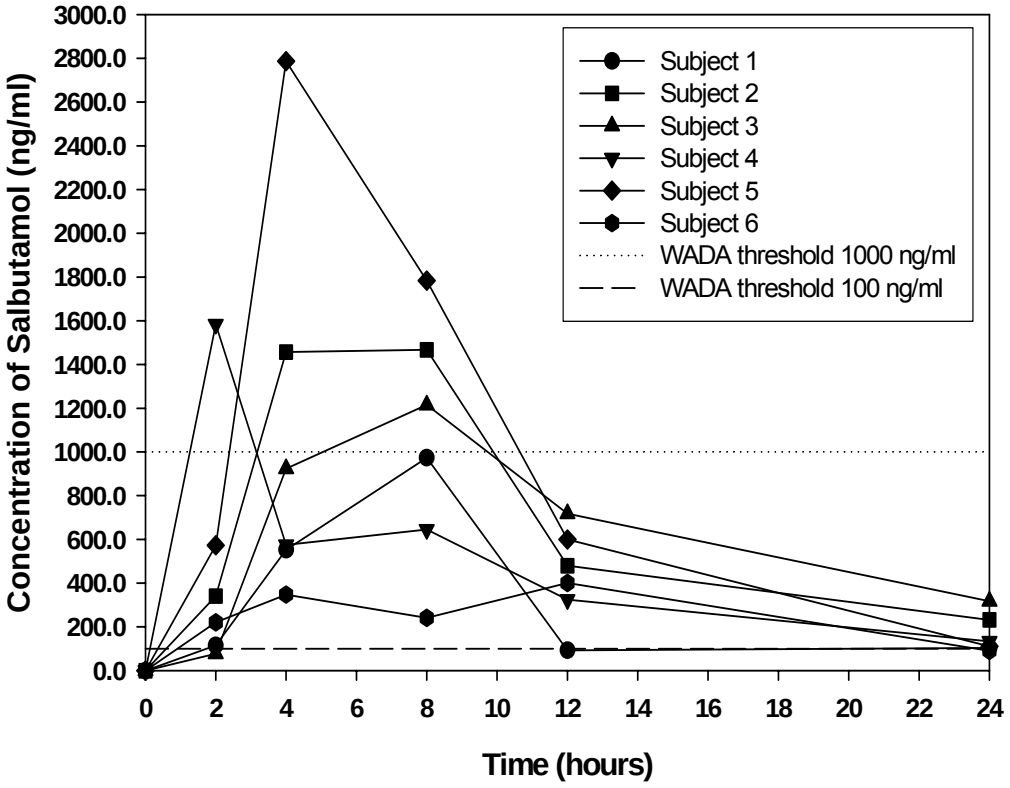

Figure 4: Urinary salbutamol concentrations in 6 male volunteers after the administration of a 2 mg tablet of salbutamol.