Urine Concentrations of Inhaled Salmeterol and its Metabolite α-Hydroxysalmeterol in Asthmatic and Non-Asthmatic Subjects

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Abstract

Salmeterol is a long-acting beta₂-agonist, which is on the WADA prohibited list, but can be used by athletes in therapeutic doses by inhalation. The prohibited list, however, contains no urinary threshold for salmeterol, which gives athletes the opportunity to inhale unlimited doses of salmeterol. In doping controls, metabolites may be used as markers for misuse of substances. No studies have determined urine concentrations of α-hydroxysalmeterol, the metabolite of salmeterol. Furthermore, the metabolism and excretion of salmeterol may vary between asthmatics and non-asthmatics. We determined the serum and urinary concentrations of salmeterol and its metabolite α-hydroxysalmeterol after inhalation of 100 µg salmeterol in ten asthmatics and ten non-asthmatics. Blood samples were collected at baseline and ½, 1, 2, 3, 4 and 6 hours after the administration of salmeterol. Urine samples were collected at baseline and 4, 8 and 12 hours after administration. The urinary concentration of salmeterol following enzymatic hydrolysis of the glucuronide fraction was 0.38 ± 0.26 ng·mL⁻¹ in asthmatics and 0.38 ± 0.22 ng·mL⁻¹ in non-asthmatics, 4 hours after inhalation. The highest median serum concentration (Cmax) was 0.07 ± 0.03 ng·mL⁻¹ in asthmatics after 30 minutes (Tmax) and 0.06 ± 0.03 ng·mL⁻¹ in non-asthmatics. No statistical differences were found in Cmax or Tmax of salmeterol between asthmatics or non-asthmatics in neither the serum nor urine samples. The highest median urinary concentration of α-hydroxysalmeterol following enzymatic hydrolysis of the glucuronide fraction was 2.86 ± 1.75 ng·mL⁻¹ in asthmatics, 4 hours after inhalation of salmeterol. In non-asthmatics, it was 2.73 ± 2.08 ng·mL⁻¹. The α-hydroxysalmeterol had a significantly (p<0.05) higher concentration after 12 hours compared with non-asthmatics. In doping control, α-hydroxysalmeterol may be a more suitable marker for excessive use of inhaled salmeterol, due to its higher concentration in urine.

Keywords: α-hydroxysalmeterol; Salmeterol; Pharmacokinetics; Beta₂-agonists; Asthma; Doping

Introduction

The prevalence of asthma in the population in western countries is between 7 and 10% and has been rising over a period of twenty years [1]. Elite athletes have a higher prevalence of asthma, airway hyperresponsiveness (AHR) and airway symptoms than the general population [2-4]. It is well known that asthmatics with Exercise-Induced Asthma (EIA) respond well to anti-asthmatic pharmacological treatment such as inhaled corticosteroids, inhaled beta₂-agonists and leukotriene receptor antagonists [5,6]. Elite athletes suffering from asthma should be treated in accordance with the suggested GINA guidelines [7], and therefore, long-acting beta₂-agonists (LABA) may often be needed due to the frequent use of short-acting beta₂-agonists (SABA). However, the use of beta₂-agonists in sports is restricted by anti-doping regulations, and beta₂-agonists are on the World Anti-Doping Agency’s (WADA) prohibited list [8]. This is in part due to the performance enhancing effects beta₂-agonists may provide during physical performance, resulting in an unfair competitive advantage when used by healthy athletes and partly due to health risk associated with excessive long-term use [9,10].

As of 2012, the WADA prohibited list only requires documentation of asthma for the use of terbutaline, where as salbutamol, salmeterol and formoterol are allowed when taken by inhalation in therapeutic doses [8]. Thus, therapeutic use of salbutamol, salmeterol and formoterol only requires a declaration of use, where as earlier a therapeutic use exemption (TUE) was necessary. Consequently, the prohibited list now contains therapeutic and urinary thresholds of salbutamol and formoterol, while salmeterol only have a therapeutic threshold and no urine threshold.

Several studies have examined the blood and urine concentrations of inhaled and oral administrated salbutamol, thus providing support for setting a urinary threshold of 1000 ng·mL⁻¹ on the prohibited list [11-13]. Few studies have examined blood concentrations of salmeterol, only one has measured the urine concentrations, and none have measured its metabolite, α-hydroxysalmeterol, in the urine [14,15]. As a result, no urine concentration threshold exits on the prohibited list for salmeterol and α-hydroxysalmeterol, giving athletes an open window for supra-therapeutic use of salmeterol. Furthermore, no studies have compared urine concentrations of asthmatics with non-asthmatics after inhalation of salmeterol. Studies are needed to exclude the possibility of any differences in the metabolism of salmeterol between asthmatics and non-asthmatics.

The main purpose of the present study was therefore to measure the concentrations of the metabolite, α-hydroxysalmeterol, in urine after inhalation of 100 µg salmeterol. A secondary purpose was to determine differences in the metabolism and excretion of salmeterol and α-hydroxysalmeterol between asthmatic and non-asthmatic subjects.

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Methods and Materials

Study design

This study was an open-labeled survey. Each participant was asked to discontinue any use of medication, including beta,-agonists, 14 days before the study. At the pre-examination visit, the participants were interviewed about asthma symptoms. They filled out a questionnaire, and lung function measurements and metacholine bronchial challenges were performed to evaluate the diagnosis of asthma. Furthermore, measurements of Fractional exhaled nitric oxide (FeNO) as well as skin prick tests were performed on all participants. Written informed consents were obtained from all subjects. The protocol was approved by the local Ethics Committee (no. H-C-2009-044). The study was conducted in accordance with the protocol and the guideline for Good Clinical Practice (GCP).

Subjects

In total, ten asthmatics and ten non-asthmatics, aged 24.6 ± 3.9 yr was enrolled (Table 1). They were all male and non-smokers. The participants were physically active less than six hours per week. Asthma was defined based on asthma symptoms and a positive test showing reversible airway disease. The severity of the asthma was based on the recommendations made by the GINA guidelines [7].

All participants in the asthmatic group had been using beta,-agonists for a period of at least one year. They were excluded if they exercised more than six hours per week, suffered from other illnesses apart from asthma and allergy, were smokers or ex-smokers (≥ 10 pack/year(s)) or had suffered from an upper or lower respiratory tract infection within two weeks before the study.

Medication

On the day of the study, all participants inhaled salmeterol xinafoate corresponding to a total of 100 µg salmeterol as a single dose (Serevent Diskus 50 µg), and they were all instructed in the inhalation technique and supervised during inhalation.

Blood samples

Blood samples (9 mL) were collected from the medial cubital vein at baseline (t0) and ½, 1, 2, 3, 4 and 6 hours after the administration of salmeterol. Each tube was placed for 30 minutes at room temperature and then centrifuged at 3000 rpm, 704 x g for 15 minutes. Serum was collected in 3.6 mL cryo tubes and frozen at -80°C until analysis.

Urine samples

Urine was collected in 40 mL aliquots from all participants before the administration of salmeterol, at baseline (t0) and again 4, 8 and 12 hours after administration. The samples were immediately frozen and stored at -80°C until analysis. The 12-hour samples were obtained in the participants’ homes, frozen and stored at -20°C and delivered the next day to the department and stored at -80°C.

FeNO

Fractional exhaled nitric oxide was measured according to the recommendations of the American Thoracic Society (ATS) / European Respiratory Society (ERS) with an NO analyzer (NIOX MINO; Aerocline, Stockholm, Sweden) at a flow of 50 mLs⁻¹ [16].

Lung function measurements

Spirometry was performed in accordance with the ATS / ERS recommendations [17]. Forced expired volume in 1 s (FEV₁) and forced vital capacity (FVC) were measured using a 7-L drip wedge spirometer (Vitalograph, Buckingham, UK). The differences between the two highest values of FEV₁ and FVC should be less than 150 mL or 5%. Predicted values of FEV₁ were based on reference values according to Nysom and colleagues [18].

Questionnaires

The participants were asked to fill out various questionnaires before the clinical and physical tests. Subjects were asked about respiratory and allergic symptoms, use of medication, hospital referrals and general practitioner or specialist visits. The subjects also completed an asthma control questionnaire (ACQ) and an asthma questionnaire of life quality (mini AQLQ) [19].

Skin prick test

A skin prick test for ten standard aeroallergens (birch, grass, mugwort, horse, dog, cat, house dust mites (Dermatophagoides pteronyssinus and Dermatophagoides farinae), and molds (Alternaria iridis and Cladosporium herbarum) (Soluprick SQ System; ALK-Abelló, Hoersholm, Denmark) was performed [20]. Atopy was defined as the development of a wheal of at least 3 mm in diameter in response to at least one allergen.

Bioanalytical procedures

Quantitative determinations of salmeterol and its major metabolite, α-hydroxysalmeterol, were performed in urine and serum by the WADA accredited Norwegian Doping Control Laboratory. The analytical methods were validated according to in-house procedures which are in agreement with WADAs regulations.

All reagents used in the analysis were of analytical grade and all solvents were of HPLC grade. Water was obtained using a Millipore purification system (Millipore, Bedford MA, USA). α-Hydroxysalmeterol reference material was kindly provided by GlaxoSmithKline (Brentford, UK) with assistance from WADA.

Analysis of urine samples

A urine aliquot of 1 mL was diluted with an equal volume of water. Salmeterol-d₄, (AH Diagnostics, Aarhus, Denmark) and ractopamine-d₆, (RIVM, Bilthoven, the Netherlands) were selected as internal standards for the measurement of salmeterol and α-hydroxysalmeterol, respectively. The internal standard solution (25 µL, salmeterol-d₄ and ractopamine-d₆, each 0.1 µM.L⁻¹ in methanol) was added, and the samples were then subjected to enzymatic hydrolysis by β-glucuronidase (25 µL, E.coli K12, Roche Diagnostics, Mannheim, Germany). This was followed by solid phase extraction (SPE) on Oasis MCX columns (Waters, Milford MA, USA) according

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age(yr)</th>
<th>Height(m)</th>
<th>Weight(kg)</th>
<th>FEV₁/FVC</th>
<th>Bronchial Challenge</th>
<th>FeNO(ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthmatics</td>
<td>26.1 ± 4.4</td>
<td>1.81 ± 0.06</td>
<td>75 ± 6.7</td>
<td>0.78 ± 0.09*</td>
<td>-21.7 ± 3.7% PD in FEV₁</td>
<td>28.1 ± 9.7</td>
</tr>
<tr>
<td>Non-asthmatics</td>
<td>23 ± 2.4</td>
<td>1.81 ± 0.04</td>
<td>75.5 ± 5.9</td>
<td>0.84 ± 0.05</td>
<td>-5.9 ± 3.4% PD in FEV₁</td>
<td>14.8 ± 3.4</td>
</tr>
</tbody>
</table>

Table 1: FEV₁/FVC, forced expired volume 1 s / Forced Vital Capacity; Bronchial Challenge to metacholine, PD > 20% in FEV₁, was considered a positive test, FeNO(ppb), fractional exhaled nitric oxide. *Significant (p<0.05) difference between asthmatics and controls. Mean ± SD.
to the manufacturer’s guidelines. The extracts were evaporated to dryness under nitrogen and reconstituted in 100 µL water: acetonitrile 95:5. Instrumental analysis was performed by liquid chromatography – tandem mass spectrometry (LC-MS/MS) on a Thermo Surveyor liquid chromatography system coupled to a Thermo TSQ Quantum IonMax triple-quadrupole mass spectrometer (Thermo-Fisher Scientific, San Jose CA, USA). The injection volume was 10 µL. The chromatographic separation was performed on a Thermo Betasil C18 column (150-2.1 mm, 3 µm particle size) with mobile phases consisting of water: acetonitrile 95:5 (A) and water: acetonitrile 5:95 (B), both containing 5 mM ammonium formate. The flow rate was 200 µL·min⁻¹, and the elution employed a linear gradient starting at 100 % A, decreasing to 30 % A in 10 minutes. The mass spectrometer was operated in positive electrospray ionisation (ESI+) mode with a spray voltage of 4 kV and a capillary temperature of 330°C. Nitrogen was used as sheath and auxiliary gases at flow settings of 40 and 20 arbitrary units, respectively. Data acquisition was performed in selected reaction monitoring (SRM) mode, and the acquisition parameters are shown in Table 2.

Five-point calibration curves covered a linear range of 0.025 to 2 ng·mL⁻¹ for salmeterol and 0.05 to 7.5 ng·mL⁻¹ for α-hydroxysalmeterol. The samples were analysed in batches of approximately 40 samples, with a new set of calibrators for each batch. The limits of quantification (LOQ), intermediate precision and recovery in the urine samples are summarised in Table 3.

The urinary concentrations of salmeterol and α-hydroxysalmeterol were normalised to a urine specific gravity of 1.020 g/mL⁻¹ by using the following equation:

\[
\text{Corrected urinary concentration} = \frac{\text{measured urinary concentration}}{\text{urine specific gravity}} - 1
\]

The reported urinary concentrations represent the sum of the glucuronide conjugates (expressed as the free drug following hydrolysis) and free drug concentrations in accordance with the WADA technical document [21].

### Analysis of serum samples

The analytical procedure was identical to the procedure used for the urine, except that the enzymatic hydrolysis step was omitted. The calibration ranges in the serum were 0.025 to 1 ng·mL⁻¹ for salmeterol and 0.010 to 1 ng·mL⁻¹ for α-hydroxysalmeterol. The limits of quantification (LOQ), intermediate precision and recovery in the serum samples are summarised in Table 3.

### Statistical analyses

The statistical software program SPSS 19.0 (SPSS, Inc., Chicago, IL) was used. Normality was tested using Shapiro-Wilk test. Normally distributed continuous variables were expressed as a mean ± standard deviation (SD) and differences between asthmatics and non-asthmatics were analyzed using an independent t-test. Skewed data was presented as a median ± interquartile range. Friedman test was used to detect differences between time points within each group and wilcoxon signed-rank test with a bonferroni correction was used to detect pair wise comparisons in case of a significant Friedman test. Mann-Whitney U test was used to detect differences between groups. Values are corrected for urine specific gravity before analyses. A p-value < 0.05 was considered statistically significant.

### Results

#### Urine concentrations

The highest observed individual urine concentration of α-hydroxysalmeterol was at 4 hours reaching 8.00 ng·mL⁻¹. The urine concentration of α-hydroxysalmeterol was similar between the groups during the first 4 hours with a median concentration of 2.86 ± 1.75 ng·mL⁻¹ in asthmatics and 2.73 ± 2.08 ng·mL⁻¹ in non-asthmatics. At 8 hours after administration the median concentrations were 2.45 ± 1.88 and 1.49 ± 1.73 ng·mL⁻¹ in asthmatics and non-asthmatics, respectively. At 12 hours, there was a significant (p <0.05) difference between asthmatics and non-asthmatics (2.05 ± 1.25 vs. 0.92±0.95 ng·mL⁻¹) (Figure 1). The urine concentrations of

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Collision energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol</td>
<td>416</td>
<td>380</td>
<td>20</td>
</tr>
<tr>
<td>d3-salmeterol (ISTD)</td>
<td>419</td>
<td>383</td>
<td>14</td>
</tr>
<tr>
<td>α-hydroxysalmeterol</td>
<td>432</td>
<td>396</td>
<td>19</td>
</tr>
<tr>
<td>Ractopamine (ISTD)</td>
<td>307</td>
<td>167</td>
<td>16</td>
</tr>
</tbody>
</table>

**Table 2:** Acquisition parameters for the analysis of salmeterol and α-hydroxysalmeterol.

<table>
<thead>
<tr>
<th></th>
<th>Salmeterol</th>
<th>α-hydroxysalmeterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine limit of quantification</td>
<td>0.12 ng·mL⁻¹</td>
<td>0.14 ng·mL⁻¹</td>
</tr>
<tr>
<td>Urine intermediate precision (level 1)</td>
<td>10 % (0.20 ng·mL⁻¹)</td>
<td>12 % (0.50 ng·mL⁻¹)</td>
</tr>
<tr>
<td>Urine intermediate precision (level 2)</td>
<td>11 % (1 ng·mL⁻¹)</td>
<td>10 % (5 ng·mL⁻¹)</td>
</tr>
<tr>
<td>Recovery (Level 1)</td>
<td>88 % (0.2 ng·mL⁻¹)</td>
<td>62 % (0.5 ng·mL⁻¹)</td>
</tr>
<tr>
<td>Recovery (Level 2)</td>
<td>73 % (1 ng·mL⁻¹)</td>
<td>73 % (5 ng·mL⁻¹)</td>
</tr>
<tr>
<td>Serum limit of quantification</td>
<td>0.04 ng·mL⁻¹</td>
<td>0.05 ng·mL⁻¹</td>
</tr>
<tr>
<td>Serum intermediate precision (0.025 ng·mL⁻¹)</td>
<td>13 %</td>
<td>18 %</td>
</tr>
<tr>
<td>Serum intermediate precision (0.20 ng·mL⁻¹)</td>
<td>5 %</td>
<td>22 %</td>
</tr>
<tr>
<td>Serum intermediate precision (1 ng·mL⁻¹)</td>
<td>4 %</td>
<td>16 %</td>
</tr>
<tr>
<td>Recovery (0.025 ng·mL⁻¹)</td>
<td>97 %</td>
<td>87 %</td>
</tr>
<tr>
<td>Recovery (0.2 ng·mL⁻¹)</td>
<td>78 %</td>
<td>98 %</td>
</tr>
<tr>
<td>Recovery (1 ng·mL⁻¹)</td>
<td>56 %</td>
<td>83 %</td>
</tr>
</tbody>
</table>

**Table 3:** Limits of quantification and intermediate precision for salmeterol and α-hydroxysalmeterol in urine and serum.
α-hydroxysalmeterol were significantly (p<0.05) higher after 4 hours compared to 12 hours in both asthmatics and non-asthmatics. When not corrected for USG the median concentrations of α-hydroxysalmeterol was 2.80 ± 3.23, 2.39 ± 2.68 and 1.68 ± 1.28 ngmL⁻¹, 4, 8 and 12 hours after administration in asthmatics and 2.95 ± 3.30, 1.18 ± 1.80 and 0.58 ± 0.70 ngmL⁻¹ in non-asthmatics, with no statistical differences between the groups. The highest individual concentration was 12.3 ngmL⁻¹ when not corrected for USG.

The maximum median urine concentration of salmeterol was observed 4 hours after administration, reaching 0.38 ± 0.26 ngmL⁻¹ in asthmatics and 0.38 ± 0.22 ngmL⁻¹ in non-asthmatics. At 4 hours after administration, the highest individual urine concentration was 0.54 ngmL⁻¹ in asthmatics and 0.62 ngmL⁻¹ in non-asthmatics. The urine excretion of salmeterol was decreased at 8 hours, reaching a median concentration of 0.19 ± 0.11 ngmL⁻¹ in asthmatics and 0.14 ± 0.21 ngmL⁻¹ in non-asthmatics. Twelve hours after administration of salmeterol, the median concentration was 0.06 ± 0.15 ngmL⁻¹ in asthmatics and 0.0 ± 0.15 ng·mL⁻¹ in non-asthmatics (Figure 2). No statistically significant differences were found between the groups. The urine concentrations of salmeterol were significantly (p<0.05) higher after 4 hours compared to 8 hours for both asthmatics and non-asthmatics. When not corrected for USG, the median concentrations were 0.37 ± 0.36 ngmL⁻¹, 0.17 ± 0.10 ngmL⁻¹ and 0.11 ± 0.09 ngmL⁻¹ 4, 8 and 12 hours after administration in asthmatics and 0.25 ± 0.30 ngmL⁻¹, 0.14 ± 0.16 ngmL⁻¹, and 0.08 ± 0.13 ngmL⁻¹ in non-asthmatics. The highest observed individual urine concentration of salmeterol was 0.74 ngmL⁻¹ when not corrected for USG.

**Serum concentrations**

The serum concentration of the metabolite α-hydroxysalmeterol was below LOQ (<0.05 ngmL⁻¹) in all observations apart from one measurement, which reached 0.05 ngmL⁻¹.

The maximum individual concentration of salmeterol in serum was 0.13 ngmL⁻¹ in asthmatics and 0.13 ngmL⁻¹ in non-asthmatics. The time to maximum concentration (Tmax) was observed at 30 minutes in both groups. The highest median serum concentration also occurred at 30 minutes, reaching 0.07 ± 0.03 ngmL⁻¹ and 0.06 ± 0.03 ngmL⁻¹ for asthmatics and non-asthmatics, respectively (Figure 3). Salmeterol could not be detected from the serum at 6 hours in both groups. No statistically significant differences were found between the groups. The area under curve (AUC) was 0.12 ngmL⁻¹ h in asthmatics and 0.08 ngmL⁻¹ h in non-asthmatics.

**Discussion**

For the first time, the present study provides concentrations of the metabolite, α-hydroxysalmeterol, in the urine of both asthmatic and non-asthmatic subjects. In doping analysis, drug metabolites are often used as markers. For the basis of doping controls, α-hydroxysalmeterol could be used as a marker for excessive use of salmeterol. The higher concentrations of the metabolite make it easier to quantify during laboratory procedures making it more suitable for doping control procedures. This has also been proposed by Deventer et al. were the authors suggest α-hydroxysalmeterol as a consideration when detecting therapeutic use of inhaled salmeterol [15]. This is also supported by the sensitivity in this study were the LOQ for α-hydroxysalmeterol of 0.14 ngmL⁻¹ allowed detection and quantification of all obtained samples from the participants.

The concentration of α-hydroxysalmeterol in urine was significant higher in asthmatics at 12 hours after the inhalation of salmeterol. Several factors could be the reason for this finding. Firstly, the analytical
method has a certain degree of variability, expressed as the intermediate precision, as shown in table 3. Secondly, the inhalation technique might vary between the groups, resulting in lower drug uptakes [22,23]. However, the participants were taught to inhale properly and were supervised during administration of the drug. Lastly, habitual use of beta₂-agonists among the asthmatics could potentially induce hepatic cytochrome P450 3A4 (CYP3A4) enzyme activity, resulting in a higher rate of aliphatic oxidation of salmeterol [14,24,25]. Although, the latter seems unlikely as habitual therapeutic medication with beta₂-agonists are inhaled in very low doses (<1 mg). Therefore, the difference found between asthmatics and non-asthmatics may be of minor importance, and perhaps due to analytical variance.

In the present study, we have shown that there were no differences between the levels of salmeterol in urine and serum in subjects with asthma compared with non-asthmatics. This is important, as earlier studies concerning urine concentrations of salmeterol have been performed in non-asthmatics. The urine concentrations of salmeterol did not differ between the groups at any sampling point. A recent study by Deventer et al. found urine concentrations of salmeterol between 0.10 and 1.27 ngmL⁻¹ in healthy subjects after inhalation of 100 µg salmeterol xinafoate [15] that peaked 1 to 3 hours after administration. The first sampling point in this study was after 4 hours, thus explaining the lower concentrations observed. The concentrations of salmeterol in the urine after 8 hours were only slightly above the LOQ of 0.12 ngmL⁻¹, and well below after 12 hours.

Our findings of salmeterol in serum corresponds with earlier findings, where concentrations of 0.1 to 0.2 ngmL⁻¹ salmeterol were observed during the first 15 minutes after inhalation of 50 µg salmeterol [14,26]. The serum concentrations of salmeterol peaked at 30 minutes after administration in both groups, and our findings indicate low and often undetectable amounts of salmeterol in serum after inhalation of therapeutic doses. Salmeterol was only detectable during the first two hours after administration and was below the LOQ in most of the remaining observations. The analytical method applied is therefore limited to quantifying serum concentrations of salmeterol only during the first hours after administration of a therapeutic dose. Serum concentrations of the metabolite α-hydroxysalmeterol were all below LOQ apart from one observation, which showed an amount of 0.05 ngmL⁻¹ in 30 minutes after administration.

The analytical procedures developed in the present study were found to be fit for the intended purpose, and they could easily be adapted for routine doping control. However, the very low concentrations of salmeterol and α-hydroxysalmeterol present in biological fluids pose a great analytical challenge, and the desired level of sensitivity could not be fully achieved. Consequently, salmeterol and α-hydroxysalmeterol were undetectable in some of the samples collected in this study. Furthermore, as shown in table 3, the recovery of salmeterol from serum appears to decrease at higher concentrations in a concentration-dependent manner. This is likely due to incomplete elution from the SPE columns or incomplete dissolution after the SPE step. As the internal standard corrects for variations in recovery, the analytical linearity is maintained and the validity of the measurements remains unaffected. The issue was not investigated any further.

In agreement with other studies, we found single samples showing higher urine concentrations when these weren’t corrected for USG [13,27]. Hence, the highest observed concentration of both salmeterol and the metabolite were 0.74 and 12.3 ngmL⁻¹ when uncorrected and 0.62 and 8.0 ngmL⁻¹ when corrected, respectively. This difference is of major importance when it comes to analytical doping procedures of substances where a urinary threshold exists. Accordingly, Elers et al. showed that inhalation of the maximum permitted daily dose of salbutamol (1600 µg) could result in a false positive doping test. When not corrected for USG the study found one sample exceeding the current urinary threshold of salbutamol of 1.000 ngmL⁻¹. Consequently, the concentration of salbutamol in the sample was 1.082 ngmL⁻¹, when uncorrected and 746 ngmL⁻¹ when corrected for USG [13]. Similar findings were observed in a group of elite athletes after inhalation of 800 µg salbutamol, with one urine sample exceeding the threshold. Thus, the sample concentration was 1.057 ngmL⁻¹ when not corrected for USG and well below the threshold after correction with a concentration of 661 ngmL⁻¹ [27]. Correction for USG may therefore have an impact when evaluating doping cases.

Currently, only urine thresholds exist for salbutamol and formoterol on the WADA 2012 prohibited list. If a threshold should be applied for salmeterol, several factors should be taken into account. The first factor is the large inter-individual differences between subjects, showed in this and other studies [13,15]. Secondly, daily use of salmeterol may lead to an accumulation of salmeterol and α-hydroxysalmeterol in the urine. Thirdly, the amount of fluid intake on the sampling day, as well as urinary pH, may have an impact on the concentration [28]. A large intake of fluids results in hemodilution and higher urine output. No fluid intake or sweating during exercise results in hemoconcentration and hence, a higher salmeterol concentration. Therefore correction for USG is important in urine analysis. Lastly, the threshold should be high enough to ensure no risks of having false positive tests in doping controls. However, in ‘Adverse Analytical Findings’ where a sample exceeds the threshold limit, the athlete is provided with the possibility to prove that the finding are a consequence of therapeutic use through a controlled pharmacokinetic study [8].

In terms of doping abuse, it is debatable whether or not excessive use of inhaled beta₂-agonists such as salmeterol even provides any beneficial effect on performance. The use of inhaled beta₂-agonists among athletes was as high as 7.1 and 7.7% during the Olympic Games in 2006 and 2010 [10]. Yet, several studies have investigated the effects of inhaled beta₂-agonists without showing any performance enhancing effects. Though controversies still occur, current research suggests that inhaled beta₂-agonists don’t provide any enhancements in neither endurance performance nor on aerobic capacity in non-asthmatics, described better in detail in two reviews by Kinderman and Wolforth et al. [9,27,29]. However, studies exist showing positive effects of beta₂-agonists when taken orally on muscular performance and supramaximal exercise [8]. Nevertheless, therapeutic use of inhaled beta₂-agonists doesn’t appear to improve performance, and the current doping regulations seem reasonable to reduce misuse, both in asthmatic and non-asthmatic athletes. On the critical side, most of these studies have been conducted in a non-athletic population using short acting beta₂-agonists [30].

Conclusion

After inhalation of 100 µg salmeterol, the maximum individual urine concentration of α-hydroxysalmeterol was 8.00 ngmL⁻¹ at 4 hours after administration, with a median concentration of 2.86 ± 1.75 ngmL⁻¹ in asthmatics and 2.73 ± 2.08 ngmL⁻¹ in non-asthmatics. The highest urine concentration of salmeterol was 0.62 ngmL⁻¹. No difference was found in the urine concentrations of salmeterol between the groups at any sampling point. In doping control analysis, α-hydroxysalmeterol, may serve as a better marker due to its higher concentrations making it easier to detect.

Future research should focus on the use of inhaled salmeterol for longer treatment periods in order to observe a possible accumulation
of salmeterol and α-hydroxysalmeterol in the urine. Currently, only a urinary threshold exists for salbutamol (1000 ng.mL⁻¹) and formoterol (30 ng.mL⁻¹) on the prohibited list, whereas terbutaline, procaterol and salmeterol do not have thresholds [8]. Future studies are therefore necessary to determine urinary concentrations for the rest of the beta₂-agonists.

Acknowledgements

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References

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