

Research article

## Acute Impact of Inhaled Short Acting $\beta_2$ -Agonists on 5 Km Running Performance

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### Abstract

Whilst there appears to be no ergogenic effect from inhaled salbutamol no study has investigated the impact of the acute inhalation of 1600  $\mu\text{g}$ , the World Anti-Doping Agency (WADA) daily upper limit, on endurance running performance. To investigate the ergogenic effect of an acute inhalation of short acting  $\beta_2$ -agonists at doses up to 1600  $\mu\text{g}$  on 5 km time trial performance and resultant urine concentration. Seven male non-asthmatic runners (mean  $\pm$  SD; age  $22.4 \pm 4.3$  years; height  $1.80 \pm 0.07$  m; body mass  $76.6 \pm 8.6$  kg) provided written informed consent. Participants completed six 5 km time-trials on separate days (three at 18 °C and three at 30 °C). Fifteen minutes prior to the initiation of each 5 km time-trial participants inhaled: placebo (PLA), 800  $\mu\text{g}$  salbutamol (SAL800) or 1600  $\mu\text{g}$  salbutamol (SAL1600). During each 5 km time-trial HR,  $\text{VO}_2$ ,  $\text{VCO}_2$ ,  $\text{V}_E$ , RPE and blood lactate were measured. Urine samples (90 ml) were collected between 30-180 minutes post 5 km time-trial and analysed for salbutamol concentration. There was no significant difference in total 5 km time between treatments (PLA  $1714.7 \pm 186.2$  s; SAL800  $1683.3 \pm 179.7$  s; SAL1600  $1683.6 \pm 190.7$  s). Post 5 km time-trial salbutamol urine concentration between SAL800 ( $122.96 \pm 69.22$   $\mu\text{g}\cdot\text{ml}^{-1}$ ) and SAL1600 ( $574.06 \pm 448.17$   $\mu\text{g}\cdot\text{ml}^{-1}$ ) were not significantly different. There was no improvement in 5 km time-trial performance following the inhalation of up to 1600  $\mu\text{g}$  of salbutamol in non-asthmatic athletes. This would suggest that the current WADA guidelines, which allow athletes to inhale up to 1600  $\mu\text{g}$  per day, is sufficient to avoid pharmaceutical induced performance enhancement.

**Key words:** Anti-Doping, WADA code, asthma, treatment, athlete care.

### Introduction

Between 2002 and 2010, the International Olympic Committee (IOC) required athletes to present evidence of current asthma or exercise induced bronchoconstriction (EIB) in order to use inhaled  $\beta_2$ -Agonists. Initially this was conducted independently by the IOC for the Olympic Games and followed by a global requirement, via the World Anti-Doping Agency (WADA), incorporating it into the Therapeutic Use Exemption (TUE) certificate system. These regulations, guided by the IOC Medical Commission (IOC-MC), were based on health not doping (performance enhancing) concerns for athletes in light of a marked increase in the notification for the use of inhaled short acting  $\beta_2$ -agonist from 3.7% at the Atlanta Olympic Games, 1996, to 5.7% at the Sydney Olympic Games,

2000 (Fitch et al., 2008). Examining the prevalence of asthma and EIB in Great British Olympic athletes across two summer Olympic cycles (2000 and 2004) we provided support for the health justification of adding inhaled short acting  $\beta_2$ -agonist to the prohibited list (Dickinson et al., 2005). Our data demonstrated that the requirement for a TUE for the use of inhaled short acting  $\beta_2$ -agonist had no impact on the proportion of Great British Olympic team athletes presenting with asthma, EIB or AHR between the 2000 Sydney Olympic Games and the 2004 Athens Olympic Games (~21% at both) however; we identified a number of athletes with false positive diagnoses and athletes who had not been previously identified. Accordingly, we concluded, as have others, that the requirement of demonstrable evidence through the TUE process improves the quality of care for athletes (Couto et al., 2013). Furthermore, data from our lab (Dickinson et al., 2006a; 2006b) and others (Anderson et al., 2003; Parsons et al., 2007; Rundell et al., 2004,) has demonstrated the improved diagnostic sensitivity and specificity of incorporating indirect airway challenges into the process of diagnosing an athlete with asthma, EIB or AHR.

The weight of evidence supports the improved health care of athletes following the introduction of the TUE process for inhaled short acting  $\beta_2$ -agonist (Fitch et al. 2008; Couto et al. 2013). In recent years it has been argued that the requirement of a TUE is not warranted due to the limited evidence to suggest doses of inhaled  $\beta_2$ -agonist (200 – 800  $\mu\text{g}$ ) have a significant ergogenic effect. A meta-analysis, incorporating the small numbers of studies that do exist, reported no performance improvement in endurance performance from up to 800  $\mu\text{g}$  of inhaled short acting  $\beta_2$ -agonist (Pluim et al., 2011). However the requirement of needing a TUE to use inhaled  $\beta_2$ -agonist therapist may also lead to athletes requiring inhalers for asthma/EIB not taking them in order to not risk a doping violation.

Whilst there appears to be no ergogenic effect from inhaled salbutamol at low doses, no study has investigated the impact of inhaling the World Anti-Doping Agency (WADA) daily upper limit of 1600  $\mu\text{g}$  (~16 inhalations of a standard salbutamol inhaler; WADA, 2013) on endurance running performance. Furthermore, only a limited number of studies have examined the salbutamol elimination in urine of inhaled doses as high as 1600  $\mu\text{g}$  and have reported urine concentrations close to the WADA upper limit of 1000  $\text{ng}\cdot\text{ml}^{-1}$ . Of note, these studies have failed to compare high dose administration of short acting  $\beta_2$ -

agonist with an intermediate dose (800 $\mu$ g) and placebo. They have also focussed on multiple dosing regimens such as 4 x 400  $\mu$ g of salbutamol as opposed to a single high dose (Elers et al., 2011). Whilst athletes are usually prescribed 200-400  $\mu$ g of inhaled salbutamol our experience suggests that athletes are often prescribed inhaled salbutamol *pro re nata* (i.e. on an as needed basis) that could be interpreted as a clearance to inhale unlimited amounts of salbutamol to combat respiratory symptoms. Indeed, recently a Rugby League player escaped a doping violation after he inhaled over 1600  $\mu$ g over the course of a match and then tested positive in the post-match anti-doping test. The player's defence was based on the prescription of inhaled salbutamol was on an 'as needed basis' with no guidance on an upper limit to its use. Accordingly, in practice 16 inhalations in a short period of time prior to and during competition may occur in poorly controlled, less informed or unscrupulous athletes. Yet these athletes are working within the recommended limit stated on the 2014 Prohibited List. Furthermore, the impact of exercising in a hot environment, resulting in significant dehydration, may have a profound effect on urine concentrations.

To date, no studies have examined the impact of a hot environment on combined Salbutamol administration (up to 1600  $\mu$ g) and endurance running performance, or on the resultant salbutamol elimination in urine. Accordingly, the purpose of this study was to contribute to the understanding of the potential ergogenic effect of inhaled short acting  $\beta_2$ -agonists at doses up to and including the maximal dose as stipulated on the WADA 2014 Prohibited List (WADA, 2014) on endurance exercise. In addition, we examined the salbutamol elimination in urine following exercise in temperate (18°C) and hot (30°C) environments.

## Methods

Prior to the commencing the study ethical approval was obtained from Liverpool John Moores University Local Ethics Committee (ethics no: 09E18GW). Seven male runners (mean  $\pm$  SD; age 22.4  $\pm$  4.3 years; height 1.80  $\pm$  0.07 m; body mass 76.6  $\pm$  8.6 kg) volunteered and provided written and verbal informed consent. All participants were free from asthma, EIB and AHR confirmed by no previous history of disease and presenting with a negative Eucapnic Voluntary Hyperpnoea (EVH) challenge (Anderson et al., 2001). All participants were free from chest infection for at least 4 weeks prior to assessment; they were not taking any medication and there were no other health or medical contradictions to them taking part in the study as confirmed by information provided on a physical activity readiness questionnaire. All participants were actively engaged in endurance running training (>45 minutes continuous running) at least 3 times per week. Participants were required to complete six 5 km time-trials; 3 in a temperate environment (18°C, 40% Relative Humidity (RH)) and 3 in a hot (30°C, 40% RH) environment. Fifteen minutes prior the initiation of each 5 km time-trial participants inhaled one of the following treatments, via a pocket chamber, in a randomised, double-

blind design:

Temperate (18°C, 40% RH):

Treatment 1: 16 inhalations of placebo (PLA)

Treatment 2: 8 inhalations of 100  $\mu$ g salbutamol, 8 inhalation of placebo (SAL800)

Treatment 3: 16 inhalations of 100  $\mu$ g salbutamol (SAL1600)

Hot (30°C, 40% RH)

Treatment 4: 16 inhalations of placebo (PLA)

Treatment 5: 8 inhalations of 100  $\mu$ g salbutamol, 8 inhalation of placebo (SAL800)

Treatment 6: 16 inhalations of 100  $\mu$ g salbutamol (SAL1600)

### 5 km time trial

Participants were familiarised to running on a non-motorised treadmill (Woodway Curve, Woodway, USA) prior to initiating the 5 km time-trials. Familiarisation runs took place over a distance of 5 km on at least two occasions. Participants progressed to the recorded 5 km time-trials once they felt comfortable pacing themselves on the non-motorised treadmill over the 5 km distance.

Each time-trial was conducted under controlled laboratory conditions: Temperate (18°C, relative humidity 40%), and Hot (30°C, relative humidity 40%). Prior to starting the time-trial participants were fitted with a heart rate monitor (Polar RS400; Polar Electro Oy, Kempele, Finland) and connected to a breath-by-breath gas analyser via a face mask (Oxycon Pro, Jagear, Wuerzberg, Germany). Over the course of the 5 km time-trial the following were measured: time, average heart rate (HR), oxygen consumption ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), minute ventilation ( $\text{V}_E$ ), respiratory exchange ratio (RER) and rating of perceived exertion (RPE). Two minutes following the completion of the 5 km time-trial capillary blood lactate was measured (Lactate Pro, Arkray KDK, Japan).

During the 5 km time-trial participants were only given feedback on the distance they had covered. They were blinded to all other feedback such as time and HR. Participants were encouraged to complete the time-trial as fast as possible with prizes offered to the five fastest times. During the time-trial consistent positive encouragement was given to each participant.

### Maximal flow-volume loops

Three maximal flow-volume loops were measured (MicroLab Spirometer ML3500, Cardinal Health, Chatham Maritime, UK) at baseline, 10 minutes after inhalation of PLA, SAL800 and SAL1600 and then 10 minutes post-5 km time-trial. On each occasion maximal flow volumes were measured according to the European Respiratory Society criteria (Miller et al., 2005). At each time point Forced Expiratory Volume in One Second ( $\text{FEV}_1$ ), Forced Vital Capacity (FVC), Peak Expiratory Flow (PEF) and Forced Expiratory Flow between 25 % and 75 % of FVC ( $\text{FEF}_{25-75}$ ) were measured.

### Urine collection

Prior to the commencement of each trial subjects were

asked to provide a urine sample in order to void themselves of urine, after which placebo or Salbutamol was administered as described above. Subjects were instructed to collect the first sample of urine passed following completion of the laboratory-based tests in line with in-competition anti-doping procedures as outlined in the World Anti-Doping Code International Standard for Testing 2012. Urine samples were provided by participants between 30 and 180 minutes following the completion of the 5 km time trials. Consumption of water during this period was encouraged *ad libitum* to ensure diuresis. From the sample provided by each subject a 20 ml aliquot of urine was stored at  $-80^{\circ}\text{C}$  until analysis.

### Urinalysis

All urinalysis was performed at HFL Sport Science (Fordham, UK) an independent drug surveillance laboratory and former WADA-accredited laboratory. Sample preparation involved the addition of 200 ng of Salbutamol- $\text{D}_3$  (NMI) as an internal standard to 1 ml of urine. Following the addition of 2 ml of 0.1M phosphate buffer pH 6.8 and 100  $\mu\text{l}$  of E. Coli enzyme ( $\beta$ -glucuronidase) solution the mixture was incubated overnight at  $37^{\circ}\text{C}$ . Strata XC 60 mg solid phase extraction cartridges (Phenomenex, Macclesfield, UK) were conditioned with 3 ml of methanol followed by 3 ml of reagent grade water. Following centrifugation at 3500 rpm for 5 min the samples were applied to the cartridges. The cartridges were then washed with 3 ml of 0.1M acetate buffer pH 9.0 followed by 3 ml of reagent grade water, 3 ml of 0.1M HCl, 3 ml of methanol and 3 ml of diethyl ether. The cartridges were then dried for 5 min under vacuum and samples were eluted into glass vials with two, 1 ml of basic drug elution solvent (160 ml ethyl acetate, 34 ml propan-2-ol and 6 ml 34% ammonia solution). Samples were then evaporated to dryness at ambient temperature using a centrifugal vacuum concentrator (Genevac Ltd, Ipswich, UK) and reconstituted in 10  $\mu\text{l}$  of isopropanol followed by 200  $\mu\text{l}$  of basic reconstitution solution (495 ml of 0.1 acetic acid mixed with 5 ml Benzyldimethylphenyl Ammonium). Samples were centrifuged at 3000

rpm for 10 min prior to LCMS submission. Samples were injected onto a Thermo Scientific Accela HPLC system coupled to a Thermo Scientific LTQ Orbitrap Discovery Mass Spectrometer (Thermo Fisher Scientific, Waltham, USA). Chromatographic separation was performed on a Waters Atlantis T3 column (2.1 x 100 mm, particle size 3  $\mu\text{m}$ ; Waters Ltd, Elstree, UK) at  $35^{\circ}\text{C}$ . The mobile phase was a gradient system of 0.1% acetic acid aqueous solution containing uracil ( $300\text{ ng}\cdot\text{ml}^{-1}$ ) and 0.1% acetic acid in acetonitrile containing uracil ( $300\text{ ng}\cdot\text{ml}^{-1}$ ) set at a flow rate of  $0.4\text{ ml}\cdot\text{min}^{-1}$ .

The urine salbutamol concentrations reported correspond to the sum of the free and glucuronide conjugates. The samples were analysed over the calibration range of 10 to  $2000\text{ ng}\cdot\text{ml}^{-1}$ . The lower limit of quantification was accepted as the lowest standard on the calibration curve ( $10\text{ ng}\cdot\text{ml}^{-1}$ )

### Statistical analysis

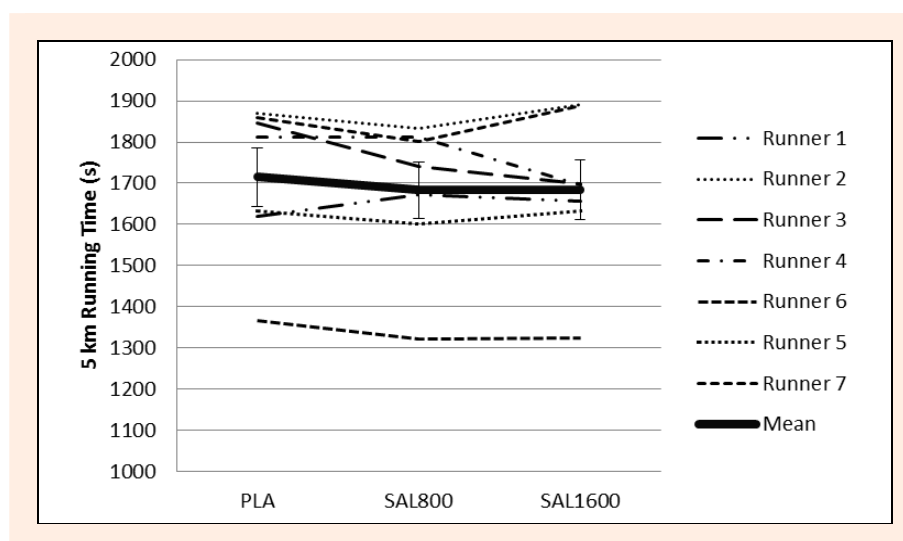
Total time to complete the 5 km time-trial under each of the conditions was analysed by a repeated measures ANOVA. Similar analyses were undertaken for HR,  $\text{VO}_2$ ,  $\text{VCO}_2$ , VE, RER, RPE, blood lactate, FEV1, FVC, PEF and  $\text{FEF}_{25-75}$ . A p-value of  $\leq 0.05$  was deemed significant for all analysis.

### Results

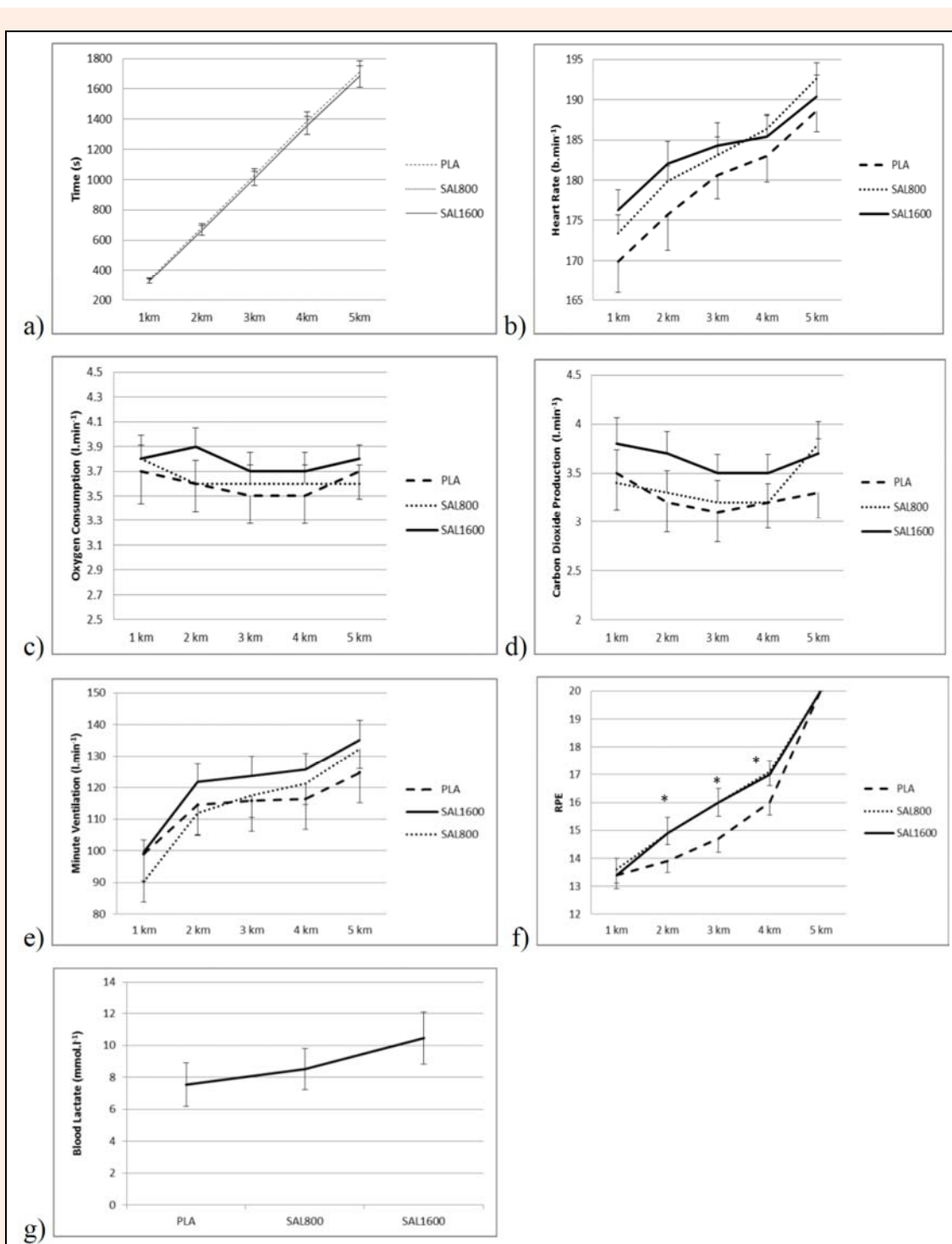
Throughout all trials participants reported no side effects from inhalation of up to  $1600\text{ }\mu\text{g}$  inhaled salbutamol.

#### Performance trial (hot: $18^{\circ}\text{C}$ , 40% RH)

All seven participants completed all three 5 km time-trials. No significant difference was noted for overall completion time between trials (see Figure 1). Furthermore, over each 1 km split no significant difference between conditions for mean: time; HR;  $\text{VO}_2$ ;  $\text{VCO}_2$ ;  $\text{V}_E$ ; and RER were observed (see Figure 2). There was no significant difference between conditions in the post 5 km time-trial lactate values (see Figure 2). At 2, 3 and 4 km RPE was significantly increased under the SAL800 and



**Figure 1.** Individual and mean  $\pm$  SE 5 km time-trial performance under each condition in a temperate environment ( $20^{\circ}\text{C}$ , 40% RH).



**Figure 2.** Mean  $\pm$  SE for time, HR,  $\text{VO}_2$ ,  $\text{VCO}_2$ ,  $\text{V}_E$  and RPE at each 1 km split of the temperate (18°C) 5 km time-trial (a-f) and post-5 km time-trial blood lactate (g). \* = SAL800 and SAL1600 significantly greater than PLA.

SAL1600 when compared to PLA (Figure 2e). There was no significant interaction between Maximal Flow Volume measures and condition 10 minutes post-inhalation or post-5 km time-trial (see Table 1).

#### Performance trial (hot: 30°C, 40% RH)

All seven participants completed all three 5 km time-trials. No significant difference was noted for overall completion time between trials (see Figure 3). Furthermore, over each 1 km split no significant time difference

between conditions was observed (see Figure 4a.). Average  $\text{VO}_2$  during the 1<sup>st</sup> km was significantly greater ( $p = 0.046$ ) during PLA ( $3.941 \pm 0.490 \text{ l}\cdot\text{min}^{-1}$ ) when compared with SAL800 ( $3.591 \pm 0.544 \text{ l}\cdot\text{min}^{-1}$ ) and SAL1600 ( $3.516 \pm 0.416 \text{ l}\cdot\text{min}^{-1}$ ) trials, however overall mean: HR;  $\text{VO}_2$ ,  $\text{VCO}_2$  and RPE during the 5 km time-trial were not significantly different between trials (see Figure 4). There was no significant difference between conditions in the post 5 km time-trial lactate values (see Figure 4).

**Table 1.** Maximal flow-volume values at baseline, 10 minutes post-treatment and post-5 km time-trial. Data are means ( $\pm$ SD).

		Placebo	SAL800	SAL1600
<b>Baseline</b>	FEV <sub>1</sub> (l)	4.6 (.9)	4.6 (.9)	4.6 (.9)
	Predicted FEV <sub>1</sub> (%)	106.0 (13.5)	106.1 (14.5)	106.0 (14.8)
	FVC (l)	5.4 (.9)	5.3 (1.0)	5.2 (1.0)
	Predicted FVC (%)	103.4 (12.4)	102.0 (13.6)	101.3 (13.4)
	FEV <sub>1</sub> %	86.4 (3.4)	87.4 (3.4)	87.6 (3.4)
<b>Post Salbutamol</b>	FEV <sub>1</sub> (l)	4.6 (.9)	4.7 (.9)	4.7 (.9)
	FVC (l)	5.3 (.9)	5.3 (.9)	5.2 (1.0)
	FEV <sub>1</sub> %	87.1 (3.4)	89.1 (4.1) *	89.4 (4.1) *
<b>Post 5 km time-trial</b>	FEV <sub>1</sub> (l)	4.6 (.9)	4.7 (1.0)	4.7 (1.0)
	FVC (l)	5.1 (1.0)	5.1 (1.0)	5.1 (1.0)
	FEV <sub>1</sub> %	89.9 (5.4)	91.0 (4.7)	92.0 (4.7)

\*= significantly greater than PLA.

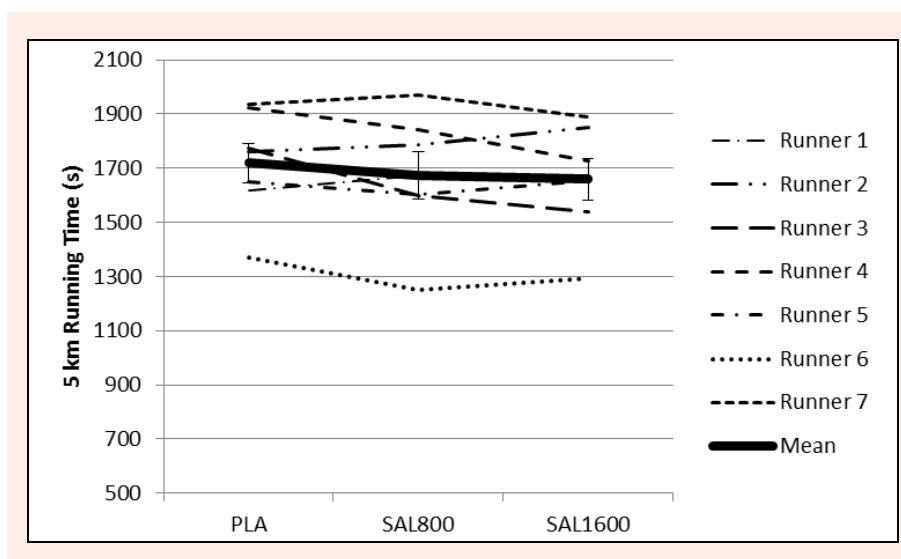
### Urine analysis

Following inhalation of 800  $\mu$ g of Salbutamol and a 5 km time-trial under temperate (18°C) and hot (30°C) ambient conditions mean  $\pm$  SD urine concentrations were 122.96  $\pm$  69.22 ng·ml<sup>-1</sup> and 138.83  $\pm$  98.11 ng·ml<sup>-1</sup>, respectively (see Figure 5). Following inhalation of 1600  $\mu$ g of Salbutamol and a 5 km time-trial, mean  $\pm$  SD urine concentrations were 574.06  $\pm$  448.17 ng·ml<sup>-1</sup> and 270.32  $\pm$  183.90 ng·ml<sup>-1</sup> under temperate and hot ambient conditions, respectively (see Figure 5). Whilst there were no significant differences between drug urine concentrations under either condition there was a high degree of inter-individual variation following the inhalation of 1600  $\mu$ g of Salbutamol (see Figure 5). Only one sample reached the WADA upper limit of 1000 ng·ml<sup>-1</sup> (1190 ng·ml<sup>-1</sup>; in the temperate environment) however; the sample failed to reach the WADA decision limit of 1200 ng·ml<sup>-1</sup>.

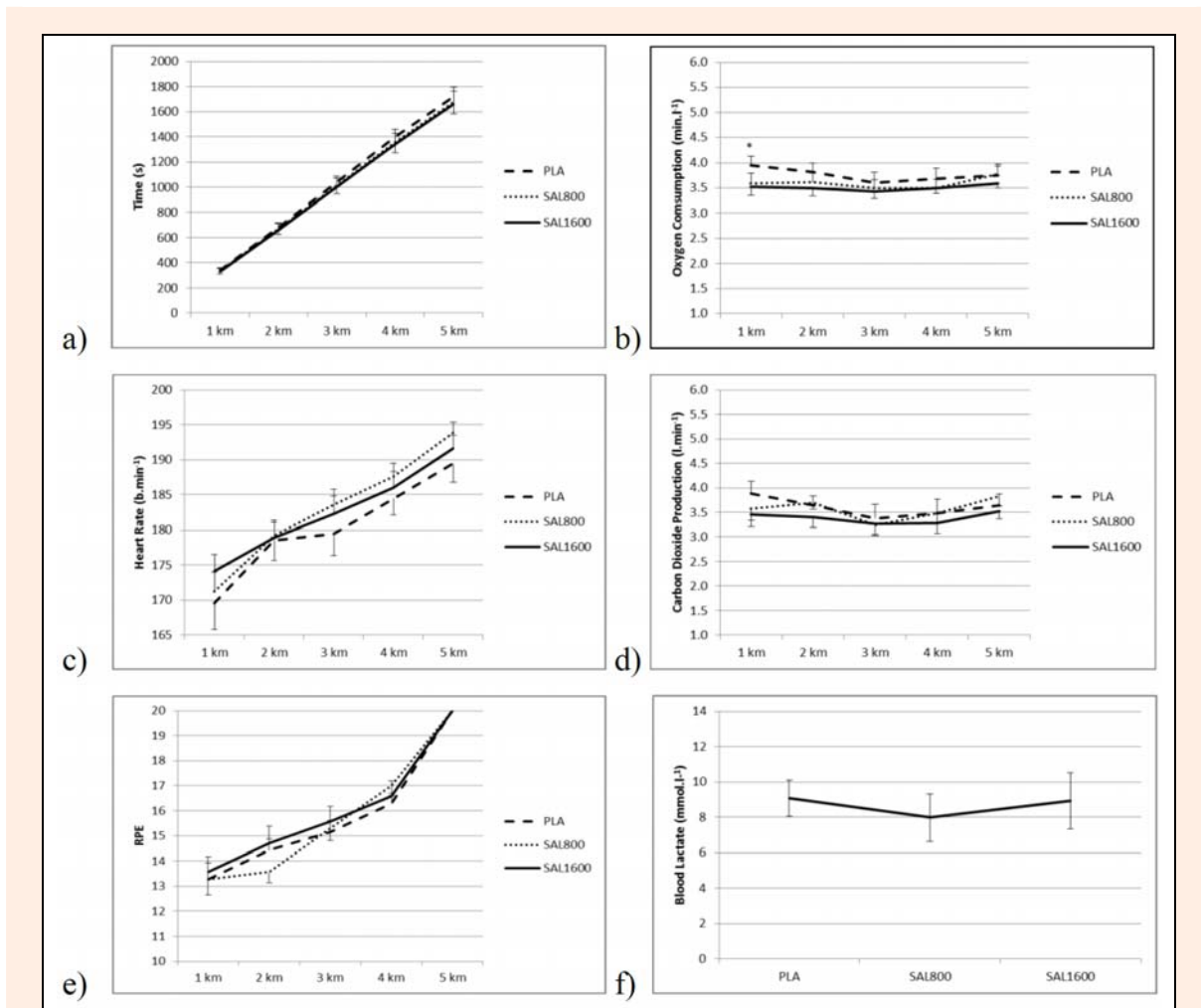
### Discussion

This is the first study to examine the impact of inhaled salbutamol at a dose of 1600  $\mu$ g *versus* 800  $\mu$ g and placebo on time-trial endurance running performance. Furthermore, this study is the first to examine the salbutamol elimination in urine following inhalation of salbutamol at a doses of 1600  $\mu$ g and 800  $\mu$ g and competitive endurance

performance in temperate (18°C; 40% RH) and hot (30°C; 40% RH) environments. The results from the current study suggests that inhaling up to 1600  $\mu$ g of salbutamol 15 minutes prior to a 5 km time-trial does not result in any performance improvement or change in physiological function. Previous studies that have focused on inhaled salbutamol have generally focused on non-specific performance trials such as time to exhaustion or physiological markers of performance (Decorte et al., 2008; Sporer et al., 2008). A meta-analysis of previous studies has demonstrated no improvement following acute inhalation of up to 800  $\mu$ g of salbutamol on running time to exhaustion, VO<sub>2max</sub>, peak power, 20 km cycling time-trial and total work during a 30 s Wingate test (Pluim et al 2011). A recent study by Koch et al. (2013) demonstrated no performance enhancement in 10 km cycling time trial performance following 400  $\mu$ g inhaled salbutamol in athletes with and without a positive EVH challenge. Our data complements previous research by focusing on running time-trial performance and adds to the current body of knowledge by reporting on the current upper WADA limit of 1600  $\mu$ g per day of inhaled salbutamol. Further to our data, a recent study by Elers et al. (2011) suggested that inhaling an acute dose of up to 4000  $\mu$ g of salbutamol resulted in no improvement in cycling time to exhaustion or oxygen uptake kinetics. Accordingly, from a



**Figure 3.** Individual and mean  $\pm$ SE 5 km time-trial performance under each condition in a hot environment (30°C, 40% RH).

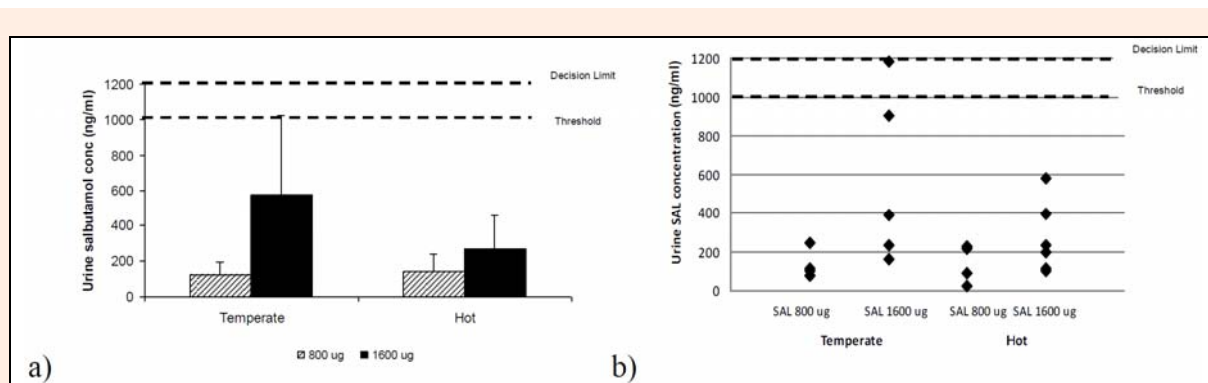


**Figure 4.** Mean  $\pm$ SE for time,  $VO_2$ , HR,  $VCO_2$ , and RPE at each 1 km split of the 5 km time-trial (a-e) and post-5 km time-trial blood lactate (f) during 5 km time-trial in the hot environment (30°C).

performance perspective the current WADA upper limit of 1600  $\mu$ g per day, even in single acute doses, appears appropriate for endurance events given the absence of improvement in performance in non-asthmatic athletes.

The main action of inhaled salbutamol is to act as a bronchodilator to reverse the bronchoconstriction of airway smooth muscle. This results in the asthmatic airway becoming dilated resulting in reduced airway resistance, leading to improvements in  $V_E$  and exercise performance

(Haverkamp et al., 2007). It has been suggested that inhaled salbutamol may result in a performance improvement by causing a significant bronchodilation in the airways of non-asthmatics leading to an improved  $V_E$  and increased oxygen uptake during exercise. In our study we observed a non-significant 0.1 litre improvement in  $FEV_1$  10 minutes post-salbutamol inhalation, which did not result in an improvement in  $V_E$  during the 5 km time-trials following inhalation of 800  $\mu$ g or 1600  $\mu$ g of



**Figure 5.** a) Mean  $\pm$ SD and b) individual urine SAL concentration (ng·ml<sup>-1</sup>) post 5 km time trial under temperate (20°C) and hot (30°C) conditions following inhalation of 800  $\mu$ g or 1600  $\mu$ g SAL.

salbutamol when compared to placebo. Previous studies have demonstrated non-significant improvements in FEV<sub>1</sub> of 0.2 l following inhalation of 800 µg, which did not result in greater V<sub>E</sub> or improved endurance performance (Decorte et al., 2008). Therefore there is no evidence available that up to 1600 µg of inhaled salbutamol results in significant bronchodilation, improved V<sub>E</sub> or improved endurance performance.

Further study is required in a larger cohort to ensure an avoidance of Type II error. A power calculation would suggest that to detect a change in 1% in sprint performance a sample size of 48 participants would be required. Furthermore our participants were not elite endurance athletes. Elite athletes could not be included in this study as they would have been at risk of a doping violation. Therefore we cannot claim our results directly represent the effects of inhaled salbutamol in elite endurance athletes.

The current urinary threshold imposed by WADA is intended to enable differentiation between the use of oral and inhaled Salbutamol and also approved therapeutic use and misuse. Oral use is associated with performance enhancement since it typically represents doses in the region of 10-fold greater than those following inhalation. Nevertheless, there has been limited research to examine this association. From an endurance exercise perspective only Collomp et al. (2000) has demonstrated enhanced performance whereby short-term oral administration of salbutamol (12 mg/day for 3 weeks) improved time to exhaustion during sub-maximal cycling exercise. Caruso et al. (1995) and Martineau et al. (1992) have demonstrated an increase in muscle strength following prolonged oral administration of salbutamol. Indeed protein synthesis and muscle hypertrophy have been shown to be an effect of β<sub>2</sub>-agonist use in animal models, particularly long acting β<sub>2</sub>-agonists such as Clenbuterol. However, research is clearly required to examine further the claim that oral administration has a positive impact on sports performance.

There exists some ambiguity in terms of the therapeutic use of inhaled Salbutamol. Whilst the recommended maximal dosing regimen for Salbutamol is 100 µg to 400 µg up to four times daily, it is typically prescribed *pro re nata* (when required) which may add to the confusion. Individuals encouraged to administer Salbutamol *pro re nata* may dose over and above the maximal recommended daily dose of 1600 µg either intentionally or inadvertently, however, in both instances, individuals intent to dope for performance enhancement purposes may be nil. Such circumstances may lead to the current threshold being unintentionally breached and thus bring about an adverse analytical finding (AAF). Clearly individuals administering inhaled Salbutamol up to, and above the 1600 µg dose indicates uncontrolled asthma. Desensitisation or tolerance are experienced by those regularly administering inhaled Salbutamol which not only increases the risk of unsuccessful treatment in an emergency but also increases the likelihood of further overdosing in an attempt to control EIB.

The current study demonstrates the possibility of a urinary Salbutamol concentration above the current

threshold following therapeutic use. However, whilst the WADA Prohibited List threshold is 1000 ng·ml<sup>-1</sup> (WADA, 2014), according to the International Standards for Laboratories Technical Document (WADA, 2012) Salbutamol should only be reported as an AAF when detected at a concentration greater than 1200 ng·ml<sup>-1</sup>, a level referred to as the 'Decision Limit'. On this basis the urinalysis of the current study would not warrant any sample to be reported as an AAF. This finding was true for a hot (30°C, 40% RH) as well as a temperate environment (18°C, 40% RH). The range of salbutamol concentrations in our study are similar to Sporer et al. (2008) who reported urine concentrations of salbutamol up to 800 ng·mL<sup>-1</sup> 60 minutes post time trial following inhalation of 800 µg salbutamol. Elers et al. (2012) reported urine salbutamol concentrations peaked between 0-4 hours. They reported peak salbutamol concentration was 1057 ng·mL<sup>-1</sup> following 800 µg inhaled salbutamol. Nevertheless, in our study the inter-individual variation was high in both temperate and hot environments and combined with the low subject numbers caution is advised and future studies should aim to examine the impact of high dose Salbutamol (1600 µg) administration on urine concentration following endurance performance to establish the likelihood of the Decision Limit being breached. In line with current anti-doping practice the current study did not normalise drug concentrations for urine specific gravity. Elers et al. (2012) demonstrated that when urine samples are corrected for specific gravity no urine samples following inhalation of 800 µg Salbutamol breached the WADA Prohibited List threshold of 1000 ng·ml<sup>-1</sup>. Normalising urine samples for specific gravity may be considered by WADA consider in the future.

A limitation to our study is the variability in actual dose inhaled. Whilst the use of a chamber aimed to reduce this limitation it remains possible that some participants with low urine concentration inhaled lower doses of Salbutamol. In addition future work should investigate whether there is a relationship between body weight and the urinary concentration of salbutamol. A lighter athlete may be at a greater risk of breaching the threshold when administering high doses compared to a heavier athlete. Such findings would have implications to the care athletes receive in the future.

## Conclusion

This study has demonstrated that there is no improvement in performance following the inhalation of up to 1600 µg of Salbutamol in non-asthmatic athletes in temperate or hot environments. This would suggest that the current WADA guidelines, which allows athletes to inhale up to 1600 µg is sufficient to avoid pharmaceutical induced enhancement in 5 km running performance. However, such high doses not only suggest poor management of asthma but also increase the risk of an athlete contravening the current urinary threshold.

## Acknowledgment

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## Key points

- Inhaling up to 1600  $\mu\text{g}$  of Salbutamol does not result in improved 5 km time trial performance.
- The position of Salbutamol on the World Anti-Doping Agency list of prohibited appears justified.
- Athletes who use up to 1600  $\mu\text{g}$  Salbutamol in one day need to review their therapy as it would suggest their respiratory condition is not under control.

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