Abstract

Terbutaline is a prohibited drug except for athletes with a therapeutic use exemption certificate; terbutaline's effects on endurance performance are relatively unknown.

Purpose: To investigate the effects of two therapeutic (2mg; 4mg) inhaled doses of terbutaline on 3km running time-trial performance.

Methods: Eight males (24.3±2.4yrs; 77.6±8kg; 179.5±4.3cm) and eight females (22.4±3yrs; 58.6±6kg; 163±9.2cm) free from respiratory disease and illness provided written informed consent. Participants completed 3 km running time-trials on a non-motorised treadmill on three separate occasions following placebo, 2 mg or 4 mg inhaled terbutaline, in a single-blind, repeated-measures design. Urine samples (15mins post-exercise) were analysed for terbutaline concentration. Data were analysed using one-way repeated measures ANOVA, significance was set at $p<0.05$ for all analyses.

Results: No differences were observed for completion times (1103±201; 1106±195; 1098±165s; P=0.913) for the placebo trial, the 2mg inhaled trial and the 4mg inhaled trial, respectively. Lactate values were higher (P=0.02) following 4mg terbutaline (10.7±2.3mmol·L^{-1}) vs. placebo (8.9±1.8mmol·L^{-1}). FEV_1 values were greater following inhalation of 2mg (5.08±0.2; P=0.01) and 4mg terbutaline (5.07±0.2; P=0.02) compared to placebo (4.83±0.5L) post-inhalation. Urinary terbutaline concentrations were mean (306±288ng·mL^{-1}; 435±410ng·mL^{-1}; P=0.2) and peak (956ng·mL^{-1}; 1244ng·mL^{-1}) following 2mg and 4mg inhaled terbutaline, respectively. No differences were observed between the male and female participants.

Conclusions: Therapeutic dosing of terbutaline does not lead to an improvement in 3 km running performance despite significantly increased FEV_1. Our findings suggest that athletes using inhaled terbutaline at high therapeutic doses to treat asthma will not gain an ergogenic advantage during 3 km running performance.

Introduction

Short-acting β_2-agonists are used therapeutically by athletes with asthma related conditions to prevent and/or reverse the bronchoconstriction of the airways, leading to restoration of airway function.[1–5] The majority of athletes treat symptoms of exercise-induced bronchoconstriction (EIB) through the use of salbutamol, making it the most commonly used inhaled β_2-agonist in these individuals.[4] However other β_2-agonists, such as terbutaline, are available which is a suitable alternative to salbutamol, should an athlete not respond appropriately to salbutamol treatment.[6–10] Athletes that are subject to World Anti-

Doping Agency (WADA) regulations, who require alternative β_2-agonist therapy can apply for a therapeutic use exemption certificate (TUE) in order to use inhaled terbutaline.[11]

The prohibited status of terbutaline is due, in part, to the inability to distinguish between therapeutic inhaled and therapeutic oral doses (with all oral β_2-agonists being banned under the WADA code), given that an oral dose far exceeds the inhaled dose in terms of the systemic bioavailability when given therapeutically.[12,13] In some athletes the need for the use of terbutaline is justified, however there are currently no measures in place to prevent an athlete with a legitimate TUE for terbutaline from using the medication at a supratherapeutic dose with impunity.[13]

The current WADA guidelines monitor the use of the inhaled short-acting β_2-agonists, salbutamol and formoterol via a urinary threshold limit, above which will present an adverse analytical finding (AAF).[11] For salbutamol this limit is 1000 $ng \cdot mL^{-1}$ with a decision limit of 1200 $ng \cdot mL^{-1}$ and for formoterol this limit is 40 $ng \cdot mL^{-1}$ with a decision limit of 50 $ng \cdot mL^{-1}$, with any levels over this presenting an AAF. The current guidelines for use indicate that no more than 1600 µg salbutamol can be inhaled in a 24 hour period and within this no more than 800 µg can be inhaled in a 12 hour period, with the equivalent for formoterol being 54 µg over a 24 hour period.[11] If a threshold for terbutaline could be determined, this would enable it to be monitored in much the same way as both salbutamol and formoterol, preventing an athlete with a TUE for terbutaline from potentially using the medication at a supratherapeutic dose. Recently Jacobson et al.,[13] presented the case for establishment of dosing thresholds for terbutaline, these dosing thresholds are extremely important given recent evidence of ergogenic effects of supratherapeutic dosages of inhaled terbutaline on sprint and power performance, muscle strength and muscle hypertrophy, as well as inducing muscle phenotype alterations.[8,9,14,15]

The establishment of a urinary threshold for terbutaline has proven to be difficult to attain, recently Dyreborg et al.,[16] examined high-dose (4 mg) inhaled versus oral (10 mg) terbutaline, finding that the bioavailability and pharmacokinetics vary distinctly between routes of administration. Peak urinary concentration of 4 mg inhaled terbutaline occurred 2 hours post-inhalation and peak urinary concentration of 10 mg oral terbutaline occurred 6 hours post-ingestion, interestingly there was also no significant difference between urinary levels of inhaled vs oral terbutaline at the 6 hour stage. Similar work was previously performed by Elers et al.,[12] in which inhaled (2 mg) and oral (10 mg) terbutaline were examined, the study found that although there was a significant difference between urine concentrations dependent upon route of administration, no

threshold was able to be established due to high variability between individuals. It is therefore important to assess urinary levels of terbutaline for doping control purposes.

Evidence exists that the use of terbutaline at a supratherapeutic dose has the potential to be ergogenic.[17,18] These purported effects are due to the fact that short-acting β_2-agonists (a class of sympathomimetic amines) are able to activate the β_2 adrenergic receptors within the body, which are mainly present on bronchial smooth muscle.[19–21] Activation of the β_2 adrenergic receptors reverses the constriction of bronchial smooth muscle during bronchoconstriction. These same β_2 receptors are also present on cardiac smooth muscle and skeletal muscle.[21,22] Adrenergic activation of skeletal muscle has the potential to improve musculoskeletal function and thus has the potential to be ergogenic during exercise performance.[23] Recent investigations suggest an acute supratherapeutic inhaled dose (15 mg) of terbutaline may have ergogenic action in sprint cycling performance.[8,9,14,18] This 15 mg dose is approximately eight times the recommended therapeutic dose for inhaled terbutaline and in athletes with a TUE this would not be permitted according to the WADA code,[11] however current regulations would not be able to accurately detect this misuse of terbutaline, due to a lack of urinary thresholds with which to monitor terbutaline use. Given the ergogenic potential of supratherapeutic inhaled terbutaline, it remains to be determined whether athletes using terbutaline therapeutically to treat asthma symptoms could also experience an ergogenic effect, traditionally the therapeutic dose of inhaled terbutaline is between 1-2 mg, however studies have shown therapeutic use as high as 4 mg.[10,16]

The aim of the present study was to examine the potential ergogenic action of 2 mg and 4 mg inhaled terbutaline on exercise performance during a 3 km running time-trial and to measure urinary thresholds of terbutaline post-exercise performance.

Methods

Following ethical approval from the Liverpool John Moores University research ethics committee (Ethics No. P11SPS044), eight males (age: 24.3 ± 2.4 years; weight: 77.6 ± 8 kg; height: 179.5 ± 4.3 cm) and eight females (age: 22.4 ± 3 years; weight: 58.6 ± 6 kg; height: 163 ± 9.2 cm) volunteered to participate in the study, providing their written informed consent. All participants were in good health, non-smokers and took part in sport and exercise activities for at least 3 hours per week. No participant had previously been diagnosed with asthma and/or EIB, all participants were free from chest infection for at least two weeks prior to testing. Participants presented with a negative

eucapnic voluntary hyperpnoea (EVH) challenge.[24,25] No participants competed at a level where they were subject to regular anti-doping tests. Participants were informed about the nature and the risks of the experimental procedures before providing written informed consent.

3 km Time-Trial

The 3 km time-trials were conducted on a non-motorised curved treadmill (Woodway Curve, Woodway, USA). Participants were familiarised to running on a non-motorised treadmill prior to initiating their recorded 3 km time-trials. Familiarisation runs took place on at least two occasions and participants progressed to the recorded 3 km time-trials only once they felt comfortable pacing themselves on the non-motorised curved treadmill over a 3 km distance (Figure 1).

Each participant was required to perform a 3km time-trial on three occasions in a randomised, single blind, repeated measures design with a minimum of 7 days between trials. Participants were instructed to follow the same 24-hour dietary intake prior to each trial and were instructed to abstain from caffeine for 6 hours before attending. Prior to completing the 3 km time-trial participants completed baseline maximal flow-volume manoeuvre in accordance with ERS/ATS criteria.[24] Following baseline spirometry participants inhaled either eight inhalations of non-active inhalant (placebo), four inhalations of non-active inhalant plus four inhalations of 0.5 mg terbutaline (2 mg) or eight inhalations of 0.5 mg terbutaline (4 mg). Participants received the inhaled terbutaline via turbuhaler (Bricanyl, Turbuhaler, AstraZeneca, Canada), participants were advised to inhale at a steady flow-rate for 2 seconds until full inhalation and to hold each inhalation for 10 seconds, a minimum of 1 minute was required between each subsequent inhalation. Ten minutes post-inhalation spirometry was repeated, before the completion of a standardised warm-up (5 minutes on a motorized treadmill at 10 kph). The 3km time-trials were performed under controlled laboratory conditions of 18°C, 20.9% O_2 and 40% humidity.

During the time-trial participants wore a heart rate monitor (Polar RS400; Polar Electro Oy, Kempele, Finland) and face-mask connected to a breath-by-breath gas analyser (Oxycon Pro, Jaeger, Wurzberg, Germany). Every 0.5 km the following variables were measured: time (s), heart rate (HR), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), respiratory exchange ratio (RER) and rating of perceived exertion (RPE).[26] Two minutes following the completion of the 3 km time-trial a finger-tip capillary blood sample was collected to measure blood lactate concentration

(Lactate Pro, Arkray KDK, Japan) followed by spirometry and collection of a post-exercise urine sample (Figure 1).

During the 3 km time-trial participants were only given feedback on the distance they had covered. They were blind to all other feedback such as time and HR. Participants were encouraged to complete the time-trial as fast as possible, a-priori power calculations for the 3 km running time-trial predicted that for an expected completion time of 1100 seconds, with a standard deviation of (14%) 154 seconds, a sample size of 8 would be sufficient to significantly ($P<0.05$) predict a 2.5% 27 second change in performance with 80% power.

Urinalysis

Collected urine-samples were measured for pH and osmolality before 30 ml of each sample was distributed into a Nalgene bottle (Thermo Fisher Scientific, Leicestershire, UK) prior to freezing the sample at -80 ºC until urinalysis. All urinalysis was performed at HFL Sport Science (Fordham, United Kingdom), an independent drug surveillance laboratory and former WADA-accredited laboratory. All samples were packaged in dry ice during transportation to prevent thawing. The laboratory used a validated proprietary analytical method. In brief, urine samples were thawed, centrifuged and subaliquotted prior to addition of a deuterated internal standard (Terbutaline D_3; CDN Isotopes via QMX Laboratories Ltd, Thaxted, UK). Following overnight enzymatic hydrolysis with β glucuronidase from E. Coli (type 1X-A; Sigma Aldrich, Dorset, UK), sample clean-up was performed using solid phase extraction (Strata XC 30 mg 96-well plate; Phenomenex, Macclesfield, UK). After elution, samples were evaporated to dryness, reconstituted and analysed using an AB Sciex 4000 QTrap mass spectrometer (AB Sciex, Warrington, UK), with a Waters Acquity UPLC system (Waters Ltd, Elstree, UK). Chromatographic separation was achieved using a Waters Acquity HSS T3 Column (2.1 x 100 mm, particle size 1.8 µm) and gradient solvent programme using methanol and water, both containing 10 mM ammonium formate.

Sample concentrations were measured using a calibration line containing Terbutaline at different concentrations (10 to 3000 $ng \cdot ml^{-1}$) which were extracted and analysed in the same batch. Quality control samples were tested along with samples to confirm assay performance.

Sample Correction

All urine concentrations of terbutaline were corrected to a urine specific gravity of 1.02 prior to analysis using the following equation[12]:

Corrected urine concentration = terbutaline urine concentration x (0.02/(urine specific gravity -1)).

Statistical Analysis

Statistical analysis incorporated one-way repeated measures analysis of variance (ANOVA) to compare between trial conditions during time-trial performance and two-way ANOVA to compare spirometry measurements between conditions at different time-points, a Bonferroni correction was applied to correct for multiple comparisons. Significance was set at $P < 0.05$ for all analyses. All data were reported as mean (±SD) unless otherwise stated. Statistical analysis was performed using the statistical package for the social sciences (SPSS v21.0, IBM, New York).

Results

Sixteen participants successfully completed all trials, participant demographics and lung function screening values are shown in Table 1. No adverse side-effects were reported by any of the participants during the study.

There were no significant differences in completion time between trials either within the combined group (1103 ± 194 s; 1106 ± 118 s; 1098 ± 160 s; P = 0.9) or when groups were split according to gender: female (1249 ± 149.3 s; 1257 ± 112 s; 1215 ± 96 s; P = 0.37) and male (956 ± 102 s; 955 ± 113 s; 982 ± 122 s; P = 0.28) for PLA, 2 mg and 4 mg trials respectively (Figure 2).

Post time trial blood lactate was greater following 4 mg inhaled terbutaline (10.7 ± 2.3 $mmol \cdot L^{-1}$) when compared to the placebo trial (8.9 ± 1.8 $mmol \cdot L^{-1}$; P = 0.02; Figure 3). There were no differences in gas exchange variables for VO_2 (49.1 ± 7.7; 49.3 ± 5.2; 48.9 ± 5.1) VCO_2 (50.3 ± 5.9; 52.5 ± 5.5; 52.1 ±4.5) or RER (1.08 ± 0.1; 1.09 ± 0.05; 1.12 ± 0.07), for placebo, 2 mg inhaled and 4 mg inhaled terbutaline, respectively.

Exercising heart rate (HR) did not differ (P=0.95) between trial conditions, ratings of perceived exertion (RPE) values did not differ between trials at any time-point during performance (Figure 4).

There was a significant difference in FEV_1 between trial conditions post-inhalation of 2 mg and 4 mg terbutaline (Table 2). There were no differences between FEV_1 values in the placebo trial following terbutaline administration or following time-trial completion, there was no difference in baseline lung function values between conditions. There was a significant

difference in post inhalation FEV_1 values compared to placebo (P=0.007; P=0.003) for both 2 mg and 4 mg inhalation trials, respectively (Table 2; Figure 5). Interestingly, the difference in FEV_1 post time-trial between conditions was not significant (P=0.06) (Figure 5), possibly due to a slightly raised FEV_1 following exercise in the placebo trial.

There was no significant difference (P=0.195) in urine concentration between either the 2 mg inhalation or the 4 mg inhalation post time-trial in males or females with mean ± SD for the pooled groups (306 ± 288 $ng \cdot mL^{-1}$; 435 ± 410 $ng \cdot mL^{-1}$) and the peak values (956 $ng \cdot mL^{-1}$; 1244 $ng \cdot mL^{-1}$) for 2 mg inhaled terbutaline and 4 mg inhaled terbutaline, respectively (Figure 6).

Discussion

This study demonstrates that inhaled terbutaline (up to 4 mg) does not lead to improved 3 km running time-trial performance in recreationally active individuals. This is despite an observed small improvement in FEV_1 and an increase in post-exercise lactate (4 mg terbutaline only) when compared to placebo.

Our study is in agreement with others that suggest there is no significant effect on endurance performance following a high dose of inhaled terbutaline.[8,9] Previous work investigating the effects of oral supra-therapeutic doses of terbutaline (8 mg) failed to show an ergogenic effect on endurance performance and maximal sprint cycling performance.[7] Further experiments performed by Kalsen et al.,[8] examined the effects of high-dose (15 mg) inhaled terbutaline on 300 kcal cycling time-trial performance, there was no difference in completion times (1054 ± 125 s; 1072 ± 145 s) for placebo vs 15 mg inhaled terbutaline, respectively. These results are comparable to the present study in which completion times were (1102 ± 125 s; 1098 ± 109 s) in the pooled groups for placebo vs 4 mg inhaled terbutaline, respectively. This evidence supports a lack of ergogenic potential for terbutaline in moderate duration (~1100 s) endurance running and cycling performance.

Hostrup et al.,[9] reported that high-dose (15 mg) inhaled terbutaline increased muscle strength, and maximal sprint cycling performance but did not enhance endurance cycling performance. In line with these findings, further examination of this acute dose of 15 mg inhaled terbutaline was performed by Kalsen et al.,[14] investigating the effects on maximal 10s sprint cycling performance, with the finding that the observed increase in power output was also associated with increased levels of plasma lactate. They concluded that for a short period of time, terbutaline can counteract a reduction in ATP in type II muscle

fibres, further enhancing maximal sprint potential. The general consensus from Kalsen et al.,[14] and Hostrup et al.,[9] was that 15 mg inhaled terbutaline promotes a shift towards anaerobic carbohydrate metabolism during exercise, which may lead to greater power production in short-term anaerobic activity and greater fatigability over longer duration aerobic activity.[8,9,14,17,18]

Following the 4 mg inhaled terbutaline condition we observed an increase in post-exercise lactate (10.7 ± 2.3 mmol·L^{-1}) when compared to placebo (8.9 ± 1.8 mmol·L^{-1}). This may be, in part, due to enhanced Ca^{2+} release and increased contractile properties of skeletal muscle following terbutaline administration.[14,17,18] Hostrup et al.,[18] suggest that this enhanced contractility of skeletal muscle leads to elevated glycolytic activity during high-intensity exercise. These findings are in accordance with the findings of Kalsen et al.,[8] when investigating the effect of high-dose (15 mg) terbutaline on steady state exercise and also 300 kcal time-trial cycling performance, where lactate accumulation was higher during steady state exercise and was found to be attributable to higher rates of glycogenolysis and glycolysis, with no concomitant improvement in endurance performance. In association with the findings of Kalsen et al.,[8] it is possible that the lack of ergogenic effect seen in both our study and the study by Sanchez et al.,[7] can be explained by an earlier onset of fatigue during endurance performance due to enhanced glycolytic activity induced by terbutaline.[9,14]

The improvements seen in other studies with regard to sprint and power performance, could be due to greater potentiation of adrenergic receptors at very high dosages, according to Baker et al.,[27] a combination of selective affinity and intrinsic efficacy (ability to induce a response) dictate the strength of response at a given receptor. A highly selective partial agonist of the β_2-receptor such as terbutaline, with high intrinsic efficacy, given at a supra-therapeutic dose would have the ability to bind to the β_2-receptors in many types of tissue, increasing the ergogenic potential of the drug.[27] This could be one factor that could support the ergogenic effects found in those studies examining 15 mg inhaled terbutaline for strength and power performance.[9,14,17,18] With this in mind, the distribution of the high therapeutic dose (4 mg) in the present study, would likely have been lower than that of the 15 mg inhaled dose studies, therefore there could have been a lower potency of the β_2-agonist. Given that the present study's evidence stems from recreationally active individuals, it is likely that these results are transferrable to highly trained individuals, (i.e. the physiological response would be the same in both groups). Although this is a limitation, ethically, it would not have been possible to perform this study in an elite population, due to the athletes' responsibility to undertake out-of-competition testing.

A TUE is needed for the use of inhaled terbutaline during competition, largely due to the inability to distinguish between route of administration and total dose administered.[12,16,28] In the present study we were able to measure urine concentrations of 2 mg and 4 mg doses of terbutaline, interestingly our values for 2 mg (305.5±288.3 $ng \cdot ml^{-1}$) inhaled terbutaline are lower than those found in a previous investigation by Elers et al.,[12] for 2 mg inhaled terbutaline (472±324 $ng \cdot ml^{-1}$) and our values after 4 mg inhaled terbutaline (435.4±409.8 $ng \cdot mL^{-1}$) are comparable to the values after 10 mg oral terbutaline in the Elers et al.,[12] study (402±663 $ng \cdot ml^{-1}$). Interestingly, these values for both varying dosages and alternate routes of administration have very similar mean values, further highlighting the difficulty in distinguishing between therapeutic and supra-therapeutic use of terbutaline.[12] Of note, the timing of the urine sample in the Elers et al.,[12] study was at 4 hours, whereas in the present study urine samples were collected 1-hour post-inhalation. Indeed, serum concentrations of terbutaline reached a peak at the 4-hour stage in the Elers et al.,[12] study, therefore it is possible that inhaled terbutaline may not have reached peak levels in the urine at the 1-hour sample collection in the present study. The 4 mg inhaled dose was previously examined by Dyreborg et al.,[16] with peak concentrations reaching 1954 $ng \cdot mL^{-1}$ at the 2 hour stage post-inhalation, the present study found peak concentrations reaching 1244 $ng \cdot mL^{-1}$ 1 hour post-inhalation, it would have been beneficial to examine urinary levels of terbutaline at additional timepoints in the present study in order to ascertain time to maximal concentration (T_{max}) of terbutaline. A number of factors contribute to the varying levels of urinary terbutaline, recent work by Kreiberg et al.,[29] indicate varying pharmacokinetics of 4 mg inhaled terbutaline dependent upon external factors such as exercise performance and also environmental conditions, these differences exist post-correction for urine specific gravity, explanations for such variance include but are not limited to; inhalation technique, exercise intensity and hydration status.

In the investigations by Elers et al.,[12] and Dyreborg et al.,[16] significant differences were found between oral and inhaled doses, but no cut-off value could be established. If a cut-off value were able to be established then it is possible that inhaled terbutaline would be able to be monitored in much the same way as both salbutamol and formoterol, where an AAF would indicate possible supra-therapeutic inhaled use or oral administration, which have established ergogenic potential in strength and power performance.[7–9,14,17,18] Further investigation is needed to establish the ergogenic effects of therapeutic inhaled terbutaline on sprint and power performance. Recent findings also highlight that daily use of 4 mg inhaled terbutaline displays repartitioning properties, allowing for reductions in body fat and

increases in muscle mass.[30] Care is warranted with regard to the use of terbutaline in athletes with a TUE.

Practical Applications

Therapeutic use of terbutaline in athletes with a TUE will not lead to an ergogenic advantage during running-based endurance exercise. Investigations into appropriate monitoring of terbutaline are warranted in order to prevent the potential misuse of terbutaline via supratherapeutic dosing.

Conclusions

The findings of the present study suggest that therapeutic doses of inhaled terbutaline (up to 4 mg) do not improve 3 km running time-trial performance. Endurance running athletes using inhaled terbutaline via TUE, as therapy for their asthma, are therefore unlikely to experience an additional ergogenic advantage. Further research is needed investigating the effects of therapeutic inhaled doses of terbutaline during strength and power performance to fully elucidate any ergogenic potential.

References

1. Anderson SD, Daviskas E. The mechanism of exercise-induced asthma is ... *J Allergy Clin Immunol*. 2000;106(3):453-459. doi:10.1067/mai.2000.109822
2. Rundell KW, Im J, Mayers LB, Wilber RL, Szmedra L, Schmitz HR. Self-reported symptoms and exercise-induced asthma in the elite athlete. *Med Sci Sports Exerc*. 2001;33(2):208-213. doi:10.1097/00005768-200102000-00006
3. Dickinson J, McConnell A, Whyte G. Diagnosis of exercise-induced bronchoconstriction: eucapnic voluntary hyperpnoea challenges identify previously undiagnosed elite athletes with exercise-induced bronchoconstriction. *Br J Sports Med*. 2011;45(14):1126-1131. doi:10.1136/bjsm.2010.072520
4. Fitch KD. β_2-Agonists at the Olympic Games. *Clin Rev Allergy Immunol*. 2006;31(2-3):259-268. doi:10.1385/CRIAI:31:2:259
5. Driessen JMM, Gerritsma M, Westbroek J, ten Hacken NHT, de Jongh FHC. The effect of nebulized salbutamol or isotonic saline on exercise-induced bronchoconstriction in elite skaters following a 1,500-meter race: study protocol for a randomized controlled trial. *Trials*. 2013;14(1):204. doi:10.1186/1745-6215-14-204

6. Sanchez AMJ, Collomp K, Carra J, et al. Effect of acute and short-term oral salbutamol treatments on maximal power output in non-asthmatic athletes. *Eur J Appl Physiol*. 2012;112(9):3251-3258. doi:10.1007/s00421-011-2307-3

7. Sanchez AMJ, Borrani F, Le Fur MA, et al. Acute supra-therapeutic oral terbutaline administration has no ergogenic effect in non-asthmatic athletes. *Eur J Appl Physiol*. 2013;113(2):411-418. doi:10.1007/s00421-012-2447-0

8. Kalsen A, Hostrup M, Karlsson S, Hemmersbach P, Bangsbo J, Backer V. Effect of inhaled terbutaline on substrate utilization and 300-kcal time trial performance. *J Appl Physiol*. 2014;117(10):1180-1187. doi:10.1152/japplphysiol.00635.2014

9. Hostrup M, Kalsen A, Bangsbo J, Hemmersbach P, Karlsson S, Backer V. High-dose inhaled terbutaline increases muscle strength and enhances maximal sprint performance in trained men. *Eur J Appl Physiol*. 2014;114(12):2499-2508. doi:10.1007/s00421-014-2970-2

10. Prior J, Nowell R, Cochrane G. High-dose inhaled terbutaline in the management of chronic severe asthma: comparison of wet nebulisation and tube-spacer delivery. *Thorax*. 1982;37:300-303.

11. WADA. The World Anti-doping Code - Prohibited List 2018. *www.wada-ama.org*. 2018;(JANUARY). https://www.wada-ama.org/sites/default/files/prohibited_list_2018_en.pdf.

12. Elers J, Hostrup M, Pedersen L, et al. Urine and serum concentrations of inhaled and oral terbutaline. *Int J Sports Med*. 2012;33(12):1026-1033. doi:10.1055/s-0032-1311590

13. Jacobson GA, Hostrup M. Terbutaline: level the playing field for inhaled β 2 -agonists by introducing a dosing and urine threshold. *Br J Sports Med*. 2017;51(18):1323-1324. doi:10.1136/bjsports-2016-096453

14. Kalsen A, Hostrup M, Soderlund K, Karlsson S, Backer V, Bangsbo J. Inhaled beta2-Agonist increases power output and glycolysis during sprinting in men. *Med Sci Sports Exerc*. 2016;48(1):39-48. doi:10.1249/MSS.0000000000000732

15. Hostrup M, Onslev J, Jacobson GA, Wilson R, Bangsbo J. Chronic β2-adrenoceptor agonist treatment alters muscle proteome and functional adaptations induced by high intensity training in young men. *J Physiol*.

2018;596(2):231-252. doi:10.1113/JP274970

16. Dyreborg A, Krogh N, Backer V, Rzeppa S, Hemmersbach P, Hostrup M. Pharmacokinetics of oral and inhaled terbutaline after exercise in trained men. *Front Pharmacol*. 2016;7(JUN):1-10. doi:10.3389/fphar.2016.00150

17. Hostrup M, Kalsen A, Onslev J, et al. Mechanisms underlying enhancements in muscle force and power output during maximal cycle ergometer exercise induced by chronic β_2 -adrenergic stimulation in men. *J Appl Physiol*. 2015;119(5):475-486. doi:10.1152/japplphysiol.00319.2015

18. Hostrup M, Kalsen A, Ortenblad N, et al. β2-adrenergic stimulation enhances Ca2+ release and contractile properties of skeletal muscles, and counteracts exercise-induced reductions in Na+-K+-ATPase Vmax in trained men. *J Physiol*. 2014;592(24):5445-5459. doi:10.1113/jphysiol.2014.277095

19. Johnson M. The β -Adrenoceptor. *Am J Respir Crit Care Med*. 1998;158(supplement_2):S146-S153. doi:10.1164/ajrccm.158.supplement_2.13tac110

20. Qing F, Rhodes CG, Hayes MJ, et al. In vivo quantification of human pulmonary beta-adrenoceptor density using PET: comparison with in vitro radioligand binding. *J Nucl Med*. 1996;37(8):1275-1281.

21. Brodde OE, Michel MC. Adrenergic and muscarinic receptors in the human heart. *Pharmacol Rev*. 1999;51(4):651-690.

22. Sato S, Shirato K, Tachiyashiki K, Imaizumi K. Muscle plasticity and β2-adrenergic receptors: Adaptive responses of β2-adrenergic receptor expression to muscle hypertrophy and atrophy. *J Biomed Biotechnol*. 2011;2011. doi:10.1155/2011/729598

23. Collomp K, Le Panse B, Candau R, Lecoq AM, De Ceaurriz J. Bêta-2 agonistes et performance sportive. *Sci Sport*. 2010;25(6):281-290. doi:10.1016/j.scispo.2010.08.002

24. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-338. doi:10.1183/09031936.05.00034805

25. Anderson SD, Argyros GJ, Magnussen H, Holzer K. Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. *Br J Sports Med*. 2001;35(5):344-347. doi:10.1136/BJSM.35.5.344

26. Borg GAV. Psychophysical bases of perceived exertion. *Med Sci Sport Exerc*. 1982;14(5):377???381. doi:10.1249/00005768-198205000-00012

27. Baker JG. The selectivity of β-adrenoceptor agonists at human β1-, β2- and β3-adrenoceptors. *Br J Pharmacol*. 2010;160(5):1048-1061. doi:10.1111/j.1476-5381.2010.00754.x

28. Krogh N, Rzeppa S, Dyreborg A, et al. Terbutaline accumulates in blood and urine after daily therapeutic inhalation. *Med Sci Sports Exerc*. 2017;49(6):1236-1243. doi:10.1249/MSS.0000000000001199

29. Kreiberg M, Becker V, Jessen S, et al. Influence of exercise in normal and hot ambient conditions on the pharmacokinetics of inhaled terbutaline in trained men. *Scand J Med Sci Sport*. 2017;27(7):692-703. doi:10.1111/sms.12697

30. Jessen S, Onslev J, Lemminger A, Backer V, Bangsbo J, Hostrup M. Hypertrophic effect of inhaled beta $_2$ -agonist with and without concurrent exercise training: A randomized controlled trial. *Scand J Med Sci Sports*. 2018;(May):1-9. doi:10.1111/sms.13221

Tables

Table 1: Mean (±SD) Participant Demographics and Lung Function at Baseline and % Change in Lung Function Post-EVH in Males and Females.

Group	Height (cm)	Weight (kg)	Age (yrs)	Baseline FEV_1 (L)	% Predicted FEV_1	Baseline FVC (L)	% Predicted FVC	FEV_1/FVC Ratio	Baseline PEF (L)	% Predicted PEF	Post-EVH % Fall in FEV_1
Males (n=8)	179.5 (4.3)	77.6 (8)	24.3 (2.4)	5.2 (0.2)	114 (4.6)	5.9 (0.6)	110.5 (8.2)	0.83 (0.05)	580.6 (57.9)	96 (10)	5.1 (6.1)
Females (n=8)	163 (9.2)	58.6 (6)	22.4 (3)	3.6 (0.5)	108.9 (13.4)	3.93 (0.5)	105.3 (12)	0.92 (0.03)	439.1 (75.7)	102.8 (17.8)	3.8 (1.6)

FEV_1 - Forced Expiratory Volume in 1 Second; EVH - Eucapnic Voluntary Hyperpnoea; FVC – Forced Vital Capacity; PEF – Peak Expiratory Flow

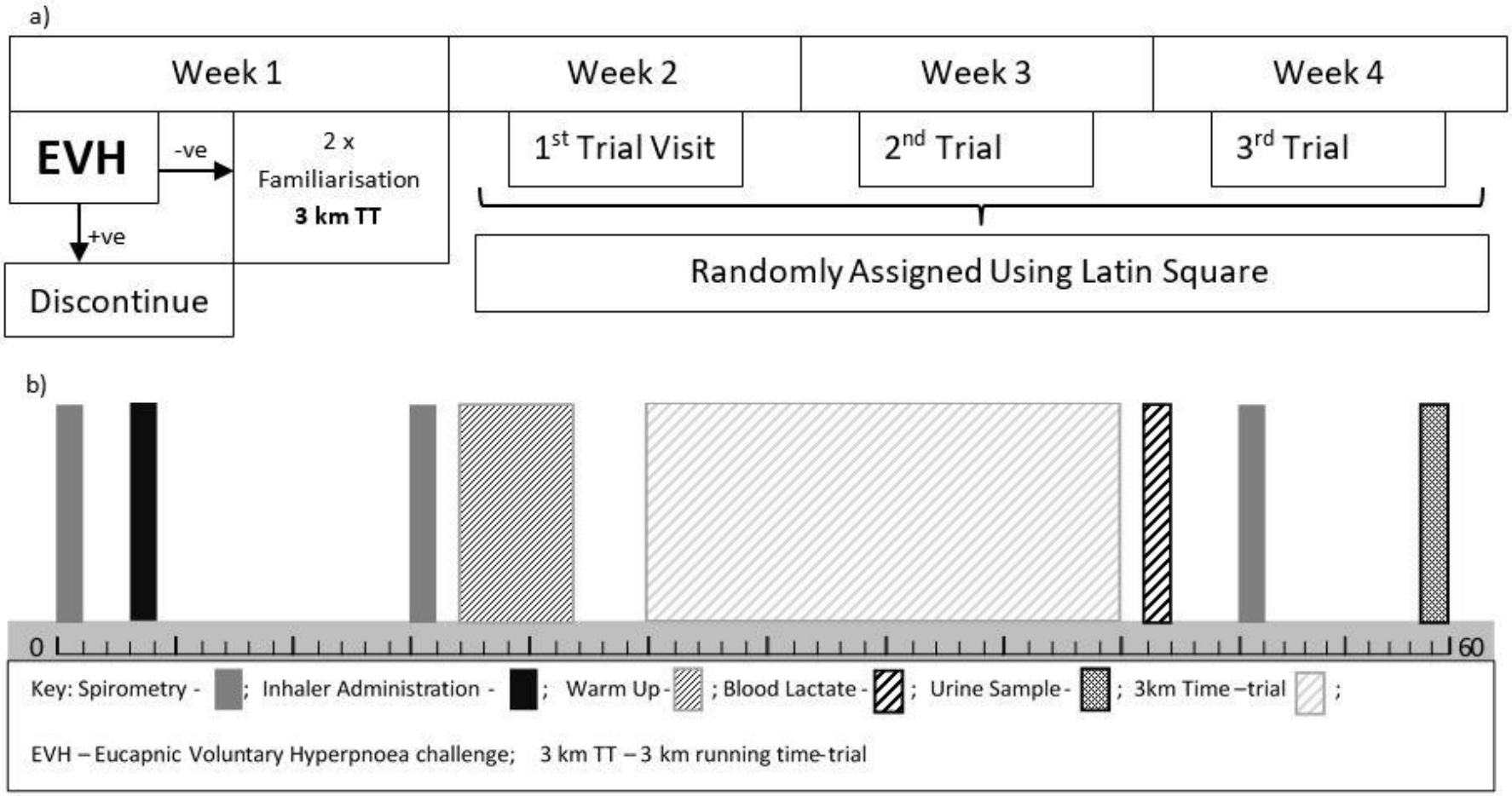
ECCS – *European Community for Coal and Steel Reference Values for Predicted Lung Function*

Table 2: FEV_1 (L) for trial conditions at baseline, post inhaler and post time trial in the pooled group

Time point	Placebo	2 mg	4 mg
Baseline	4.81 ± 0.55	4.84 ± 0.54	4.80 ± 0.55
Post Inhaler	4.83 ± 0.54	5.08 ± 0.55*	5.07 ± 0.48†
Post Time-Trial	4.87 ± 0.56	5.07 ± 0.55	5.04 ± 0.49

Significantly different from placebo * P=0.01 † P=0.02

FEV_1 – Forced Expiratory Volume in 1 Second



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587 **Figure 1**: a) Study duration, progression and randomisation protocol b) Schematic diagram of the test procedures during the 60-minute trial visit

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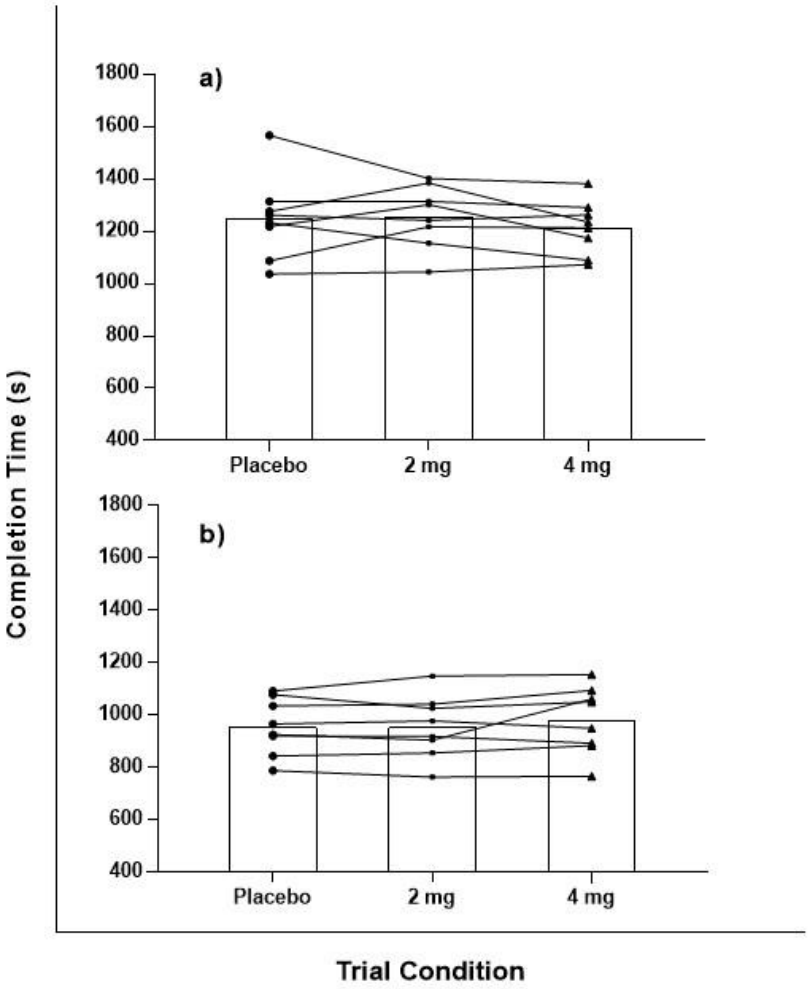

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590 **Figure 2:** *Mean and individual 3km running time-trial completion times for a) females and b) males following placebo, 2 mg inhaled terbutaline and 4*
591 *mg inhaled terbutaline trial conditions.*

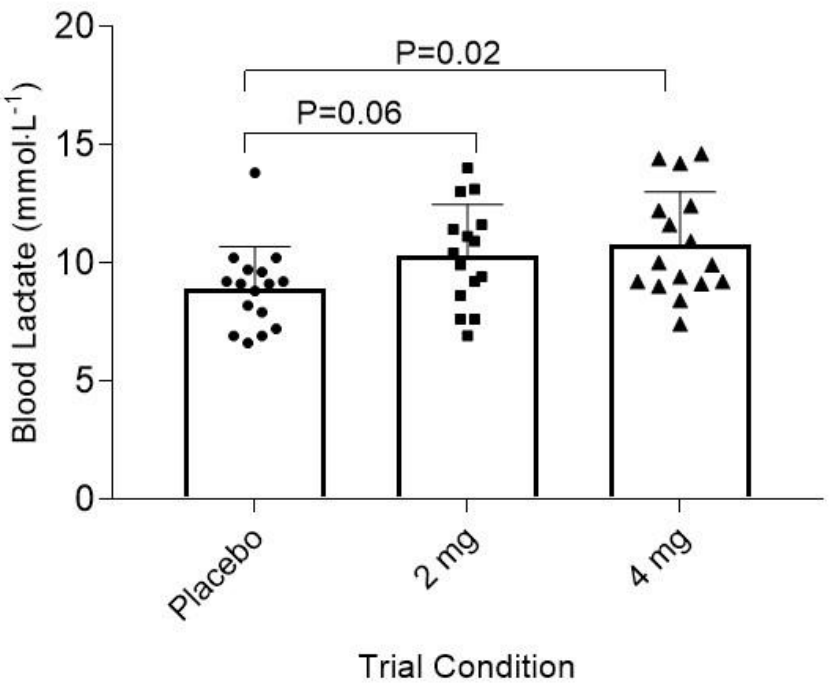
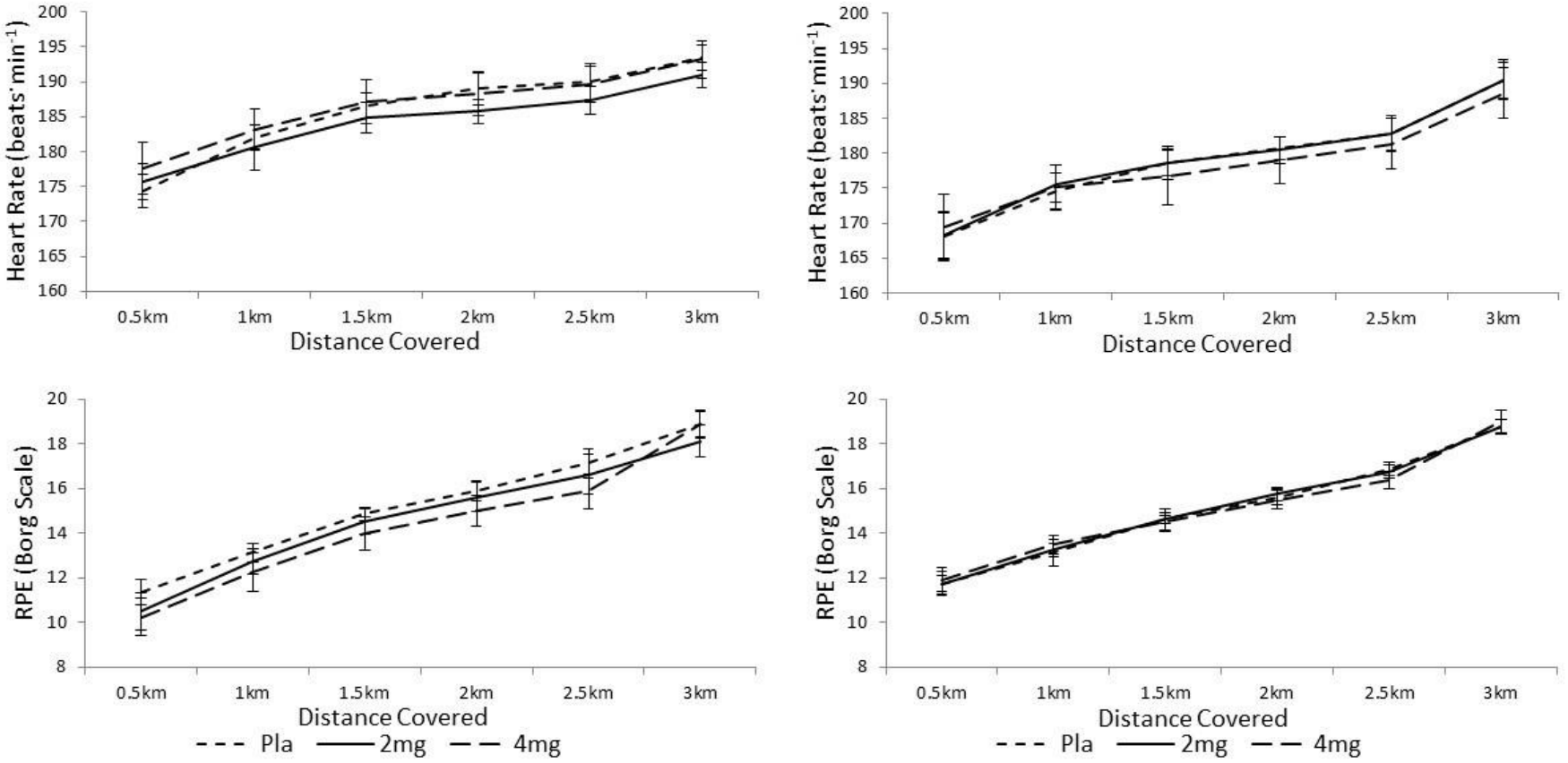


Figure 3: *Mean (±SD) Lactate values post 3km running time-trial for placebo, 2 mg inhaled terbutaline and 4 mg inhaled terbutaline conditions.*



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599 **Figure 4:** *Exercising values (Mean ± SD) for: Heart Rate (HR) in a) females b) males and rating of perceived exertion (RPE) in c) females d) males during*
600 *each of the three trial conditions, placebo, 2 mg inhaled terbutaline and 4 mg inhaled terbutaline.*

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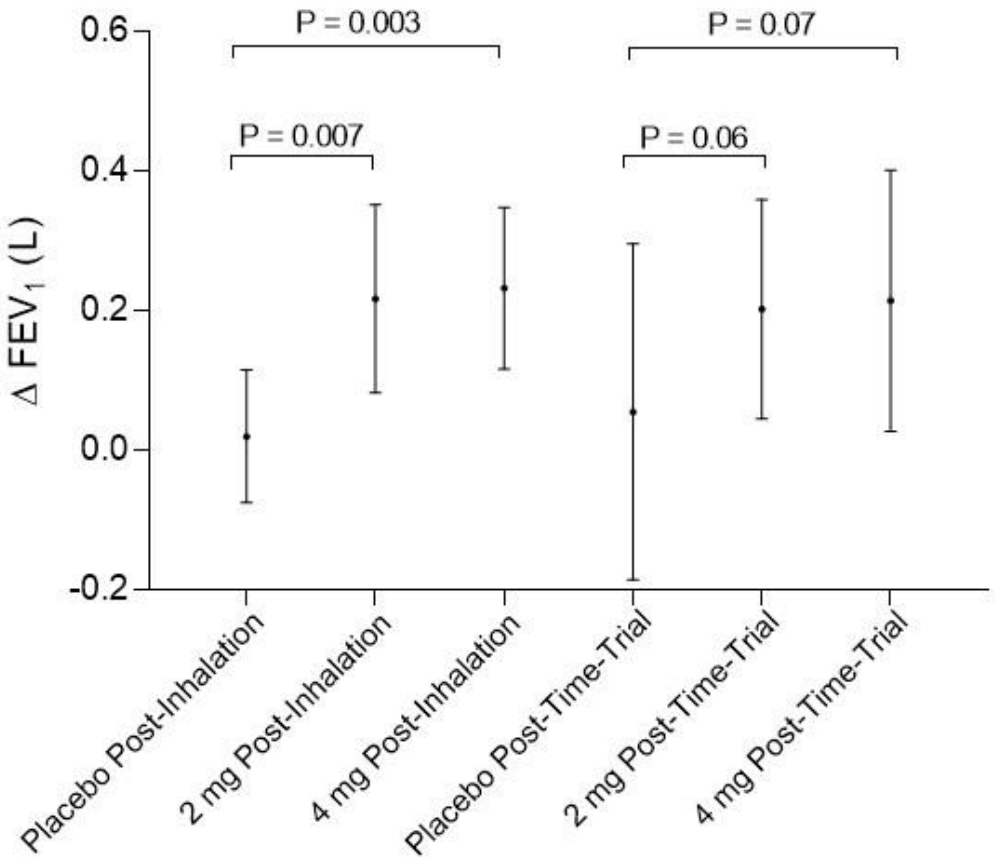

Figure 5: *Mean (± SD) change in FEV_1 from baseline post-inhalation and post-time-trial completion for placebo, 2 mg inhaled terbutaline and 4 mg inhaled terbutaline.*

ΔFEV_1 – Change in FEV_1 compared to baseline

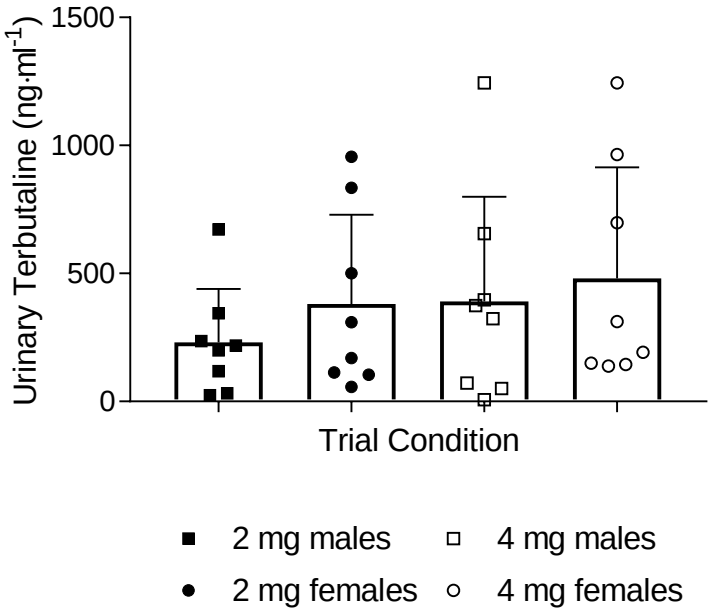

Figure 6: *Individual peak and mean (± SD) urinary concentrations 1 hour post terbutaline inhalation in the 2 mg inhaled and 4 mg inhaled terbutaline trials in males and females.*