

The impact of acute and chronic administration of short-acting β_2 -agonists on urinary pharmacokinetics and athletic performance

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Abstract

Exercise Induced Bronchoconstriction (EIB) is common amongst elite athletes. Short-acting β_2 -agonists represent the first-line treatment of EIB, however; limited data currently exists examining the ergogenic and pharmacokinetic impact of chronic short-acting β_2 -agonist administration. Furthermore, the ergogenic impact of acute and chronic administration of short-acting β_2 -agonists in asthmatic individuals is unknown. Whilst the short-acting β_2 -agonist salbutamol is permitted in and out of competition due to a known pharmacokinetic response, no urinary threshold has been established for the use of the alternative short-acting β_2 -agonist terbutaline.

The purpose of study 1 was to investigate the ergogenic potential of the WADA upper daily limit of $1600 \mu\text{g}\cdot\text{day}^{-1}$ salbutamol every day for 6 weeks versus placebo, alongside combined resistance and endurance training. Findings highlighted improvements in; 1 repetition maximum (1RM) bench press (Baseline: 65.6 ± 5.4 kg vs. 64.3 ± 4.9 kg – 6 weeks: 70.3 ± 4.9 vs. 72.5 ± 5.4 kg) and leg press (Baseline: 250 ± 26.9 vs. 217.9 ± 19 kg – 6 weeks: 282.5 ± 22.5 vs. 282.8 ± 18.3 kg); vertical jump test (Baseline: 53.5 ± 4.1 vs. 50.4 ± 2.1 cm – 6 weeks: 55 ± 3.5 vs. 52.4 ± 1.7 cm); 3 km running time-trial performance (Baseline: 988.7 ± 68.7 vs. 1040.5 ± 66.3 s – 6 weeks: 947.5 ± 54.9 vs. 1004.3 ± 70.5 s); isokinetic dynamometry (Baseline: 196.1 ± 47.3 vs. 184.6 ± 35.0 n.m. – 6 weeks: 179.5 ± 48.9 vs. 195.2 ± 28.9 n.m.); and body composition (Baseline: 32.1 ± 13.9 vs. 34.9 ± 10.4 mm – 6 weeks: 32.4 ± 14.5 vs. 34.5 ± 10 mm) for both the salbutamol group and the placebo group, respectively, over the 6 week period, with no difference observed between groups, indicating long-term therapeutic use of salbutamol at the WADA upper daily limit has no ergogenic effect. Of note, one participant exceeded the urinary threshold, presenting with an adverse

analytical finding (AAF) showing that the upper daily limit can lead to AAF's in susceptible individuals.

Athletes who respond poorly to salbutamol treatment are able to apply for the use of the short-acting β_2 -agonist terbutaline via a therapeutic use exemption (TUE) certificate. Urinary upper limits are unknown for terbutaline and as such it is prohibited at all times without the presentation of a TUE. The purpose of study 2 was to investigate the urinary excretion of terbutaline following single and repeated use of inhaled or oral terbutaline. The aim of the study was to establish a differential distinction between routes of administration which could assist the WADA with regard to anti-doping policy and procedure. Results demonstrated a significant difference in urine concentration of terbutaline between inhaled and oral administration for female Caucasian (670.1 ± 128.3 vs. 361.8 ± 43.8 ng·ml⁻¹; $P=0.019$; 680.8 ± 91 vs. 369.9 ± 41.9 ng·ml⁻¹; $P=0.006$), male Afro-Caribbean (343.18 ± 45 vs. 231.3 ± 32.95 ng·ml⁻¹; $P=0.044$; 389.73 ± 67.4 vs. 212.4 ± 50.3 ng·ml⁻¹; $P=0.008$) and male Asian (266.4 ± 23.7 vs. 143.3 ± 22 ng·ml⁻¹; $P=0.004$; 379.5 ± 50.4 vs. 197.5 ± 38.6 ng·ml⁻¹; $P=0.000$) groups for single (5 mg oral vs. 2 mg inhaled) and repeated (4 x 5 mg oral vs. 8 x 1 mg inhaled) administration trials, respectively. No difference was observed in male Caucasians. High intra- and inter-individual variability between samples meant that a clear distinction between routes of administration could not be established. The study was able to identify an upper urinary threshold following inhaled administration of 1284.3 ng·ml⁻¹ and an upper urinary threshold following oral use of 2376.3 ng·ml⁻¹ which may inform the process of distinguishing between inhaled and oral use.

Athletes are permitted to use inhaled terbutaline therapeutically through the TUE process. The purpose of study 3 was to investigate the ergogenic effect of terbutaline at high (2 mg and 4 mg) therapeutic inhaled doses on 3 km running time-trial performance in males and females. The

study found that inhaled terbutaline, when used at the highest therapeutic dose, has no impact upon 3 km time-trial performance in males (956.3 s vs. 982 s) and females (1249 s vs. 1214.7 s) for placebo vs. 4 mg inhaled terbutaline, respectively.

The majority of studies investigating the ergogenic potential of salbutamol have been in healthy individuals. It is not yet understood whether the exercise response differs in asthmatic individuals. The purpose of study 4 was to investigate the use of inhaled salbutamol (400 µg) during a 3 km running time-trial in eucapnic voluntary hyperpnoea positive (EVH+ve) and negative (EVH-ve) individuals, in a low humidity environment. Results demonstrated increased FEV₁ in both groups following salbutamol inhalation, which did not translate to improved performance. No performance differences were found between salbutamol and placebo (Sal: 1012.7 ± 50 vs. 962.1 ± 37.5 s – Pla: 1002.4 ± 46.5 vs. 962 ± 28.8 s) in the EVH+ve group vs. the EVH-ve group, respectively.

This thesis is the first to investigate the effects of long-term use of salbutamol at the WADA upper daily limit on exercise performance. It is also the first study to establish upper urinary thresholds for terbutaline use, and the effects of therapeutic inhaled terbutaline on exercise performance. The effect of salbutamol on exercise performance at low humidity in asthmatic individuals has also never previously been investigated. Overall, the findings from this thesis support previous research that inhaled β₂-agonist use does not provide any ergogenic potential. With β₂-agonists being an essential therapy for the treatment of EIB their current position on the WADA List of Prohibited Substances and Methods is appropriate. Further research is warranted to fully elucidate the upper urinary threshold for terbutaline to inform WADA and support the re-introduction of terbutaline as a therapeutic tool in the treatment of EIB in athletes.

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List of Abbreviations

Δ	–	change
\dot{V}_E	–	minute ventilation
$\dot{V}O_2$	–	oxygen consumption
μ	-	micro
μg	–	micrograms
AAF	–	adverse analytical finding
AC	–	adenylate cyclase
AHR	–	airway hyper-responsiveness
ATP	–	adenosine tri-phosphate
ATS	–	American Thoracic Society
B	–	B-lymphocyte
BTS	–	British Thoracic Society
Ca^{2+}	-	calcium
cAMP	–	cyclic adenosine monophosphate
CD34+	-	haematopoietic progenitor cell antigen
cm	–	centimetres
D	–	dendritic cell

DoU	–	declaration of use
E	–	eosinophil
ECM	–	extra-cellular matrix
ECP	–	eosinophil cationic protein
EIA	–	exercise-induced asthma
EIB	–	exercise-induced bronchoconstriction
EVH	–	eucapnic voluntary hyperpnoea
EVH+ve	–	eucapnic voluntary hyperpnoea positive
EVH-ve	-	eucapnic voluntary hyperpnoea negative
F	–	fibroblast
FC	–	female Caucasian
FEF ₂₅₋₇₅	–	forced expiratory flow rate between 25-75%
FeNO	–	fractional exhaled nitric oxide
FEV1	–	forced expiratory volume in 1 second
FVC	–	forced vital capacity
g	–	gram
GB	–	Great Britain
GI	–	gastro-intestinal
Gi	–	G-protein i

GM-CSF	–	granulocyte macrophage – colony stimulating factor
GPCRs	–	G-protein coupled receptors
Gq	–	G-protein q
h	–	hours
HPLC	–	high performance liquid chromatography
HR	–	heart rate
HSP-20	–	heat shock protein – 20
ICS	–	inhaled corticosteroid
IgE	–	immunoglobulin E
IOC	–	International Olympic Committee
IOC-MC	-	International Olympic Committee – Medical Commission
Km	–	kilometres
LT	–	leukotriene
LTC	–	leukotriene C ₄
M	–	macrophage
m	–	milli
MA	–	male Asian
MAC	–	male afro-Caribbean
MBP	–	major basic protein

MC	–	male Caucasian
Mc	–	mast Cell
mg	–	milligrams
ml	–	millilitres
MLCK	–	myosin light-chain kinase
mm	–	millimetres
MVV	–	maximum voluntary ventilation
n	–	nano
N	–	neutrophil
N. m.	–	newton metres
NA	–	noradrenaline
PAF	–	platelet activating factor
PDE	–	phosphodiesterase
PEF	–	peak expiratory flow
PG	–	prostaglandin
PKA	–	protein kinase A
PRN	–	<i>pro re nata</i>
RER	–	respiratory exchange ratio
RM	–	repetition maximum

RPE	–	rating of perceived exertion
RyR	–	ryanodine receptor
s	–	seconds
SAN	–	sino-atrial node
SBM	–	Subepithelial basement membrane
SD	–	standard deviation
SE	–	standard error
SR	–	sarcoplasmic reticulum
T	–	T-lymphocyte
TGF	–	transforming growth factor
TGF- β	–	transforming growth factor β
T _{H2}	–	T-helper 2 lymphocyte
TNF	–	tumor necrosis factor
TUE	–	therapeutic use exemption
vs	–	versus
WADA	–	World Anti-Doping Agency
β_2 R	–	β_2 -Receptor

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1. Introduction

1.1 Introduction

In 2002 The International Olympic Committee (IOC) established the requirement for athletes to present evidence of current asthma, exercise induced asthma (EIA), exercise induced bronchospasm (EIB) or airway hyper-responsiveness (AHR) through the Therapeutic Use Exception (TUE) certificate process. These regulations, guided by the IOC Medical Commission (IOC-MC), were based on health and not anti-doping (performance enhancing) concerns for athletes in light of a marked increase in the notification by athletes for the use of inhaled short acting β_2 -agonist from 3.7% in Atlanta, 1996, to 5.7% in Sydney, 2000 (Fitch *et al.*, 2008). Dickinson *et al.* (2005) provided support for the health justification of adding inhaled short acting β_2 -agonists to the prohibited substances list when reporting data from the Sydney, 2000, and Athens, 2004, Great Britain Olympic Teams (Team GB; Dickinson *et al.*, 2005).

The data from Dickinson *et al.* (2005) demonstrated that the establishment of a TUE for inhaled short acting β_2 -agonists had no impact on the proportion of Team GB presenting with asthma, EIA, EIB or AHR (c.21% at both Olympic Games) however; the use of the TUE identified a number of athletes with false positive diagnoses and athletes who had not been previously identified. Accordingly, they concluded, as have others, that the requirement of demonstrable evidence through the TUE process improves the quality of care for athletes. Furthermore, Rundell *et al.*, (2004) has demonstrated the

improved diagnostic sensitivity and specificity of objective, bronchial provocation tests of airway function as part of the TUE process.

Whilst the weight of evidence supports the improved health care of athletes following the introduction of the TUE process for inhaled short acting β_2 -agonists (Dickinson *et al.*, 2005) WADA removed the need for a TUE for salbutamol in 2010, replacing it with the need for declaration of use (DoU). The position of salbutamol was further relaxed in 2012 to allow athletes to use salbutamol for the treatment of respiratory symptoms, monitored with a urinary concentration threshold limit of 1000 ng.ml^{-1} (WADA Prohibited List, 2015). Due to their potential performance enhancing properties (Pluim *et al.*, 2011), all orally administered β_2 -agonists are banned for use by athletes in and out of competition (WADA, 2015). There are a number of inhaled β_2 -agonists that are permitted in the form of salbutamol, salmeterol, and formoterol that can be used by athletes who have asthma, EIA, EIB or AHR. In contrast, terbutaline remains on the restricted list and can only be used through the therapeutic use exemption (TUE) process (WADA 2015), which requires the athlete to submit objective evidence of asthma/EIA/EIB/AHR and a detailed history of their condition. The regulations for the use of salbutamol, salmeterol and formoterol are relatively relaxed when compared with terbutaline.

In contrast to the improved health care of athletes, little is understood of the ergogenic effect of inhaled short acting β_2 -agonists. β_2 -adrenergic

stimulation of various organs plays an important role in adaptation to exercise. Increased transport capacity through an increase in cardiac output, increased availability of substrates for energy metabolism by increases in lipolysis and glycogenolysis and increased skeletal muscle contractility associated with increased activity of the sympathetic nervous system are all, in part, mediated by β -adrenergic receptor stimulation (Hoffman, 2001). Despite this only a small number of studies have examined the ergogenic effect of inhaled short acting β_2 -agonists.

A recent review highlighted there is limited evidence to suggest that short acting β_2 -agonists provide any ergogenic effect (Price *et al.*, 2014). However, the majority of previous studies have investigated the impact of inhaled salbutamol on endurance running, cycling and swimming performance in Caucasian males (Meeuwisse *et al.*, 1992; van Baak *et al.*, 2000; Decorte *et al.*, 2008; McKenzie *et al.*, 1983; Koch *et al.*, 2013; Koch *et al.*, 2014; Elers *et al.*, 2010). Of the small number of studies that have investigated the impact of short acting β_2 -agonists on sprint/power performance there is contrasting evidence for an ergogenic effect (Signorile *et al.*, 1992; Sporer *et al.*, 2008; Decorte *et al.*, 2013; Decorte *et al.*, 2008; Kalsen *et al.*, 2013; Van Baak *et al.*, 2000). Furthermore, previous research investigating inhaled salbutamol has primarily focused on acute administration of a single therapeutic and supra-therapeutic dose. Research investigating long-term daily administration of salbutamol at the maximum WADA permitted dose, is therefore warranted.

The majority of available evidence for the ergogenic effect of inhaled β_2 -agonists has focused on salbutamol. Very few studies have investigated the ergogenic effects of terbutaline (Larsson *et al.*, 1997; Unnithan *et al.*, 1994; Sanchez *et al.*, 2013; Kalsen *et al.*, 2014; Hostrup *et al.*, 2014). The study by Larsson *et al.* (1997) reported no significant effect on running time to exhaustion performed at 10°C in elite athletes following the administration of 3 mg of inhaled terbutaline, another study by Sanchez *et al.* (2013) also investigating oral terbutaline found no significant effects on performance. Kalsen *et al.* (2014) and Hostrup *et al.* (2014) both investigated supra-therapeutic inhaled terbutaline finding improvements in strength and power performance yet no improvements in endurance performance. The lack of research on the ergogenic effects of inhaled terbutaline is a key factor in the decision by WADA for a full TUE for inhaled terbutaline.

Research investigating the differentiation between oral and inhaled salbutamol (Elers *et al.*, 2010; Berges *et al.*, 2000) has managed to successfully identify differences between routes of administration. However, limited research is available examining the differentiation of inhaled and oral administration of terbutaline. Roig, *et al.* (2002) attempted to distinguish between oral and inhaled doses of terbutaline through analysis of terbutaline concentration in urine. Although different trends were observed after oral and inhaled doses in total terbutaline, total free terbutaline concentrations and in ratios between its enantiomers, differences observed were not sufficiently

significant to establish cut-off values in order to clearly distinguish between routes of administration.

The dosing strategy employed by Roig *et al.*, (2002) has limited ecological validity as it failed to replicate the dosing strategy that may be adopted by an elite athlete which would be a standardized dose used *PRN*. More recently Elers *et al.*, (2012b) examined terbutaline use via both oral and inhaled administration with more ecologically valid dosing strategies. The findings indicate that, although there was a significant difference between values, they were unable to identify a cut-off value that could distinguish between routes of administration. Further research investigating urinary concentrations of inhaled and oral terbutaline, along with the ergogenic potential of therapeutic doses of inhaled terbutaline are therefore warranted.

This thesis will add to the current body of knowledge associated with the ergogenic effect and pharmacokinetics of short acting β_2 -agonists, and in the process assist WADA in the implementation of regulations on the use of inhaled short acting β_2 -agonists and assist in the resolution of contested doping violations. Firstly, by investigating the legitimacy of the chronic administration of the WADA daily upper limit of 1600 μg salbutamol per day. Secondly, by investigating the urinary concentrations of inhaled and oral terbutaline to establish cut-off limits for anti-doping purposes. Thirdly, investigate the effects of a therapeutic dose of inhaled terbutaline on endurance performance. Finally, investigate the potential for ergogenic

effects of salbutamol in eucapnic voluntary hyperpnoea (EVH) positive individuals along with the effectiveness of salbutamol at offsetting any detriment in either lung function or performance that may be experienced by mild EVH positive (EVH+ve) individuals, exercising in a low humidity environment.

2. Review of the literature

2.1 Asthma

Asthma is a chronic inflammatory disorder of the conducting airways which causes airway hyper-responsiveness (AHR) and recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning (Barnes, 2011). These episodes are associated with widespread but variable airflow obstruction that is reversible, either spontaneously or with treatment. Asthma is heterogeneous with respect to immunopathology, clinical phenotypes, response to therapies and natural history.

Accordingly, asthma is being redefined as a collection of different endotypes rather than a single, specific disease with a unifying pathogenic mechanism (Barnes, 2011). Asthma is purported to affect an estimated 300 million people worldwide (Braman *et al.*, 2006; Masoli *et al.*, 2004) with recent estimates suggesting that around 15% of the UK population (5.4 million people) are asthmatic, leading to increasing National Health Service (NHS) expenditure of up to 1 billion pounds per annum (Asthma U.K., www.asthma.org.uk accessed December 2014).

2.1.1 Pathophysiology of asthma

The characteristic mechanisms of asthma include activation of mast cells, infiltration of eosinophils and infiltration of T helper 2 (T_H2) lymphocytes into the airway epithelium (Barnes, 2008). The activation of mast cells in the airway epithelium (e.g. by allergens), releases inflammatory mediators into the extra-cellular matrix (ECM), including histamine, leukotriene (LT) D₄, and prostaglandin (PG) D₂. The release of these mediators leads to bronchoconstriction, microvascular leakage and plasma exudation in susceptible individuals (Skidgel *et al.*, 2012). Figure 2.1 depicts the downstream cascade of cellular mechanisms caused by activation of the mast cell in both a healthy and a damaged airway epithelium, with the latter also showing increased eosinophil infiltration and activation.

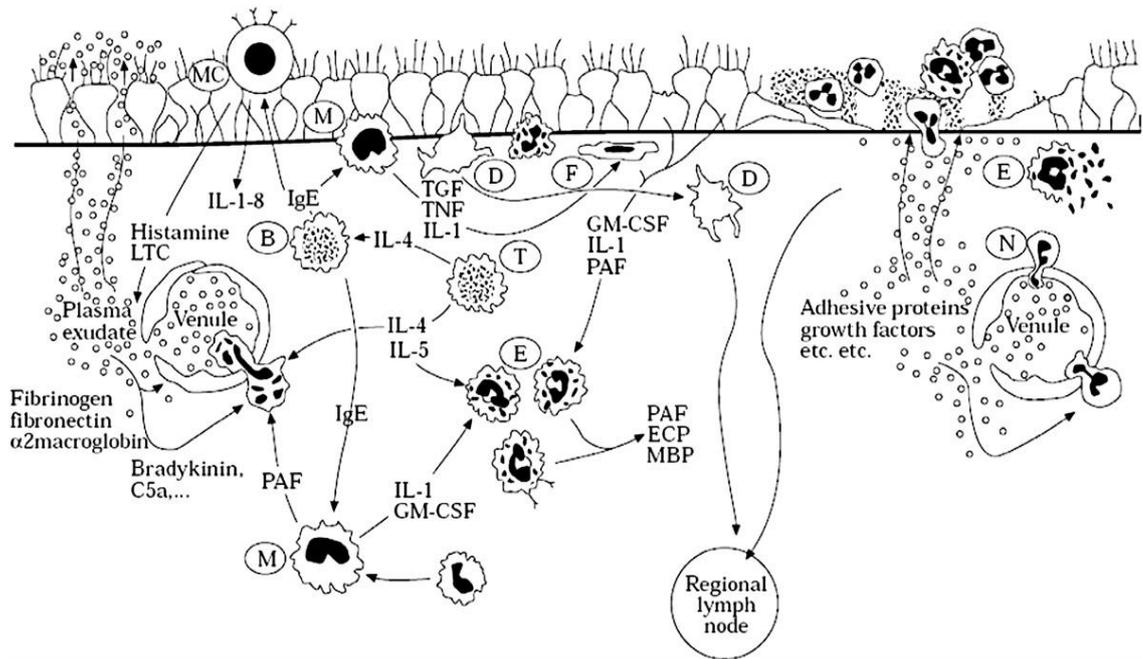
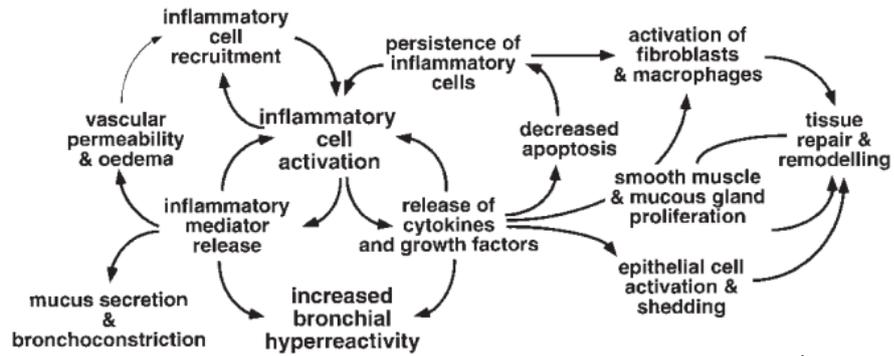


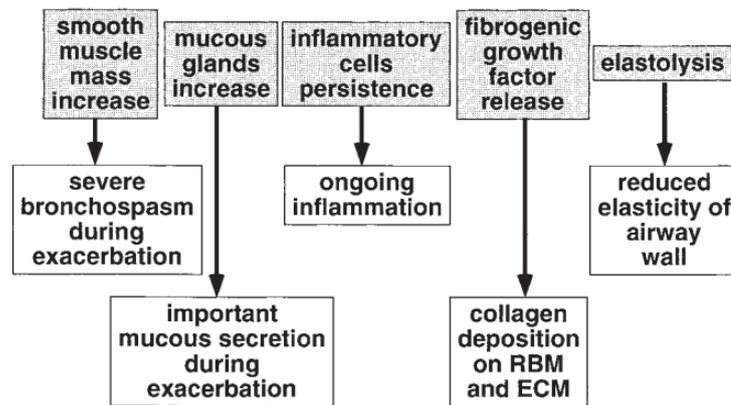
Figure 2.1 The airway epithelium showing the process of exudative inflammation in the intact epithelium (left) and damaged epithelium (right). Plasma derived adhesive proteins and solutes are contributing to the milieu of the lamina propria, epithelium and mucosal surface. MC – Mast Cell, M – Macrophage, E – Eosinophil, N – Neutrophil, F – Fibroblast, D – Dendritic Cell, B – B-lymphocyte, T – T-lymphocyte, GM-CSF – Granulocyte Macrophage Colony Stimulating Factor, LTC – Leukotriene C₄, TGF – Transforming Growth Factor, TNF – Tumor Necrosis Factor, IL – Interleukin, IgE – Immunoglobulin E, PAF, Platelet-activating factor; ECP, eosinophil cationic protein; MBP, major basic protein (Anderson & Kippelen 2008)

2.1.2 Chronic adaptations in asthma

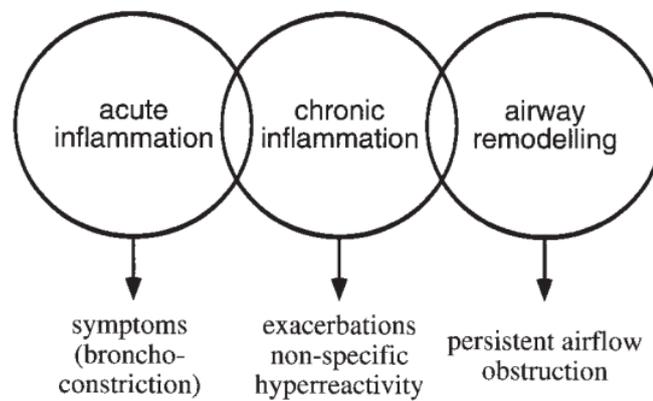
Chronic features of asthma are; mucus hyperplasia, fibroblast proliferation and airway remodelling. The chronic exposure to the stimulus causes increased infiltration and activation of eosinophils, neutrophils and mast cell degranulation (Figure 2.1). In addition, Holgate, (2008) explains that the chronic adaptations with frequent asthma exacerbations include smooth muscle cell hyperplasia, epithelial mesenchymal transition and fibroblast stimulation via CD34+ cells and transforming growth factor β (TGF β) in the repair of damaged epithelial tissue (Figure 2.2).



a)



b)



c)

Figure 2.2: Diagram of the mechanisms for structural changes showing; a) Mechanisms for acute and chronic inflammation in asthma and the remodelling process; b) Clinical consequences of airway remodelling in asthma; c) Link between pathologic mechanisms and clinical consequence in asthma (Bousquet *et al.*, 2000)

Although historically asthma has been thought to be a T_H2 mediated disease it is now largely agreed that a number of different phenotypes exist, each with differing mechanisms of action and each with differing degrees of severity dependent upon the specific mechanism (Boulet *et al.*, 2014). Table 2.1 highlights the varying phenotypes of asthma, their identification and treatment (Wenzel *et al.*, 2012).

Table 2.1: Asthma phenotypes, identification and treatment (Adapted from Wenzel *et al.*, 2012)

Asthma phenotypes in relation to characteristics					
Phenotype	Natural History	Clinical and physiological features	Pathobiology and biomarkers	Genetics	Response to therapy
Early-onset allergic	Early onset; mild to severe	Allergic symptoms and other diseases	Specific IgE; T _H 2 cytokines; thick SBM	17q12; T _H 2-related genes	Corticosteroid responsive; TH2-targeted
Late-onset eosinophilic	Adult onset; often severe	Sinusitis; less allergic	Corticosteroid refractory eosinophilia; IL-5		Responsive to antibody IL-5 and cysteinyl leukotriene modifiers; corticosteroid refractory
Exercise-induced		Mild; intermittent with exercise	Mast cell activation; T _H 2 cytokines; cysteinyl leukotrienes		Responsive to cysteinyl leukotriene modifiers, beta-2 agonists and antibody to IL-9
Obesity-related	Adult onset	Women are primarily affected; very symptomatic; AHR less clear	Lack of T _H 2 biomarkers; oxidative stress		Responsive to weight loss, antioxidants and possibly to hormone therapy
Neutrophilic		Low FEV ₁ ; More air trapping	Sputum neutrophilia; T _H 17 pathways; IL-8		Possibly responsive to macrolide antibiotics

2.1.3 Diagnosis of asthma

The predominant feature of asthma is airway smooth muscle contraction, resulting in obstructed airways during expiration and reduced forced expiratory volume in 1 second (FEV₁) due to decreased flow rate through the lower airways. The most widely used test for lung function is spirometry (Dwyer *et al.*, 2012), where measurements of FEV₁, forced vital capacity (FVC) and their ratio (FEV₁/FVC) are measured. When airway smooth muscle constricts there is a marked fall in FEV₁, a greater time to FVC, and an FVC value that remains relatively unchanged, leading to a disparity between FEV₁/FVC from resting values (Quanjer *et al.*, 1993).

In order to obtain an accurate diagnosis of asthma, the physician should perform a consultation with the patient and obtain a full symptoms history and physical examination, only when combined with evidence of airway hyper-responsiveness and airway reversibility is this the most effective way of diagnosing asthma (Dwyer *et al.*, 2012). Objective evidence of bronchoconstriction can be obtained following either direct or indirect bronchoprovocation challenges, or the assessment of reversibility to bronchodilator medication. These are the most effective methods of determining Airway Hyper-responsiveness (AHR). Following the direct or indirect bronchoprovocation challenges (Table 2.2) there will be a significant reduction in expiratory airflow in susceptible individuals due to bronchial smooth muscle contraction, leading to reduced FEV₁, which can be reversed via the administration of β_2 -agonists in controlled asthma.

Table 2.2: Direct and indirect stimuli used to measure bronchial responsiveness. Direct stimuli cause bronchoconstriction through action on effector cells, indirect stimuli cause bronchoconstriction through action on cells which interact secondarily with effector cells (adapted from Borges *et al.*, 2011).

Direct Bronchoprovocation Stimuli		
Methacholine	Histamine	Acetylcholine
Carbachol	Prostaglandin D ₂	Leukotriene C ₄ /D ₄ /E ₄
Indirect Bronchoprovocation Stimuli		
Hypertonic aerosols (Saline/Mannitol)	Hypotonic aerosols	Exercise
Eucapnic voluntary hyperpnoea	Bradykinin	Adenosine
Propranolol	Metabisulphite	Tachykinins

2.1.4 Management of asthma

A number of therapeutic strategies have been employed in an attempt to control the symptoms associated with asthma, including β_2 -agonists, inhaled corticosteroids (ICS), and other anti-inflammatory agents. An overview of these current therapies has been summarized in Table 2.3. The degree of airway responsiveness is aided by the administration of corticosteroids to reduce airway inflammation and the β_2 -Agonists allow for airway smooth muscle relaxation, therefore bronchodilator therapies and inhaled corticosteroids are the preferred first choice of therapy for symptom relief in asthmatic individuals (McFadden *et al.*, 1994).

Table 2.3: Therapies for control of asthma and their mode of action.

Therapy	Mode of Action
β_2 – agonists	<ul style="list-style-type: none"> • Increase levels of cAMP to relax bronchial smooth muscle through the inhibition of myosin light chain kinase. (Donohue, 2004)
Corticosteroids	<ul style="list-style-type: none"> • Act upon the glucocorticoid receptor to elicit a number of anti-inflammatory mechanisms to aid in the reduction of airway inflammation (Barnes & Adcock, 2009)
Leukotriene receptor antagonists	<ul style="list-style-type: none"> • Inhibit antigen-induced contraction of bronchial smooth muscle • Inhibit eosinophil activity • Reduce sputum eosinophilia • Reduce exhaled nitric oxide levels • Improve allergen-induced decline in FEV₁ (Horwitz <i>et al</i>, 1998)
Anti-cholinergic therapies	<ul style="list-style-type: none"> • Inhibit muscarinic receptors (M₁, M₂ and M₃ subtypes, respectively) decreasing the effects of vagal stimulation on the lung. (Donohue, 2004)
Mast cell stabilizers	<ul style="list-style-type: none"> • Prevent the release of the mediators of type I allergic reactions, such as histamine, from sensitized mast cells. (O’Byrne, 2004)
PDE ₄ Inhibitors	<ul style="list-style-type: none"> • Prevent the hydrolysis of cAMP, leading to bronchodilation and reduced inflammation • Inhibition of cell trafficking • Activation of inflammatory cells (Adcock <i>et al</i>, 2008).

2.2 Asthma and the elite athlete: Exercise-induced bronchoconstriction

The transient narrowing of the airways, which limits expiration and occurs during or following exercise is termed, exercise-induced bronchoconstriction (EIB) (Anderson *et al.*, 2005). EIB is reversible either spontaneously or via therapeutic intervention (Anderson *et al.*, 2005). According to Anderson *et al.*, (2005) EIB is usually classed as a 10% reduction in forced expiratory volume in 1 second (FEV₁) from the pre-exercise value and is most commonly reported in people who are already clinically recognised asthmatics, usually stemming from either atopy or increased sputum eosinophilia. The term exercise-induced asthma (EIA) is mainly used when EIB occurs and there is also a previous history of physician diagnosed asthma (e.g. atopic).

2.2.1 Pathophysiology of EIB

According to Parsons *et al.* (2013) the pathogenesis of EIB occurs following a modest period of high-intensity exercise sufficient to markedly increase minute ventilation, resulting in a prototypical response consisting of bronchoconstriction, occurring predominantly following the cessation of hyperpnoea and usually lasting between 30-90 minutes if left untreated. Susceptibility to EIB can vary significantly between individuals, occurring variably in subsets of individuals with clinically defined asthma (McFadden *et al.*, 1994), individuals without a known history of asthma (Molphy *et al.*, 2014) and also elite athletes (Dickinson *et al.*, 2011).

If the environment in which a sporting activity takes place has increased numbers of pollutants and is extremely dry/cold, this can lead to increased need for the air to be filtered, warmed and humidified. The problem therefore, is that due to the high ventilatory demand of these activities, there is a loss of the protective effects of nasal breathing as they switch to mouth breathing (~ >100 L/min) to achieve higher ventilation (Rundell *et al.*, 2015). This places a greater strain on the respiratory system as the lower airways are recruited to warm and humidify the air, and pollutants in the air are deposited in the airways (Rundell *et al.*, 2014; Anderson *et al.*, 2008).

The overall effect of these stressors is that, in susceptible individuals, airways can become hyper-responsive, causing bronchoconstriction and airway hyper-responsiveness (AHR), resulting in EIB (Fitch *et al.*, 2012). In subjects that are susceptible to EIB, there are increases in levels of exhaled nitric oxide (Scollo *et al.*, 2000), leukotrienes (Carraro *et al.*, 2005; Hallstrand *et al.*, 2005) mast cell expression (Hallstrand *et al.*, 2010) and epithelial shedding into the lumen (Hallstrand *et al.*, 2005). The signalling cascades and potential mechanisms for the process of airway smooth muscle contraction in response to exercise are described in figure 2.3.

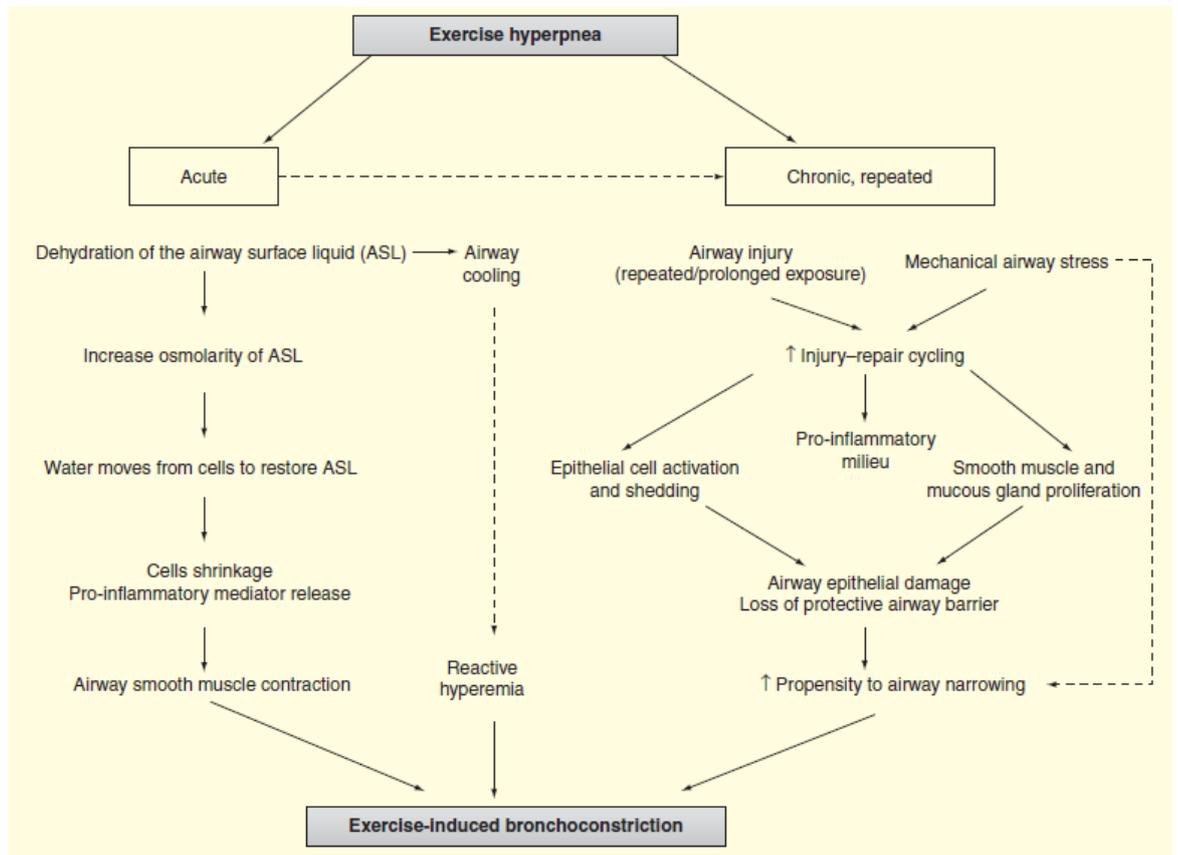


Figure 2.3: Schematic showing the proposed cascade of exercise-induced changes to the airway epithelium leading to exercise-induced bronchoconstriction in susceptible individuals (Adapted from Price *et al.*, 2014c)

2.2.2 Prevalence of Exercise-Induced Bronchoconstriction

Exercise induced bronchoconstriction is estimated to occur in up to 90% of asthmatics (McFadden *et al.*, 1994) and in up to 10% of people without a known history of asthma (Gotshall *et al.*, 2002). It is important that EIB can be identified in order to decrease the likelihood of any adverse events occurring during exercise (Backer *et al.*, 2007). Recent work has indicated that bronchoconstriction induced by hyperpnoea can have prevalence as high as 13% in previously undiagnosed individuals (Molphy *et al.*, 2014).

The occurrence of EIB is greater in elite athletes than in the general population (Dickinson *et al.*, 2005; Fitch, 2006; Parsons *et al.*, 2007). There are certain sports where the prevalence of asthma or EIB is extremely high. Sports with high EIB prevalence include swimming (76%), cross-country skiing (winter athletes) (42%) and rowing (31%) (Bougalt *et al.*, 2011; Pohjantahti *et al.*, 2005; Dickinson *et al.*, 2011). This higher prevalence can be due to increased ventilatory demand of the activity (Parsons *et al.*, 2007), increased numbers of pollutants in the sporting environment (Rundell *et al.*, 2013) and harsh low humidity or cold environments (Sue-Chu *et al.*, 2012).

2.2.3 Diagnosing EIB

When diagnosing EIB the American Thoracic Society (Parsons *et al.*, 2013) state that a diagnosis is made based upon changes in lung function post-exercise, not based upon the presence of symptoms. This is due to the low sensitivity and specificity of self-reported symptoms in establishing EIB (Parsons *et al.*, 2007; Hallstrand *et al.*, 2002; Rundell *et al.*, 2001).

In addition to measuring a fall in FEV₁ post-exercise it is also accepted that an objective bronchoprovocation challenge can be used (Parsons *et al.*, 2013). There are two methods of challenge testing dependent upon whether the bronchial smooth muscle is challenged directly (e.g. methacholine challenge) or indirectly (e.g. eucapnic voluntary hyperpnoea challenge, mannitol challenge, hypertonic saline)

An indirect airway challenge is a means of provoking the airways in a manner which will elicit bronchoconstriction in susceptible individuals. Spirometry is performed both pre- and post- an indirect airway challenge, with EIB identified following a drop in lung function (FEV₁), with the value of the drop needed to categorise EIB differing between challenge modalities (Holzer *et al.*, 2002).

2.2.3.1 Direct airway challenges

Direct airway challenges provoke the airway smooth muscle directly; these tests are useful in determining whether an individual is susceptible to airway smooth muscle contraction, an example of which is the methacholine challenge (Holley *et al.*, 2012). The methacholine challenge involves the cumulative inhalation of 5 increasing dosages of methacholine. At each stage FEV₁ is measured to detect a fall of 20% from baseline levels. Methacholine concentrations inhaled consist of 0.0625, 0.25, 1, 4 and 16 mg·mL⁻¹. Methacholine acts upon airway smooth muscle directly and a fall of 20% or more will only occur in susceptible individuals who exhibit AHR (Holley *et al.*, 2012).

2.2.3.2 Indirect airway challenges

Examples of indirect airway challenges used to identify EIB are the laboratory based exercise challenge, the sport-specific exercise challenge, the eucapnic voluntary hyperpnoea (EVH) challenge and the dry powder mannitol challenge. Each of these challenges have varying degrees of specificity, sensitivity and validity dependent upon how closely they match the original stimulus for EIB in the individual being tested (Anderson *et al.*, 2010).

The exercise challenge involves the completion of a short bout of high intensity exercise sufficient to significantly increase minute ventilation to roughly 21 x baseline FEV₁ (Parsons *et al.*, 2013). It is recommended that the air is dry, a nose clip should be worn and that heart rate reaches roughly 80-90 predicted max (~220-age). It is also recommended that exercise should continue at this high level for at least 4-6 minutes and sport-specific exercise is preferable.

Anderson *et al.* (2001) state the EVH challenge consists of baseline measurements of FEV₁, recorded from three maximal voluntary flow-volume manoeuvres. The challenge requires participants to attain target minute ventilation (\dot{V}_E) of 85% of their predicted maximal voluntary ventilation rate (MVV) (FEV₁ x30) for 6 minutes. During the 6 minutes, participants breathe air from a compressed gas cylinder containing 21% Oxygen, 5% Carbon Dioxide and 74% Nitrogen. The gas is delivered to each participant via a gas cylinder, reservoir and a two-way valve. \dot{V}_E is recorded by calculating the volume of air passing through a dry gas meter every minute. After the 6 minute EVH challenge, two consecutive maximal voluntary flow-volume loops were measured at 3, 5, 7, 10 and 15 minute time-points, with the highest of the two FEV₁ values recorded. A fall in FEV₁ greater than 10% on two successive occasions post-challenge results in a positive test.

With reference to Holley *et al.* (2012) it is noted that EVH is superior to methacholine for detecting EIB in non-athletes, this consolidates the findings

of Pedersen *et al.* (2008) who concluded that EVH is preferable to both methacholine and exercise challenge in elite swimmers.

EIB can be identified by the individual response to the indirect airway challenge and this term denotes the phenomena of the airways constricting in association to an exercise-related stimulus (Parsons *et al.*, 2013). An individual that has other environmental triggers (e.g. atopy), alongside a previous history of associated symptoms would then have sufficient information to be accurately diagnosed by a consulting physician of exhibiting exercise-induced asthma (EIA).

To further improve the validity of the EIB diagnosis, markers of inflammation can be measured. An example of which is the fractional exhaled nitric oxide (FeNO) test, which measures the amount of nitric oxide present in exhaled air. This value will rise in association with increased inflammatory markers in the airway such as eosinophils. Therefore FeNO is an indirect marker of airway inflammation. Care is warranted, however, when using FeNO as an indirect marker of airway inflammation due to the susceptibility of the test to be altered by dietary nitrate consumption (Olin *et al.*, 2001).

2.2.4 Treatment of EIB

Treatment for EIB can involve the administration of short-acting β_2 -agonists, long-acting β_2 -agonists, inhaled corticosteroids or combination therapy. Exactly which treatment methods are needed fall at the discretion of the consulting physician, given an adequate patient symptoms history and broncho-provocation challenge/reversibility test result. In some instances a physician may deem it necessary to incorporate other medications into the treatment regimen such as leukotriene receptor antagonists, theophyllines or chromones. The British Thoracic Society (BTS) recommend that immediately prior to exercise, short-acting β_2 -agonists are the drug of choice, with the addition that for most patients, exercise-induced asthma is an expression of poorly controlled asthma and that regular treatment including inhaled steroids should be reviewed (British Thoracic Society, 2009). These recommendations are also supported within the guidelines of the American Thoracic Society (ATS) (Parsons *et al.*, 2013).

The most common therapy for the management of EIB in the sporting population is via the administration of β_2 -agonist medications for the rapid relief of bronchoconstriction (Fitch, 2006). Short-acting β_2 -agonists act to reverse the bronchoconstriction of the airways through stimulation of the β_2 adrenergic receptor (β_2 -receptor) present on bronchial smooth muscle, allowing the muscle to relax and the airways to dilate restoring airway function (Hoffman, 2001).

Athletes are more likely to require the use of β_2 -agonists for the prevention and/or relief of bronchoconstriction caused by exercise. According to Fitch, (2006) salbutamol use accounted for around 6% of all athletes competing at the 2000 Sydney Olympic Games. Fitch, (2006) also identified that salbutamol was used c.5% of all athletes competing at the 2004 Athens Olympic Games. Indeed, Dickinson *et al.* (2005) identified that around 21% of British athletes used inhaled β_2 -agonists to protect against bronchoconstriction at the 2000 Sydney Summer Olympic Games and the 2004 Athens Summer Olympic Games. It is notable that the disparity between the figure presented by Fitch (2006) and those of the British cohort may be due, in part, to selection criteria of certain countries which can rule out asthmatic individuals. Other countries may also prefer to treat asthmatic symptoms with herbal remedies or mechanical strategies and finally, the type of sports in which the British team have the highest number of representatives, are potentially more asthmogenic in terms of ventilatory demand and environmental pollutants.

2.3 β_2 -adrenergic receptors

Adrenergic receptors are cell surface proteins which pick up signals from the sympathetic and parasympathetic nervous system and respond by altering the actions of the cell in which the receptor is located. The adrenergic receptors are G-Protein Coupled Receptors (GPCRs) that link onto heterotrimeric G proteins (Westfall *et al.*, 2011). Overall, there are three main types α_1 , α_2 and β each with three subtypes of adrenergic receptor including; α_{1A} , α_{1B} , α_{1D} , α_{2A} , α_{2B} , α_{2C} , β_1 , β_2 and β_3 receptor. Each of these adrenergic receptors are localised to different tissues within the body and act via signalling cascades following G-protein coupled activation, these signalling cascades occur to a varying extent and to differing physiological effects (Westfall *et al.*, 2011).

The β_2 -adrenergic Receptor (β_2 -receptor) is a subtype of the adrenergic receptors which, when activated by adrenaline and noradrenaline in the sympathetic nervous system, can exert their effects on muscle function and/or neurotransmitter release, dependent upon tissue localisation (Lynch *et al.*, 2008). The principal sites of the β_2 -receptor are the: heart; lung; blood vessels; bronchial smooth muscle; gastro-intestinal (GI) smooth muscle; kidney; skeletal muscle; olfactory bulb; piriform cortex; cortex; and hippocampus. The predominant effects of β_2 -receptor activation are smooth muscle relaxation and increased skeletal muscle contractility (Westfall *et al.*, 2011). Figure 2.4 illustrates the signalling cascade induced by β_2 -receptors present on airway smooth muscle.

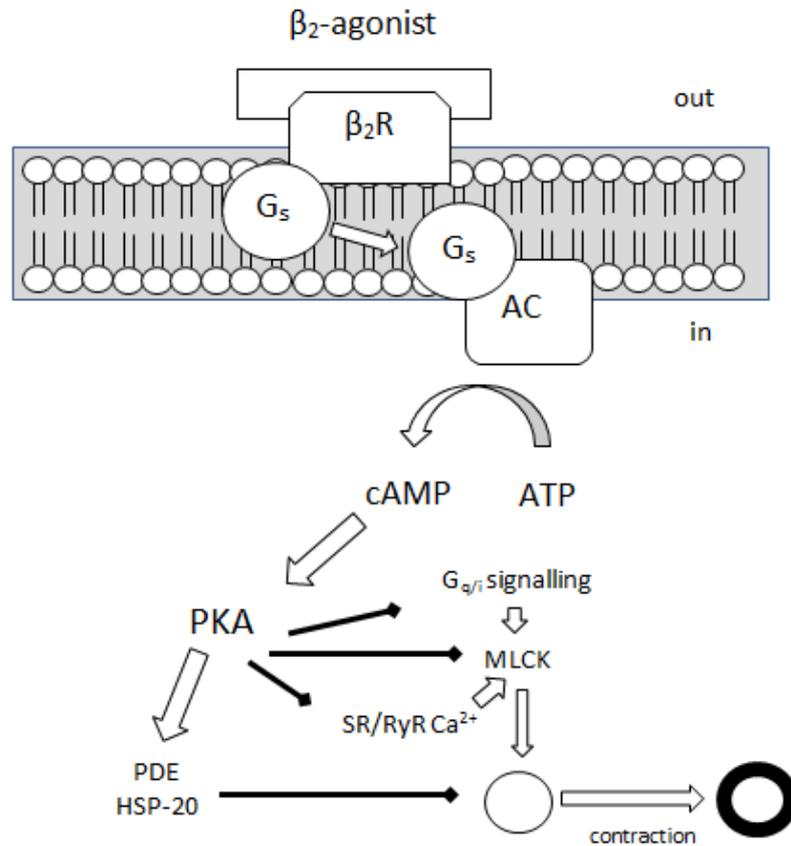


Figure 2.4: Schematic representation of the signalling cascade of β_2 adrenergic activation showing how this activates G-protein, stimulating adenylate cyclase, which in turn converts ATP to the second messenger cAMP, this then activates PKA which acts upon a number of mechanisms to cause downstream effects which include inhibition of mechanisms responsible for smooth muscle contraction.

β_2 R – β_2 Receptor; G_s – G-Protein α subunit; AC – Adenylate Cyclase; ATP – Adenosine Tri-Phosphate; cAMP – cyclic Adenosine Mono-Phosphate; PKA – Protein Kinase A; MLCK – Myosin Light Chain Kinase; PDE – Phosphodiesterase; HSP-20 – Heat Shock Protein-20; SR – Sarcoplasmic Reticulum ; RyR – Ryanodine Receptor; G_q – G Protein q; G_i – G Protein i α subunit; (Adapted from Pierce *et al.*, 2002)

The adrenergic receptors of the sympathetic nervous system can be activated by compounds that mimic the effects of adrenaline and noradrenaline (NA), such as catecholamine and sympathomimetic amines. These substances can be classified according to their mode of action which are: direct-acting; indirect-acting; or mixed-acting sympathomimetics (Lynch *et al.*, 2008). These classifications are dependent upon whether they actively stimulate the adrenergic receptor; whether they release NA from the synaptic nerve and block NA transport or block metabolising enzymes; or whether they act upon both mechanisms, respectively.

Sympathomimetic amines, which can be synthesized according to their molecular characteristics to exert their effects upon specific receptors, are important contributors to pharmacotherapy. These synthetic molecules can be used to stimulate or block signalling cascades to promote downstream actions on skeletal or smooth muscle (Bowman *et al.*, 1969). The key feature of the β_2 -receptor is that it is present in bronchial smooth muscle and is responsible for bronchial smooth muscle relaxation, making the β_2 -receptor agonist the ideal compound for the treatment of respiratory distress caused by bronchial smooth muscle contraction (bronchoconstriction) (Westfall *et al.*, 2011).

The β_2 -agonists are similar in structure to adrenaline with a single benzene ring and an amino group side-chain. Figure 2.5 provides examples including clenbuterol, salbutamol (albuterol) and terbutaline (Lynch *et al.*, 2008).

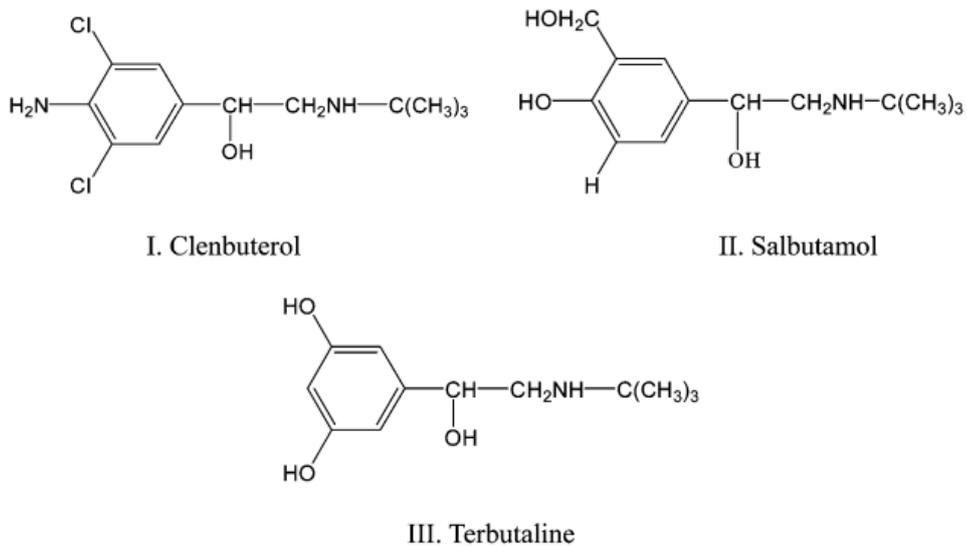


Figure 2.5: Chemical structures of the β_2 -agonists clenbuterol, salbutamol and terbutaline. (Fan *et al.*, 2013)

The long-acting β_2 -agonists such as formoterol and salmeterol have an additional benzene ring attached to the amino-group, which likely accounts for their longer duration of action (Waldeck *et al.*, 1996).

2.4 β_2 -agonists and their effects

Whilst traditionally used for the relief of respiratory distress caused by bronchoconstriction, it is widely known that some β_2 -agonists are able to increase lean mass whilst also decreasing fat mass (Lynch *et al.*, 2008). This effect is commonly known as the repartitioning effect (Emery *et al.*, 1984) with the benefits of the β_2 -agonists' anabolic properties being utilized significantly in the livestock industry to improve meat quantity and quality (Sillence *et al.*, 2004). Not surprisingly the use of β_2 -agonists such as clenbuterol, with well-known repartitioning properties became frequently used in competitive bodybuilding (Prather *et al.*, 1995) for athletes hoping to increase lean mass and decrease fat. This then led to the use of β_2 -agonists in athletes competing in sports that involve strength and power performance (Prather *et al.*, 1995) in the hope of increasing muscle mass, decreasing fat mass and improving power to bodyweight ratio.

Lynch and Ryall (2008) explain that acute administration of adrenaline has the ability to increase fast-twitch muscle fibre force production but not that of slow-twitch muscle fibre. With the β_2 -agonists being similar in structure to adrenaline it has been suggested that β_2 -agonists are able to also improve muscle force production, however a study by Ha *et al.* (1999) examining the effects of the acute administration of the β_2 -agonist terbutaline on muscle force production in rats, found that the proposed improvement in muscle contractility was due to changes in the amount of sarcoplasmic reticulum (SR) Calcium (Ca^{2+}) released and the speed of SR Ca^{2+} re-uptake rather

than changes in Ca^{2+} sensitivity or the ability of the muscle contractile proteins to generate force. This proposed mechanism is in agreement with Brodde and Michel (1999) when describing the effects of β_2 -receptor activation in the mammalian heart, which results in improved Ca^{2+} influx in the SR through phosphorylation of L-type Ca^{2+} channels and increased Ca^{2+} uptake in the SR through phosphorylation of phospholamban.

With chronic high-dose administration of β_2 -agonists there can be an increase in muscle mass associated with increased protein synthesis (Choo *et al.*, 1989) and decreased protein degradation (Benson *et al.*, 1991). The extent of the anabolic effects of β_2 -agonists largely depend upon: type of β_2 -agonist; mode of administration; dosage; frequency of administration; and duration of treatment, with animal models also exhibiting the largest increases in muscle mass following β_2 -agonist administration (Lynch *et al.*, 2008).

β_2 -agonists have been proven to have powerful lipolytic properties due to their ability to increase thermogenesis (Arch *et al.*, 1984) and impact upon adipose tissue. Adrenoreceptors present upon adipose tissue, when stimulated, can increase lipolysis and when combined with the increased energy expenditure that is associated with β_2 -agonist use, results in an overall increase in fat burning potential (Mills *et al.*, 2000).

Along with these purported mechanisms for so-called advantageous effects of β_2 -agonist administration for athletes, there are also a large range of deleterious side-effects of their use. These have far-reaching implications for the health and well-being of the athlete, who may take these substances in supra-therapeutic doses for performance enhancement. According to Lynch and Ryall (2008) since 1990 the use of β_2 -agonists for the purpose of performance enhancement has become increasingly prevalent and many of these athletes are unaware of the adverse side-effects of taking these drugs.

Some of the side-effects of β_2 -agonists in the treatment of respiratory symptoms are their ability to exert their effects upon skeletal muscle and cardiac smooth muscle leading to undesirable side-effects such as tachycardia and fine tremor (Prather *et al.*, 1995). When used via inhalation at therapeutic doses for topical administration in the lower airways, these β_2 -agonists can have minimal side-effects, however when taken orally or intravenously they can exert greater systemic effects which can have the potential to cause more notable side-effects as mentioned by Lynch and Ryall (2008) in their review of the effects of β_2 -agonists on skeletal muscle, whereby high dose β_2 -agonist administration produced an increase in skeletal muscle mass and a concomitant decrease in body fat in animal models. Prather *et al.* (1995) explain that acute side-effects associated with β_2 -agonist use include: nausea; headaches; and insomnia. With more severe side-effects resulting in muscle tremor, palpitations, muscle cramps, headache and peripheral vasodilatation. Interestingly, as highlighted by Salpeter *et al.*, (2004) in a meta-analysis of the effects of prolonged β_2 -

agonist use, there is an increased tolerance following as little as 1 week of consistent use, leading to the deleterious side effect of being unresponsive to treatment during an adverse event.

A study by Ingalls *et al.* (1996) looking at the effects of chronic high-dose administration of the β_2 -agonist clenbuterol on exercise performance in mice over a period of 8 weeks, found that although clenbuterol increased muscle mass, it had a negative effect on endurance exercise performance. Alongside decreased exercise performance there are also many studies that highlight the negative effects that the β_2 -agonist clenbuterol has on cardiac muscle hypertrophy, leading to instances of sudden death in both animal models (Duncan *et al.*, 2000) and human case studies (Kierzkowska *et al.*, 2005). In addition, chronic high dose oral administration of clenbuterol and salbutamol in rats has been shown to result in cardiac hypertrophy (Duncan *et al.*, 2000; Cepero *et al.*, 2000).

Brodde and Michel (1999) state that the relative abundance of β_2 -receptors in the heart is about 40% with the predominant receptor being the β_1 -receptor, however they also outline that drugs with a relative affinity for β_2 -receptors lose that preferential affinity with increasing concentrations and bind less selectively. They highlight that in contrast to cardiac muscle, the sino-atrial node (SAN) has a 2.5 fold higher concentration of β_2 -receptors than β_1 -receptors. These findings could be the reason why high-dose studies involving β_2 -agonists have a large correlation with cardiac abnormalities

(Duncan *et al.*, 2000; Kierzkowska *et al.*, 2005), especially with dosages that result in high systemic bioavailability of the β_2 -agonist (Duncan *et al.*, 2000; Kierzkowska *et al.*, 2005; Cepero *et al.*, 2000).

The type, dose, mode of administration and systemic bioavailability of a given β_2 -agonist all affect its mode of action and duration of action, which in turn affect its potential to cause unwanted side-effects (Lynch *et al.*, 2008). With the primary desired mode of action for β_2 -agonists in healthcare being bronchial smooth muscle relaxation, in the prevention of obstructive airways disease, it is crucial that athletes are informed about the potential for adverse events following supra-therapeutic use. It is essential that governing bodies ensure strict guidelines to oversee the legitimate use of β_2 -agonists in and out of competition, with the primary concern being the health and well-being of the athlete, with the hope of deterring potential supra-therapeutic use in the hope of gaining a competitive advantage.

2.5 Status of short-acting β_2 -agonists on the WADA Prohibited List of Substances and Methods

Throughout the past 40 years the International Olympic Committee (IOC) has changed their regulations regarding the use of short-acting β_2 -agonists in competition on a number of occasions. The constantly changing scientific literature regarding the ergogenic properties of the β_2 -agonists through differing routes of administration (oral vs. inhaled) has influentially shaped these regulations (Martineau *et al.*, 1992; Collomp *et al.*, 2010; Pluim *et al.*, 2011; Decorte *et al.*, 2008; Larsson *et al.*, 1997; Meeuwisse *et al.*, 1992; Le Panse *et al.*, 2006; Caruso *et al.*, 2005; McKenzie *et al.*, 1983; Van Baak *et al.*, 2000). The regulations imposed have been largely due to increasing concerns over possible unnecessary use of these medications by athletes and also due to health concerns regarding β_2 -agonist use (Fitch, 2006; Dickinson *et al.*, 2005; Lynch *et al.*, 2008). The specific changes in the status of the β_2 -agonists are described in table 2.4.

Table 2.4: The history of the β_2 -agonists and their guidelines for use in the athletic population since 1972 (Adapted from Fitch, 2006).

History of β_2-Agonists Guidelines for use in athletes		
1972	Permission to administer inhaled salbutamol refused by IOC-MC	✘
1975	Inhaled salbutamol and terbutaline permitted with prior notification	+
1976	Olympic team doctors notified IOC-MC of intended use of salbutamol or terbutaline	+
1980	Permission to use fenoterol by inhalation granted prior to the Moscow Olympics	+
1984	Fenoterol prohibited at the Sarajevo winter games because of metabolism to p-hydroxyamphetamine	✘
1984	Because of concerns of the effect of air pollution on bronchial airways in Los Angeles, team doctors are permitted to notify β_2 -agonists post-administration	+
1985	Biltolterol, orciprenaline (isoprotenerol) and rimiterol added as permitted β_2 -agonists.	+
1986	Notification of administration of β_2 -agonists to IOC-MC no longer required; oral β_2 -agonists reconfirmed to be prohibited.	✘
1992	Clenbuterol prohibited. Two athletes disqualified in Barcelona for using clenbuterol, β_2 -agonists listed as anabolic agents when administered systemically (orally or by injection)	✘
1993	Biltolterol, orciprenaline, and rimiterol no longer permitted β_2 -agonists. Notification of administration of permitted inhaled β_2 -agonists re-introduced.	✘
1994	Permission to administer inhaled salmeterol refused by IOC-MC.	✘
1996	Salmeterol permitted to provide prolonged protection from exercise-induced asthma.	+
2001	Formoterol permitted	+
2001	Because of concerns at the large and increasing number of athletes inhaling β_2 -agonists, as a health measure, the IOC-MC introduces the necessity for demonstrating that an athlete has asthma and/or EIB and is given a therapeutic use exemption certificate (TUE).	✘
2009	The World Anti-Doping Agency establishes regulations for all athletes to obtain a TUE for the use of β_2 -agonists.	✘
2010	Salbutamol and Salmeterol permitted via inhalation with a declaration of use.	+
2012	Salbutamol, Salmeterol and Formoterol permitted via inhalation without declaration of use. Monitoring of salbutamol and formoterol in the urine within threshold limits.	+
2012	Terbutaline remains prohibited except when used with a TUE certificate	✘
IOC-MC – International Olympic Committee Medical Commission; EIB – Exercise-Induced Bronchoconstriction ✘ - Stricter guidelines for β_2 -agonist use + - Relaxation of guidelines for β_2 -agonist use		

In 2002 the International Olympic Committee (IOC), guided by the International Olympic Committee – Medical Commission (IOC-MC) set guidelines for all athletes to provide objective evidence of bronchoconstriction via the performance of an objective bronchoprovocation challenge. These guidelines were later adopted by WADA in the 2009 Prohibited List of Substances and Methods (WADA, 2009). This was set due to increasing prevalence of β_2 -agonist use amongst athletes, which was not in correlation with the number of athletes using inhaled corticosteroids, leading to fears that athletes were not receiving adequate care and may have been over-using β_2 -agonists, leading to increased tolerance of the β_2 -agonist medication (Fitch, 2006; Anderson *et al.*, 2006a).

The exact increases are outlined by Fitch, (2006) who highlights that between the 1984 Los Angeles Olympic Games and the 1996 Atlanta Olympic Games there was a 212% increase in the use of β_2 -agonists by athletes. This figure increased by a further 151% between the Atlanta games and the 2000 Sydney Olympic Games, leading ultimately to the IOC-MC decision to change from the requirement for declared use through to the requirement for objective evidence of bronchoconstriction in 2001, with the health and wellbeing of the athlete being the main influencing factor for the decision (Fitch, 2006). This decision by the IOC-MC for the provision of demonstrable evidence of EIB led to the decision by WADA to place inhaled β_2 -agonists on the prohibited substance list in 2009, whereby their use could only be granted via a therapeutic use exemption (TUE) certificate.

The decision by the IOC-MC in 2002 to provide objective evidence of bronchoconstriction did not significantly affect the number of athletes requiring the use of short-acting β_2 -agonists at the following Olympic cycle (Fitch *et al.*, 2006; Dickinson *et al.*, 2005). The requirement to undertake a bronchoprovocation challenge was able to successfully identify previously undiagnosed individuals with bronchoconstriction and was able to successfully identify individuals that were falsely diagnosed with EIB (Dickinson *et al.*, 2005; Ansley *et al.*, 2012).

The advantages of EIB testing were evidenced in a study by Dickinson *et al.*, (2005) where the British Olympic team athletes who were currently using inhalers or were referred for screening, were tested for EIB prior to the 2004 Athens Olympic Games, via either a reversibility test, an exercise challenge or via a eucapnic voluntary hyperpnoea (EVH) challenge, which is the IOC-MC preferred method of screening for EIB (Anderson *et al.*, 2000; Holzer *et al.*, 2002; Anderson, *et al.*, 2003). The findings of the study highlight that in this cohort of elite athletes 20.7% presented with demonstrable evidence of EIB. This figure did not differ from the proportion of British athletes using β_2 -agonists (21.2%) at the previous Olympic cycle where demonstrable evidence was not required. The study concluded that screening of athletes is warranted, as it is able to maintain standards of care by highlighting previously undiagnosed individuals and also identifying any false positive

diagnoses, allowing for better control of asthmatic symptoms during training and competition.

WADA changed the status of the short-acting β_2 -agonists in 2010 to allow for the use of inhaled salbutamol and salmeterol via declaration of use (DoU) which was then relaxed further in 2012 when salbutamol, salmeterol and formoterol were permitted to be taken within recommended dosages without the requirement for a DoU (WADA 2010; WADA 2012). Further to this requirement existing urinary thresholds for salbutamol ($1000 \text{ ng}\cdot\text{mL}^{-1}$) and formoterol ($30 \text{ ng}\cdot\text{mL}^{-1}$) were outlined with the aim of distinguishing between legitimate therapeutic inhaled use and prohibited oral use (Ventura *et al.*, 2000; Elers *et al.*, 2011; Eibye *et al.*, 2013). No threshold levels were outlined for Salmeterol mainly due to the absence of any oral equivalent. Terbutaline is permitted for use by athletes only through the TUE process.

2.6 Salbutamol and the elite athlete

Salbutamol is a short-acting β_2 -agonist that is effective in the treatment of bronchoconstriction. It is predominantly administered via an inhaled dose of 100 μg per actuation; however it can also be administered orally via a 2 mg tablet.

Salbutamol is currently permitted for use by athletes and is the only short-acting β_2 -agonist permitted by WADA without TUE. Guidelines for use indicate that athletes are allowed to up to 1600 μg *pro re nata* (PRN). Urinary threshold limits for salbutamol have been established in which a concentration above 1000 $\text{ng}\cdot\text{mL}^{-1}$ constitutes an adverse analytical finding (AAF). Above this there is also a decision limit of 1200 $\text{ng}\cdot\text{mL}^{-1}$ (WADA, 2015; Elers *et al.*, 2011).

The use of salbutamol amongst the athletic population is around 5% of all athletes (Fitch, 2006). Until recently there have been mixed reports regarding the use of salbutamol and its ergogenic potential (Van Baak *et al.*, 2000; Decorte *et al.*, 2013; McKenzie *et al.*, 1983; Le Panse *et al.*, 2006; Caruso *et al.*, 1995; Caruso *et al.*, 2005). More recent findings indicate that inhaled salbutamol up to 1600 μg taken in either acute or accumulative doses over a one-off or 24 hour period does not improve endurance or strength and power performance (Pluim *et al.*, 2011; Dickinson *et al.*, 2014a; Dickinson *et al.*, 2014b; Decorte *et al.*, 2008; Sporer *et al.*, 2008).

Further to these studies, a study by Elers *et al.* (2012a) suggests inhaling an acute dose of up to 4000 µg of salbutamol results in no improvement in cycling time to exhaustion or oxygen kinetics. Accordingly, from a performance perspective the current WADA guidelines permitting athletes to inhale up to 1600 µg in a 24 hour period are appropriate as there appears to be no resultant improvement in performance in non-asthmatic athletes. However, more recent work by Kalsen *et al.* (2013) has highlighted performance improvement following combined short- (1600 µg salbutamol) and long-acting β_2 -agonists (200 µg salmeterol; 36 µg formoterol).

Decorte *et al.* (2013) have shown performance improvement in quadriceps fatigability following a supra-therapeutic (800 µg salbutamol) dose of β_2 -agonist. Interestingly the findings of Decorte *et al.* (2013) are in contrast to the findings of the same research group (Decorte *et al.*, 2008) who previously found no performance enhancement when assessing quadriceps force and fatigability with the same supra-therapeutic (800 µg salbutamol) dose of β_2 -agonist. Possible reasons for performance enhancement following higher doses is a greater systemic availability of the β_2 -agonist leading to greater activation of the β_2 -receptors, to date no study has investigated the effects of continuous daily use of β_2 -agonist at the maximum WADA daily limit, which may lead to greater systemic availability of the β_2 -agonist due to a cumulative effect of repeat administration.

In a meta-analysis of the acute performance enhancing effects of β_2 -agonists with regard to oral salbutamol, Pluim *et al.* (2011) concluded there was weak evidence to suggest acute doses of oral salbutamol would significantly improve athletes' anaerobic capacity and strength. A recent study by Sanchez *et al.* (2012) examined the impact of oral salbutamol on maximal power from either a single, acute dose (6 mg) or a daily dose ($12 \text{ mg}\cdot\text{day}^{-1}$) for three weeks. The study reported that oral salbutamol resulted in significantly improved maximal power with the one-off dose resulting in greater gains than three weeks daily intake (14% vs. 8%). The authors concluded that acute doses led to greater gains and that long term use of oral salbutamol may lead to down regulation of muscle β_2 -Adrenoreceptors resulting in a dampening of the effect of salbutamol on strength gains. The study by Sanchez *et al.* (2012) involved long-term use of oral β_2 -agonists, yet it has also been demonstrated that the same potential for dampening down of the β_2 -receptor occurs following long-term use of inhaled salbutamol (Hancox *et al.*, 2002; Salpeter *et al.*, 2004).

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 leading to a reduced airway resistance and improvements in minute ventilation (\dot{V}_E) and exercise performance (Fitch, 2006; Haverkamp *et al.*, 2007). One of the proposed ergogenic mechanisms for inhaled salbutamol is a significant bronchodilation in non-

asthmatic athletes resulting in an improved \dot{V}_E during exercise and increases in oxygen uptake. Previous research has reported improvements in \dot{V}_E following acute doses of up to 1600 μg of salbutamol in the absence of an improvement in 5 km running time-trial performance in endurance athletes or repeated sprint performance in football players (Dickinson *et al.*, 2014a; Dickinson *et al.*, 2014b). Prior to this, studies have demonstrated non-significant improvements in FEV₁ of 0.2 L following inhalation of 800 μg salbutamol, which did not result in greater \dot{V}_E or improved endurance performance (Decorte *et al.*, 2008).

Previous studies that have focused on oral salbutamol have primarily demonstrated performance gains in strength and power variables (Pluim *et al.*, 2011; Sanchez *et al.*, 2012; Caruso *et al.*, 1995; Martineau *et al.*, 1992), there remains a possibility that athletes who use oral salbutamol will benefit from improved performance. WADA have established threshold limits in the urine for the use of salbutamol, such threshold limits should act as a deterrent to any athlete that would wish to use oral salbutamol in the hope of improved performance, for fear of presenting with an AAF, which may occur following the higher oral dose.

Salbutamol is used by individuals who present with bronchoconstriction, in athletes this is predominantly exercise-induced bronchoconstriction (Anderson, 2001). Many of the studies investigating the effects of salbutamol, however, have investigated its effects in healthy individuals

(Sanchez *et al.*, 2012; Caruso *et al.*, 1995; Martineau *et al.*, 1992; Decorte *et al.*, 2008; Decorte *et al.*, 2013; Kalsen *et al.*, 2013). Within the athletic population any ergogenic potential has to be examined within those individuals who exhibit with EIB, a recent study by Koch *et al.* (2013), investigated the effects of salbutamol on cycling performance in both healthy individuals and EVH positive (EVH+ve) individuals, finding that salbutamol significantly increased lung function in both groups but that this improvement did not translate to any improvements in exercise performance. The study was repeated by the same research group (Koch *et al.*, 2014) investigating any performance improvements in females with the same findings. Given that exercise in cold, dry environments is known to be most provocative to the respiratory system (Sue-Chu *et al.*, 2012) it would be interesting to investigate the effects of salbutamol on exercise performance in both healthy and EVH+ve individuals in a low humidity environment to determine any ergogenic effects, but also to investigate the potential disadvantage that may occur in EVH+ve individuals, exercising at low humidity without the broncho-protective effects of β_2 -agonists.

Although salbutamol is the only short-acting β_2 -agonist that is permitted for use by athletes, there are also two long-acting β_2 -agonists salmeterol and formoterol that are currently permitted. If an athlete suffers from more severe asthma and is unresponsive to salbutamol, or if an athlete suffers from adverse side-effects of salbutamol use, then there is currently no other available short-acting β_2 -agonist permitted for use by athletes. It is possible however, that if an athlete provides demonstrable evidence of EIB they can

be permitted a TUE certificate for the use of the alternative short-acting β_2 -agonist terbutaline (WADA, 2015).

2.7 Terbutaline and the elite athlete

The majority of athletes treat symptoms of EIB through the use of salbutamol, however other β_2 -agonists, such as terbutaline, are available through the therapeutic use exemption (TUE) process. Terbutaline is purported to have fewer adverse side-effects than salbutamol (Sanchez *et al.*, 2013). Indeed, Sanchez *et al.* (2013) indicate that due to fewer reported side-effects than other β_2 -agonists, terbutaline may be of benefit to athletes for the relief of EIB. Terbutaline is also a fast-acting β_2 -agonist that is active for a maximum of 12 hours whereas salbutamol is active for a maximum of 6 hours, making terbutaline a desirable treatment option for the athletic population (Sanchez *et al.*, 2013).

Unlike the permitted β_2 -agonists salbutamol, formoterol and salmeterol, terbutaline is prohibited during competition except for those athletes who have a TUE (WADA, 2015). This prohibited status is largely due to the inability to distinguish between inhaled and oral use, with oral use being banned for all β_2 -agonists (WADA, 2015). Whilst investigations into threshold limits that can distinguish between inhaled and oral use of terbutaline are ongoing, it is important to outline whether there are any performance enhancing properties when taken at the therapeutic dose. Certainly there have been recent investigations into the performance enhancing effects of supra-therapeutic doses of terbutaline which have highlighted an ergogenic potential (Kalsen *et al.*, 2014; Hostrup *et al.*, 2014), yet athletes with TUE that require the use of terbutaline should only be taking a therapeutic dose

for the relief of symptoms, therefore it is this dose (0.5 – 4 mg) that warrants investigation for any ergogenic effect.

The highest acute therapeutic dose of inhaled terbutaline that is recommended, is up to 4 mg (8 x 0.5mg inhalations) (Prior *et al.*, 1982). However the dose required for therapeutic effects can be as low as a single inhalation of 0.5mg (Simpson *et al.*, 2014). With a large variation in what could be considered the ideal therapeutic dose, athletes that are using terbutaline with a TUE may feel the need to take doses towards the higher end of the spectrum in order to obtain adequate protection from the symptoms of EIB. In a study by Elers *et al.* (2012b) when trying to distinguish between inhaled and oral administration of terbutaline, a therapeutic dose of 2 mg inhaled terbutaline was chosen and compared against a supra-therapeutic dose of 10 mg oral terbutaline in order to establish differences in urinary concentrations, with a finding that no differences were apparent. With no standardised therapeutic dose outlined for the use of inhaled terbutaline in athletes with a TUE it is reasonable to assume that athletes would use single inhaled doses of either 0.5 mg, 2 mg, or 4 mg.

Hostrup *et al.* (2014) highlight the ergogenic potential of inhaled terbutaline by examining the effects of 15 mg inhaled terbutaline on muscle strength, maximal sprint performance and endurance performance in cycling. The group found significantly improved muscle strength and sprint performance but not endurance performance in trained males. Conversely, Kalsen *et al.*

(2014) also investigated the effects of 15 mg inhaled terbutaline on performance, during 300 kcal cycling time-trial, finding no significant difference in performance compared to placebo, the study did highlight that terbutaline inhalation promotes a shift towards carbohydrate metabolism during exercise.

With the requirement of a TUE for the use of inhaled terbutaline during competition being largely due to the inability to distinguish between an inhaled dose and a prohibited oral dose in urine, investigations into the urine concentrations following route of administration have been warranted (Elers *et al.*, 2012b). In a recent study by Elers *et al.* (2012b) investigating the blood and urinary concentrations of terbutaline following either an inhaled or an oral dose, it was highlighted that although significant differences were found between the doses, no cut-off value could be established between the two modes of administration.

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 adverse analytical finding (AAF) would indicate possible supra-therapeutic use or oral administration which may have ergogenic potential (Hostrup *et al.*, 2014). Indeed a recent study by Sanchez *et al.* (2013) investigating the effects of a supra-therapeutic (8mg) oral dose of terbutaline on aerobic performance found no significant difference versus placebo, highlighting the lack of ergogenic potential of

terbutaline even at high doses. In the study by Hostrup *et al.* (2014) the main findings were improved muscle strength and sprint performance. It is possible therefore, that terbutaline is only ergogenic in these types of performance tasks.

A recent study by Simpson *et al.* (2013) highlights the protective effect of a single inhaled dose of 0.5 mg terbutaline. It is possible that many athletes would only need to inhale 0.5 mg terbutaline for effective protection against EIB, however it is likely that they may choose higher doses if they thought it would be more beneficial for the prevention or relief of symptoms. Further research is warranted that could establish a cut-off threshold permitting athletes to use a single inhaled dose of 0.5 mg terbutaline, providing effective protection against EIB for the elite athlete and allowing the use of a broader range of β_2 -agonists, which would be beneficial for athletes who suffer side-effects of currently permitted medications (Sanchez *et al.*, 2013).

2.8 Summary

The use of β_2 -agonists in sporting competition has been a constant matter of debate for doping authorities. The decision to permit or restrict the use of these bronchodilating agents has been regularly altered. Athletes who experience respiratory symptoms during exercise should have adequate therapies available to prevent adverse events during training and competition. Research examining the ergogenic properties of acute administration of the β_2 -agonist salbutamol at therapeutic doses has so far been unable to ascertain any performance enhancing properties, leading to permission being granted by the IOC-MC and WADA for athletes to use inhaled salbutamol. For athletes that may have unwanted side-effects from salbutamol it is important that another short-acting β_2 -agonist is available to use during competition, such as terbutaline. The IOC-MC and WADA currently do not permit the use of terbutaline without a TUE (WADA, 2015).

The difficulty distinguishing between inhaled and oral use of terbutaline is a possible factor leading to its inclusion on the Prohibited List (WADA, 2015). The β_2 -agonist literature supports the hypothesis that the use of orally administered β_2 -agonists is potentially ergogenic due to the high systemic bioavailability following this route of ingestion. Studies that could highlight the difference between route of administration of terbutaline and the ergogenic properties of therapeutic dosages are required. This will establish a better understanding of terbutaline as a potential addition to the current

asthma medications available to the elite athlete, with the healthcare of the elite athlete being the main priority.

2.9 Statement of purpose

The purpose of the studies contained in this thesis are to assist WADA in the implementation of regulations on the use of inhaled short acting β_2 -agonists and assist in the resolution of contested anti-doping rule violations. The initial study will aim to determine the effects of chronic high-dose salbutamol on endurance, strength and power performance. Furthermore, this study aims to highlight the pharmacokinetics of long-term use. The second study aims to investigate the urinary excretion of varying dosages of terbutaline through different routes of administration. It also aims to provide a clear distinction between oral and inhaled routes and varying dosages of terbutaline through analysis of urinary concentration. Furthermore, the study will aim to look at a wider cross-section of individuals examining potential differences between gender and race. The third study will investigate the effect of varying therapeutic inhaled dosages of terbutaline on exercise performance, aiming to highlight the potential ergogenic properties of inhaled terbutaline. The final study will investigate whether inhaled salbutamol provides a health and/or 3 km time-trial performance benefit in a low humidity environment in individuals with and without a positive EVH challenge.

2.10 Hypotheses

Hypothesis 1: Long-term use of 1600 µg inhaled salbutamol per day for 6 weeks does not significantly improve performance compared to placebo.

Hypothesis 2: Long-term (1600 µg.day⁻¹ for 6 weeks) inhaled salbutamol does not lead to urinary concentrations above the WADA urinary threshold.

Hypothesis 3: An acute therapeutic dose (2 mg) of inhaled terbutaline will lead to significantly lower urine concentrations than an acute therapeutic dose (5 mg) of oral terbutaline.

Hypothesis 4: Repeated therapeutic dose (8 x 1 mg over 36 hours) inhaled terbutaline will lead to significantly lower urine concentrations than repeated therapeutic dose (4 x 5 mg over 36 hours) oral terbutaline.

Hypothesis 5: Inhaled terbutaline at two different therapeutic dosages of 2mg and 4mg does not lead to improved 3km running time-trial performance.

Hypothesis 6: EVH positive individuals exercising in a low humidity environment will have decreased 3 km running time-trial performance and decreased lung function compared to pair matched EVH negative controls.

Hypothesis 7: EVH positive individuals exercising in a low humidity environment will have improved lung function and 3 km running time-trial performance following salbutamol intervention, compared to pair matched EVH negative controls.

3. General Methods

3.1 Physical Activity Readiness Questionnaire (PARQ)

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 contraindications to them taking part in the study (such as history of any cardiovascular or metabolic disease) as confirmed by information provided on a physical activity readiness questionnaire (Appendix A).

3.2 Respiratory Symptoms History Questionnaire

Inclusion criteria required participants to have no history of physician diagnosed asthma as confirmed subjectively by the respiratory symptoms history questionnaire, previously used by Dickinson *et al.* (2011) (Appendix B).

3.3 Spirometry

All participants undertook maximal flow-volume manoeuvres using a spirometer (Microlab ML3500, Cardinal Health, Basingstoke, UK). Participants sat upright in a chair, wore a nose clip to prevent nasal

breathing and were instructed to reach maximum inhalation before placing the spirometer mouthpiece in the mouth and forcefully exhaling as hard as possible. Exhalation continued for a minimum of 6 seconds and also until they had completely emptied their lungs, indicated by a plateau in the volume/time graph on the spirometer, at which point the experimenter would signal the participant to breathe in as fast as possible to maximum inspiration, completing the maximal flow-volume manoeuvre.

A minimum of three maximum flow-volume loops were required for baseline measurements, flow-volume measurements were rejected if: the participant was deemed not to have reached maximum inspiration prior to the manoeuvre (indicated by the same start and end point of the flow-volume loop); the participant was deemed to have coughed during the manoeuvre; the participant was deemed to have performed a slow start or to have not maintained the pressure of expiration for the duration of exhalation and also if the values were not consistent (i.e. less than 5% or 150 mL variation between all three baseline values).

Flow-volume measures recorded from each maximal flow-volume loop were; Forced Expiratory Volume in one second (FEV_1), Forced Vital Capacity (FVC), FEV_1 to FVC ratio (FEV_1/FVC %), Peak Expiratory Flow (PEF) and forced expiratory flow between 25% and 75% of FVC (FEF_{25–75}). Individual maximal flow-volume loops were accepted in accordance with

European Respiratory Society American Thoracic Society criteria (Quanjer *et al.*, 1993; Miller *et al.*, 2005). Acceptability criteria are outlined in table 3.1.

Table 3.1: Maximal flow-volume loop acceptance and rejection criteria for individual efforts and for reliability between efforts (Adapted from Miller *et al.*, 2005)

Summary of within- and between- manoeuvre acceptability criteria

Within-manoevre criteria

Individual spiromgrams are acceptable if:

They are free from:

- Cough during the first second of exhalation
- Glottis closure that influences the measurement
- Early termination or cut-off
- Effort that is not maximal throughout
- Leak
- Obstructed mouthpiece

They have good starts:

- Extrapolated volume <5% of FVC or 0.15 L, whichever is greater

They show satisfactory exhalation:

- Duration of ≥ 6 s, a plateau in the volume-time curve or if they cannot or should not continue to exhale

Between-manoevre criteria

After three acceptable spiromgrams have been obtained apply the following tests:

- The two largest values of FVC must be within 0.15 L of each other
- The two largest values of FEV₁ must be within 0.15 L of each other

If both of these criteria are met the test session may be concluded

If both of these criteria are not met, continue testing until:

- Both of the criteria are met with analysis of additional acceptable spiromgrams
- A total of 8 tests have been performed (optional)
- The patient/participant cannot or should not continue

Save, as a minimum, the three satisfactory manoeuvres

FVC: Forced vital capacity; FEV₁: Forced expiratory volume in 1 second

3.4 Eucapnic Voluntary Hyperpnoea Challenge

All participants underwent a eucapnic voluntary hyperpnoea (EVH) challenge in accordance with the methods described by Anderson *et al.* (2001). Participants were instructed to avoid exercise and caffeine consumption on the day of the EVH challenge. Baseline FEV₁ was recorded from three maximal voluntary flow-volume manoeuvres. If the participant had an FEV₁ less than 80% of the predicted value or if they had an FEV₁/FVC ratio less than 70% they were deemed unsuitable to participate in the EVH challenge for health and safety reasons and were excluded from participation in any studies.

Participants were asked to attain target minute ventilation (\dot{V}_E) of 85% of their predicted maximal voluntary ventilation rate (MVV) (FEV₁ x 30) for 6 minutes (Anderson *et al.*, 2001). During the 6 minutes, participants breathed air from a compressed gas cylinder containing 21% oxygen, 5% carbon dioxide and 74% nitrogen. The gas was delivered to each participant via a gas cylinder, reservoir and a two-way valve. \dot{V}_E was recorded by calculating the volume of air passing through a dry gas meter every minute. Participants were verbally encouraged to reach MVV, however, the minimum acceptable ventilation rate for an acceptable test was 60% of the predicted MVV, if the participant failed to reach this value they were required to perform the test

again on a separate occasion. After the 6 minute EVH challenge, two maximal voluntary flow-volume loops were measured at 3, 5, 7, 10 and 15 minute time-points, with the highest of the two FEV₁ values recorded. A fall in FEV₁ greater than 10% on two successive occasions post-challenge resulted in a positive test. The data collection sheet for the EVH challenge is provided in appendix C.

3.5 Peak Oxygen Consumption ($\dot{V}O_{2peak}$)

Participants performed a standardised incremental running test to volitional exhaustion to establish peak oxygen consumption ($\dot{V}O_{2peak}$; Withers *et al.*, 2000) on a motorised treadmill (Pulsar, h/p/cosmos, Germany). The protocol consisted of one minute stages starting at 8 km·h⁻¹ and increasing every minute until a maximum speed of 16 km·h⁻¹ was attained, the gradient then increased by 1% every minute until a maximum of a 10% incline was attained. Each test was conducted under controlled laboratory conditions (temperature 20°C, relative humidity 40%), participants performed a 5 minute standardised warm-up on a motorized treadmill (10 km·h⁻¹) before performing the test. Prior to starting, the participants were fitted with a heart rate monitor (Polar RS400; Polar Electro Oy, Kempele, Finland) and connected to a breath-by-breath online gas analyser via a face mask (Oxycon Pro, Jagear, Wuerzberg, Germany). At the end of every stage and upon trial cessation the following 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). The trial ended when the participant was no longer able to continue at the desired speed or when the participant voluntarily ended the test protocol.

3.6 Three 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 over a distance of 3 km on at least two occasions. Participants progressed to the recorded 3 km time-trials once they felt comfortable pacing themselves on the non-motorised treadmill over a 3 km distance.

Each time-trial was conducted under controlled laboratory conditions (20°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 3 km time-trial the following were measured: time (s), average 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). 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).

During the 3 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.

3.7 Isokinetic Dynamometry

Prior to performing isokinetic dynamometry all participant were required to perform a familiarisation session to determine repeatability of measurements, at this familiarisation session differences between repetitions were required to be below the criterion value of 7.5% co-efficient of variation, familiarisation sessions continued until this value was met. Before completing isokinetic dynamometry participants completed a standardised 5 minute warm-up on a cycle ergometer. Participants completed peak torque assessments of knee extension and knee flexion in order to assess quadriceps and hamstrings strength. Measurements were obtained using a Biodex Dynamometer (Biodex Medical Systems, Shirley, NY, USA) in accordance with methods outlined by Wrigley and Strauss (2000). Participants were instructed to perform flexion and extension at the following rotational speeds $60^{\circ} \cdot s^{-1}$ or $240^{\circ} \cdot s^{-1}$. All participants completed a protocol consisting of 3 repetitions at $60^{\circ} \cdot s^{-1}$ followed by 45 seconds rest and a further

single repetition at the same speed, participants then had a further 45 second rest then completed 3 repetitions at $240^{\circ}.s^{-1}$, then again a further 45 seconds rest and a single repetition at the same speed, range of motion parameters were set at the start of every test, this range of motion was required for every extension and flexion in order for the acceptance of a valid test. Co-efficient of variation below 7.5% between repeated repetitions was required to ensure familiarisation with the technique, if this criterion was not met the protocol was repeated at a later date.

3.8 One Repetition Maximum (1RM) Bench Press and Leg Press

All participants were required to perform 1 repetition maximum (1 RM) tests during the second week of training to allow familiarisation with the equipment and lifting technique. On 1 RM testing days participants were required to have been free from strenuous strength training for at least 48 hours. All participants were required to complete a standardised 5 minute warm-up prior to the testing. All participants were tested for 1 RM Bench Press and Leg Press using the methods outlined by Beachle, Earle and Wathen (2008). Participants performed warm-up repetitions of 6, 4 and 2 repetitions at self-selected weights. Following this the participants moved on to 1 RM attempts with a minimum rest of between 3-5 minutes. Participants continued to perform 1RM lifts until failure, an attempt was deemed as a failure if the participant either, did not manage to meet the required range of motion (i.e. a 90° angle at the knee during leg press and the barbell within 1 cm of the

chest during bench press) or was unable to lift the weight to its original position, on two separate occasions.

3.9 Vertical Jump Test

Vertical jump height was measured using a Jump Mat (Probotics Inc, Alabama, USA) in accordance with the methods of Hatze, (1998). The participant was instructed to stand on the jump mat. From a standing position with feet shoulder width apart and arms straight out in front, the participant then performed a countermovement jump, first flexing the legs at the knee at the same time as a down-swinging arm motion, which was immediately followed by extension to push off the mat and an upward swinging motion of the arm. The participant then landed with both feet back on the mat in a standing position, if the participant landed in a squat position the measurement was rejected. Time in the air and jump height were recorded. The best of three efforts was recorded during each assessment, for reliability purposes all three jumps were required to be within 10%.

3.10 Skinfold Measurements

Skin fold measurements were taken in accordance with methods outlined by Norton *et al.* (2000). Skin fold measurements were taken at the following

recognised sites on the right hand side of the body: triceps, biceps, subscapular and supraspinale. All measurements were taken by the same technician using a single set of Harpenden skinfold callipers (Baty International, Sussex, UK). Skin fold measurements were taken from each site consecutively a total of two times. The sum of four skin folds was then calculated for both totals, the criterion for a valid measurement was a difference of less than 1 mm between the two totals. If this was not the case then the cycle of measurements was repeated until the criterion was met. The average of the two totals for the sum of four skin folds was then calculated and this average was taken as the final value.

3.11 Muscle Girth

Muscle girth measurements were taken in accordance with methods outlined by Norton *et al.* (2000) by a single, trained technician using an anthropometric tape measure. Muscle girths were measured at the arm, thigh and calf. Relaxed arm girth was measured with the right arm by the side and the participant in the anatomical position, the circumference was recorded at the radiale triceps landmark. Tense arm girth was measured with the participant holding their right arm straight out in front at shoulder height, with an angle of 45° at the elbow, the circumference was measured along the arm until the widest point was met, the participant was then instructed to bring their fist as far as possible toward the shoulder, the tense arm circumference was then measured. Calf girth was measured with the

participant in the anatomical position and the calf relaxed, measurements were taken down the calf until the widest point was met. Thigh girth was measured at the mid trochanterion–tibiale laterale landmark.

3.12 Salbutamol 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-D₃ (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 µl of E. Coli enzyme (β-glucuronidase) solution the mixture was incubated overnight at 37°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 µl of isopropanol followed by 200 µl 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 μm ; Waters Ltd, Elstree, UK) at 35°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 10-2000 $\text{ng}\cdot\text{ml}^{-1}$. Samples with salbutamol concentrations greater than the upper limit of quantification were diluted with blank human urine prior to analysis. The lower limit of quantification was accepted as the lowest standard on the calibration curve (10 $\text{ng}\cdot\text{ml}^{-1}$).

3.13 Terbutaline Urinalysis

Each urine-sample was 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 °C 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 D₃; 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 μ m) 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·ml⁻¹) which were extracted and analysed in the same batch. Quality control samples were tested along with samples to confirm assay performance.

4. The ergogenic effect of long-term use of high-dose salbutamol

4.1 Background

Between 2002 and 2010 the International Olympic Committee (IOC) established the requirement for athletes to present evidence of current asthma or exercise induced bronchoconstriction (EIB) through the Therapeutic Use Exemption (TUE) process in order to use the short acting β_2 -agonist, salbutamol. These regulations were guided by health and not anti-doping (performance enhancing) concerns (Fitch *et al.*, 2006). Previous reports have provided demonstrable evidence that the TUE process improves the quality of care for athletes (Dickinson *et al.*, 2005; Parsons *et al.*, 2007). Furthermore, it has been demonstrated that there is improved diagnostic sensitivity and specificity of incorporating indirect airway challenges into the process of diagnosing an athlete with asthma and/or EIB (Anderson *et al.*, 2003; Rundell *et al.*, 2004; Dickinson *et al.*, 2006a; Dickinson *et al.*, 2006b; Parsons *et al.*, 2007).

The weight of evidence supports the improved health care of athletes following the introduction of the TUE process for inhaled short acting β_2 -agonist. In contrast, there is limited evidence to suggest inhaled β_2 -agonists (200 – 800 μg) have an ergogenic effect. The small numbers of studies that do exist have focused mainly on endurance performance and have reported no performance effect with up to 800 μg of inhaled short acting β_2 -agonist (Pluim *et al.*, 2011). It has been demonstrated that large (up to 4000 μg)

acute doses of inhaled salbutamol do not significantly improve endurance (Dickinson *et al.*, 2014a; Elers *et al.*, 2012a), simulated association football (soccer) or repeated sprint performance (Dickinson *et al.*, 2014b). Whilst there appears to be no performance gain from acute administration of salbutamol there is limited data available investigating the ergogenic effect of chronic, daily use of salbutamol.

Sanchez *et al.* (2012) observed an improved sprint performance following 3 week daily use of oral salbutamol (12 mg.day⁻¹) however; they note that sprint performance was greater following an acute oral dose of salbutamol (6 mg) when compared with the 3 week chronic use. To date, no study has examined the ergogenic impact following the chronic use of inhaled salbutamol. Furthermore, WADA (WADA Prohibited List 2013) currently recommends a daily upper limit of 1600 µg of salbutamol *pro re nata* (as required); a dose rarely examined in the literature. A possible mechanism for the improvement in performance following prolonged use may be due to repeated dose accumulation of the β₂-agonist in the system. If there is not sufficient time for full clearance of the β₂-agonist then there will be an accumulation in the system, which may be able to stimulate the β₂-receptors more potently due to higher availability than would be observed with single administration (Hoffman, 2001).

Accordingly, the purpose of this study is to contribute to the understanding of the ergogenic effect of prolonged use of the inhaled short acting β₂-agonist

salbutamol at daily doses of 1600 µg on measures of endurance, strength and power performance in athletes.

It is hypothesized that therapeutic use of inhaled salbutamol has no effect on performance following 6 weeks use of the WADA maximum permitted (1600 µg per day) therapeutic dose for athletes.

It is also hypothesized that prolonged use of salbutamol at the WADA maximum permitted daily dose does not lead to any urinary concentrations that would present as an adverse analytical finding.

4.2 Methods

Ethical approval was obtained from Liverpool John Moores University Local Ethics Committee (ethics no: P09SPS031). Sixteen recreationally trained male athletes provided written consent and agreed to take part in the study (mean \pm SD: age 20.1 ± 1.6 years; height 179.9 ± 8.2 cm; weight 74.6 ± 9.1 kg). 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. Participants completed baseline performance challenges for endurance, power and strength. Each assessment was conducted under controlled laboratory conditions (temperature 20°C , relative humidity 40%). Following the baseline measures participants were pair-matched according to their $\dot{V}\text{O}_{2\text{peak}}$, then randomly assigned to one of two groups in a double blind design:

Placebo Group (PLA): 4 inhalations of placebo inhaler, via pocket chamber, 4 times per day for 6 weeks.

Salbutamol Group (SAL): 4 x 100 μg inhaled Salbutamol (Sandoz Ltd. Bordon, UK), via pocket chamber, 4 times per day for 6 weeks.

Participants used their inhaler as instructed daily at 08.00, 12.00, 16.00 and 20.00 hours and kept a record of their inhaler use. Over the six week intervention participants attended four (two endurance and two strength training) personal trainer led gym sessions per week. The performance tests for endurance, power and strength included 3 km running time-trial, 1RM bench press and leg press, vertical jump height and knee extension and flexion using isokinetic dynamometry, these assessments were repeated at week three and also at week six.

4.2.1 Endurance performance assessments

4.2.1.1 Peak Oxygen Consumption ($\dot{V} O_{2peak}$)

As described in general methods section 3.5.

4.2.1.2 3 km Time-Trial

As described in general methods section 3.6.

4.2.2 Strength and Power Assessments

4.2.2.1 Isokinetic Dynamometry

As described in general methods section 3.7.

4.2.2.2 One Repetition Maximum (1 RM) Bench Press and Leg Press

As described in general methods section 3.8.

4.2.2.3 Vertical Jump Test

As described in general methods section 3.9.

4.2.3 Training Protocol

Participants trained four times per week, consisting of two resistance based sessions and two endurance based sessions. Using the baseline measures

of the strength and power assessments a personalised, incremental resistance training programme was provided and weight lifted was recorded. The resistance training programme consisted of leg press, Romanian dead lift, bench press, bench pull/bent over dumbbell row, biceps curl (preacher and hammer), bench triceps dips and box jumps. The endurance sessions lasted 40 minutes and consisted of two 20 minutes steady state exercise episodes on: a treadmill; cycle ergometer; or cross-trainer. All endurance distances were recorded and a progressive increase in endurance distances was prescribed across the six weeks. Participants used HR zones to regulate the intensity of each endurance session. Typical HR zones during each session were 70-80% heart rate max for the duration of the exercise.

4.2.4 Anthropometric Measurements

4.2.4.1 Skinfold measurements

As described in general methods section 3.10.

4.2.4.2 Muscle girth measurement

As described in general methods section 3.11.

4.2.5 Urine collection

Urine samples were provided midway through the administration period. Subjects were asked to collect samples following the final daily dose of salbutamol at 20:00 h. A 20 ml aliquot was collected and stored initially overnight at 4°C and subsequently at -80°C until analysis.

4.2.6 Salbutamol Urinalysis

As described in general methods section 3.12.

4.2.7 Statistical Analysis

Changes in the performance and physiological measurements recorded in the endurance, strength and power assessments between groups (PLA or SAL) over the course of the 6 week training programme were analysed using a mixed model repeated measures ANOVA with a Bonferroni correction for multiple analyses. A p-value of ≤ 0.05 was deemed significant for all analysis.

4.3 Results

All 16 participants completed 6 weeks of either SAL or PLA and completed all endurance, strength and power assessments, there were no adverse events reported. Participant characteristics are presented in table 4.1.

4.3.1 Endurance Performance Assessments

4.3.1.1 Peak Oxygen Consumption ($\dot{V}O_{2peak}$)

Over the course of 6 week training programme $\dot{V}O_{2peak}$ improved significantly ($p=0.02$) in both PLA and SAL groups (Figure 4.1). There was no significant interaction between group and time in $\dot{V}O_{2peak}$ and HR. The SAL group had a significantly greater ($p=0.02$) change in \dot{V}_E from baseline ($139.3 \pm 22.6 \text{ l}\cdot\text{min}^{-1}$) to 6 week follow-up ($155.25 \pm 22.0 \text{ l}\cdot\text{min}^{-1}$) when compared with PLA change from baseline ($145.3 \pm 40.9 \text{ l}\cdot\text{min}^{-1}$) to 6 week follow-up ($147.13 \pm 38.1 \text{ l}\cdot\text{min}^{-1}$).

Table 4.1 Mean (\pm SD) Participant demographics and lung function values Pre- and Post- EVH challenge for: Salbutamol (SAL) and Placebo (PLA) groups.

Group	Height	Weight	Age	Baseline FEV₁	% Predicted FEV₁	Post EVH FEV₁
SAL (n=8)	180.3 (7.7)	73.6 (11.6)	20 (1.1)	4.95 (0.5)	108.1 (4.3)	4.69 (0.5)
PLA (n=8)	179.4 (9.2)	75.6 (6.2)	20.25 (2.2)	4.79 (0.7)	108 (11)	4.61 (0.7)

FEV₁ - Forced Expiratory Volume in 1 Second; EVH - Eucapnic Voluntary Hyperpnoea; MC – Male Caucasian; MAC – Male Afro-Caribbean; FC – Female Caucasian; MA – Male Asian

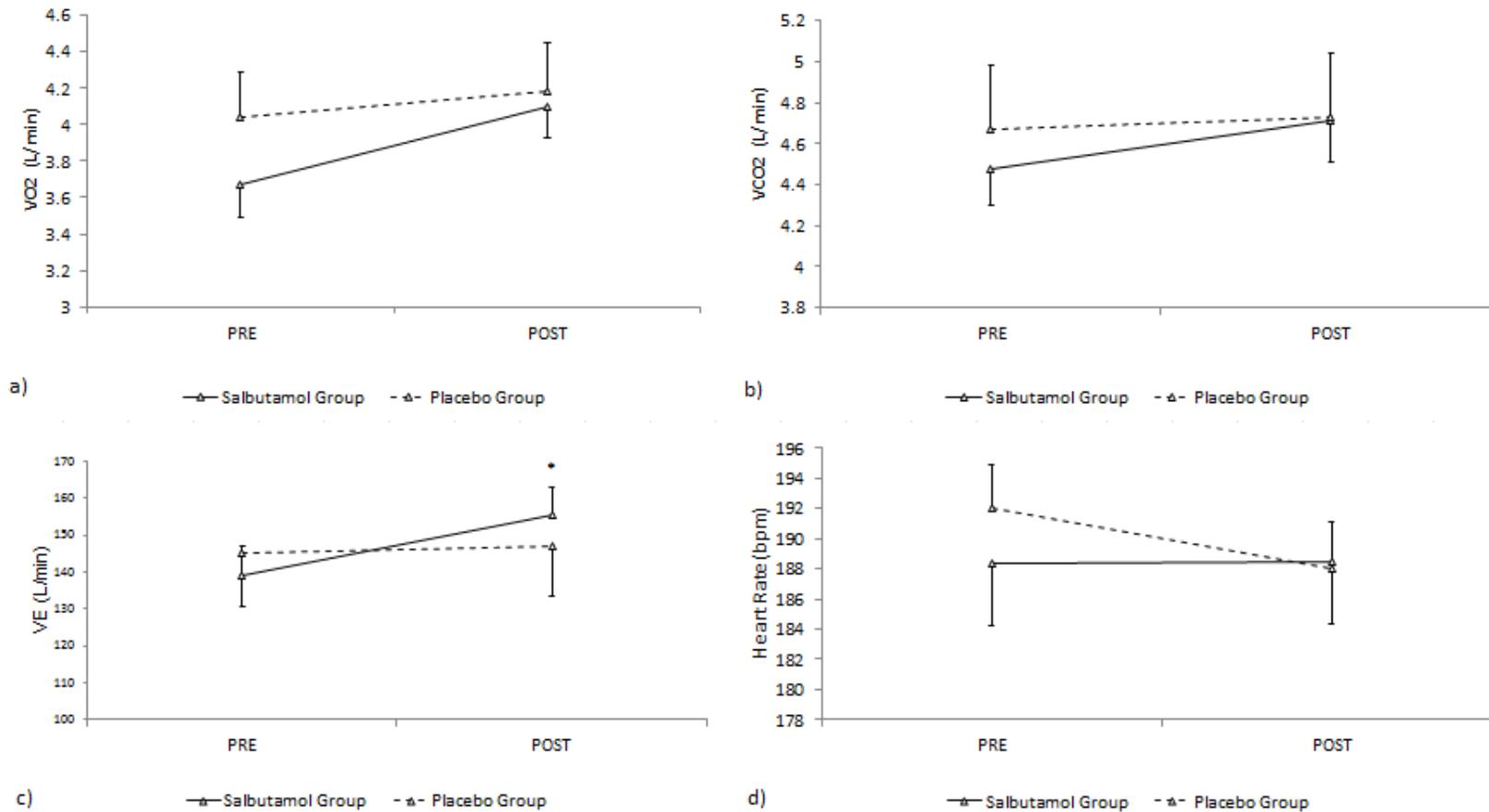


Figure 4.1: Mean (\pm SE) a) $\dot{V}O_{2peak}$, b) peak HR, c) \dot{V}_E and d) $\dot{V}CO_2$ during the $\dot{V}O_{2peak}$ assessment at baseline and at 6 week follow-up in PLA and SAL groups. (* = SAL significant increase ($p=0.02$) from baseline to 6 week follow-up).

4.3.1.2 3 km Time Trial

All participants significantly improved 3 km running time over the course of the 6 week period. There was no difference in the amount of improvement between PLA (1057.8 ± 234.1 s vs. 1005.3 ± 255.3 s) and SAL (909.2 ± 86.1 s vs. 885.8 ± 61.6 s) groups for baseline and 6 week completions times, respectively (Figure 4.2). No significant differences were noted for HR, $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E or blood lactate (Figure 4.2).

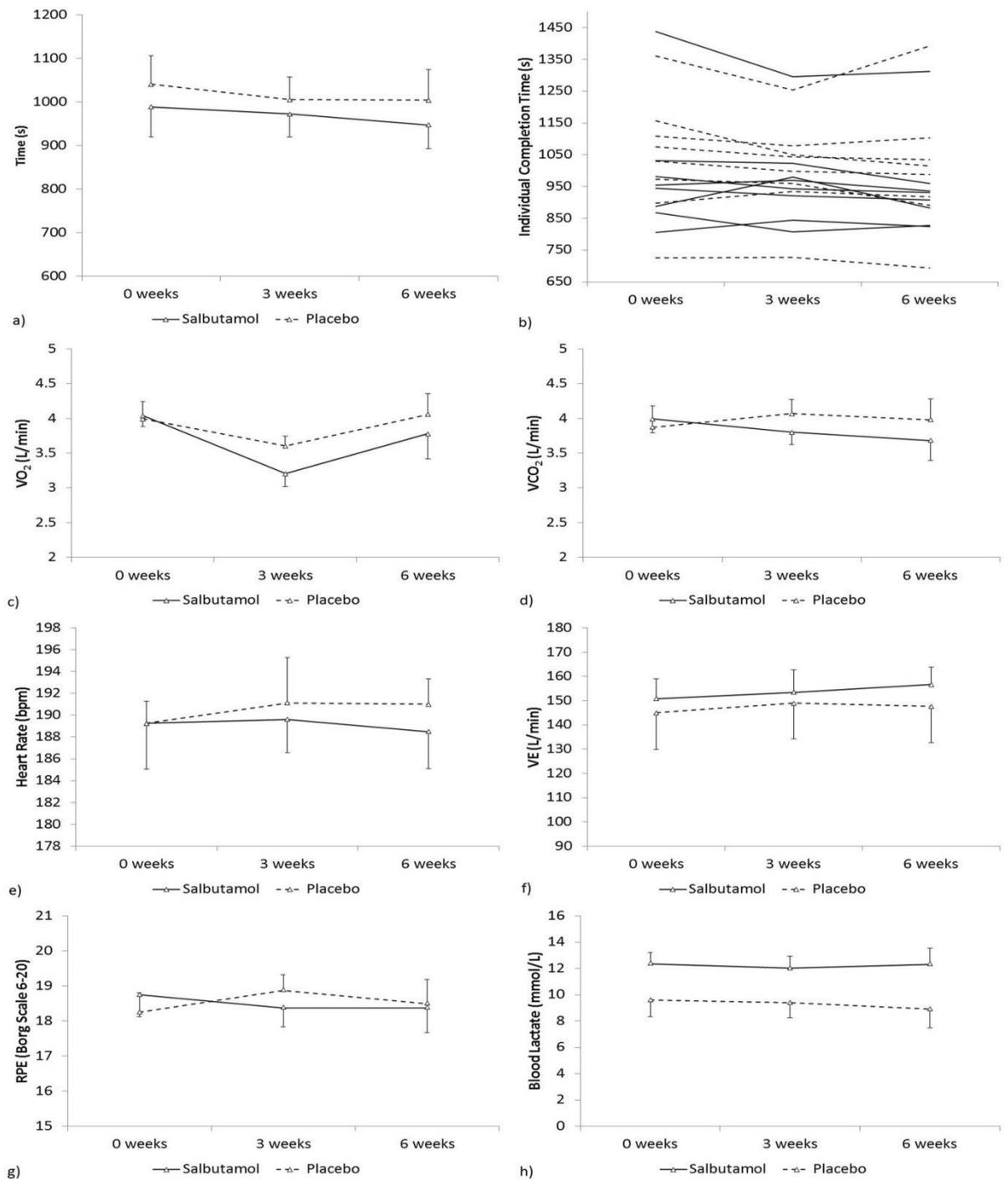


Figure 4.2: a) Mean (\pm SE) 3km completion time, b) individual completion time, c) oxygen consumption ($\dot{V}O_2$), d) carbon dioxide production ($\dot{V}CO_2$), e) heart rate (HR), f) minute ventilation (\dot{V}_E), g) rating of perceived exertion (RPE) and h) blood lactate during the 3 km time-trial between SAL and PLA at baseline, 3 weeks and 6 weeks.

4.3.2 Strength and Power Assessments

There were no significant improvements in peak torque for any peak torque measurements between baseline and 6 week follow-up tests (see Figure 4.3.). There was a significant interaction for peak torque 1RM leg extension at $60^{\circ}.s^{-1}$ between PLA and SAL ($p=0.03$) from baseline (184.6 ± 35.0 vs. 196.1 ± 47.3 n.m.) to six week follow up (195.2 ± 28.9 vs. 179.5 ± 48.9 n.m.). All other peak torque measurements for knee extension and knee flexion did not change significantly between groups. Over the course of the 6 weeks bench press and leg press 1 RM improved in both SAL and PLA ($p<0.01$). No significant changes in bench press or leg press 1 RM were observed between groups (Figure 4.3). Vertical jump height did not significantly change over the 6 week period in either PLA or SAL groups (Figure 4.4).

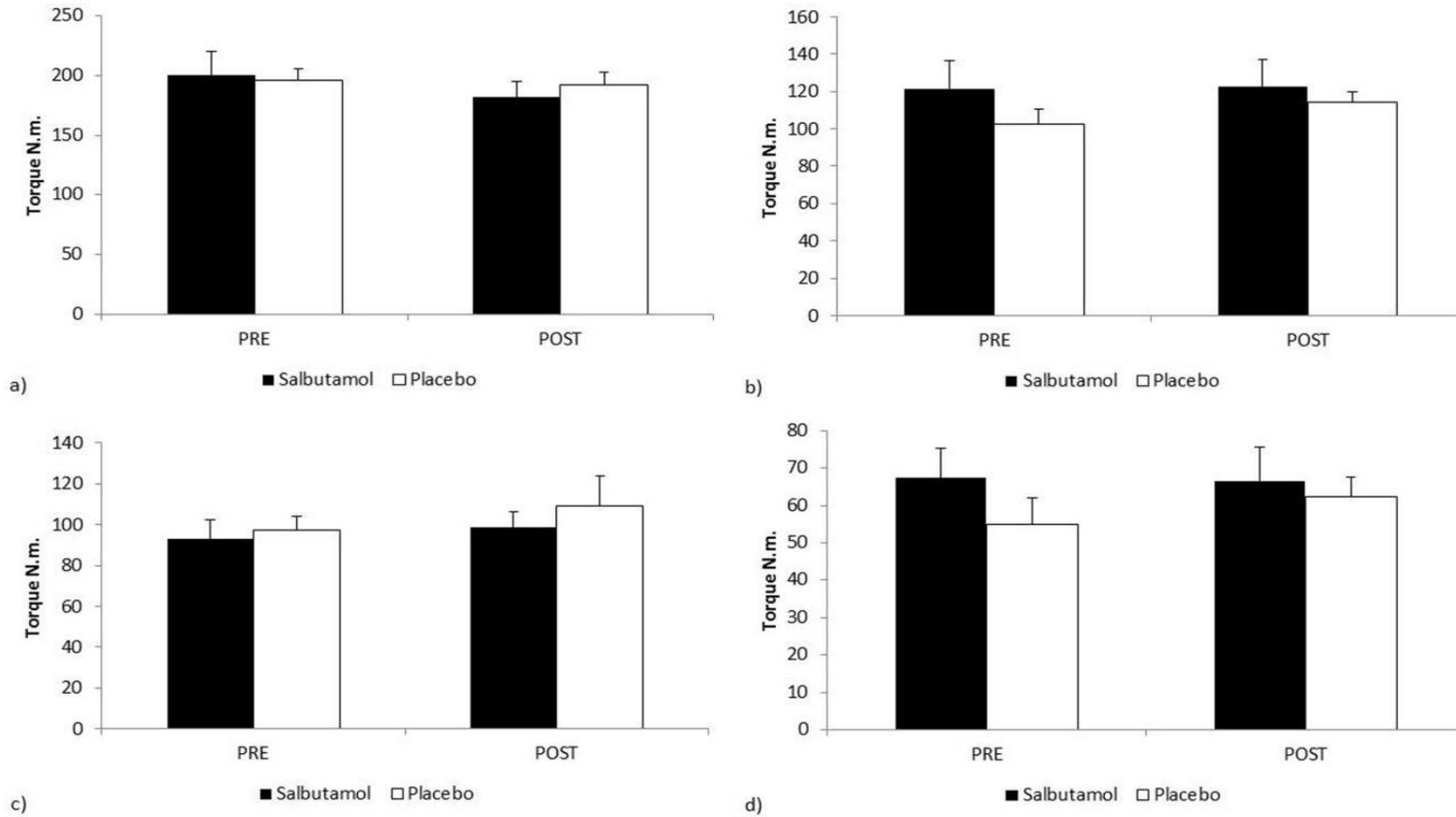


Figure 4.3: Mean (\pm SE) Peak Torque for a) 60°·s⁻¹ extension b) 240°·s⁻¹ extension c) 60°·s⁻¹ flexion and d) 240°·s⁻¹ flexion 1RM efforts between SAL and PLA at pre- and post-6 week treatment and training phase.

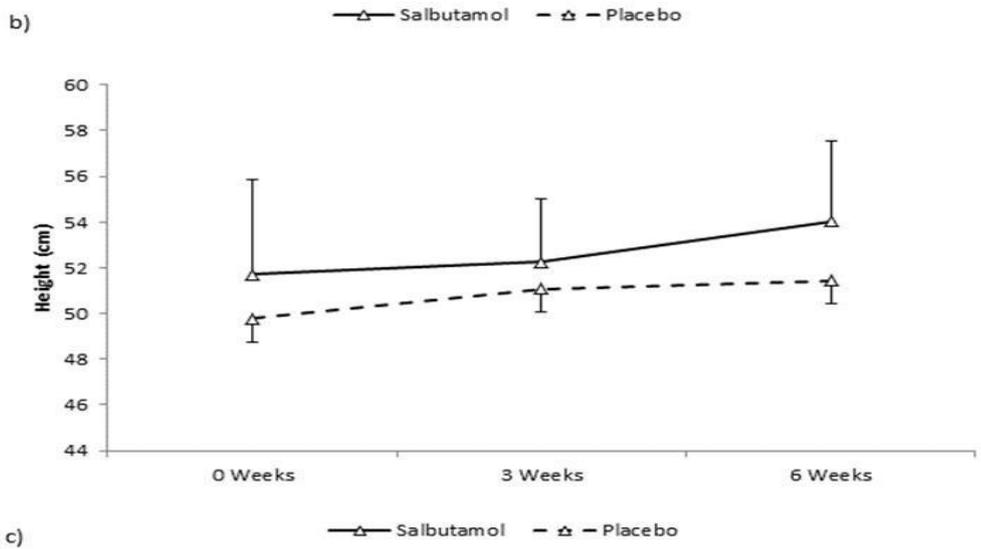
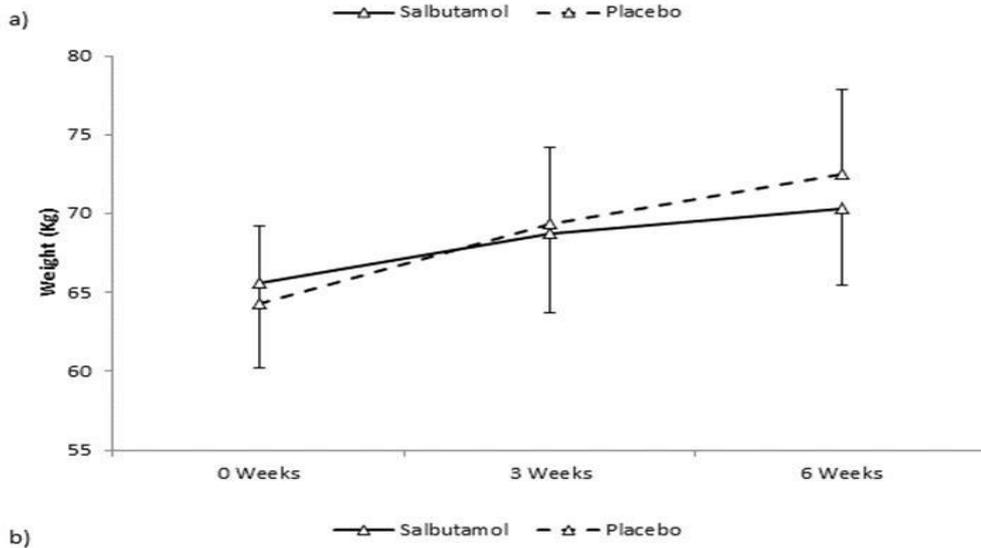
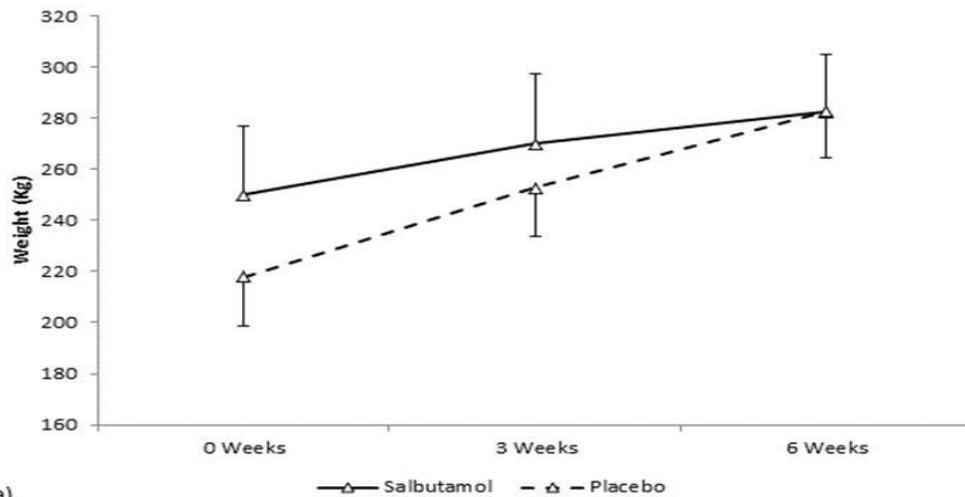


Figure 4.4: Mean (\pm SE) leg press (a) bench press (b) and vertical jump height (c) at baseline, 3 weeks and 6 weeks in PLA and SAL groups.

4.3.3 Urinary salbutamol

The mean (\pm SD) urinary salbutamol concentration at the 3 week stage of the administration period was 347.5 (\pm 361.4) ng.ml⁻¹. Whilst seven of the eight subjects from group 1 (SAL) produced samples significantly lower than the 1000 ng.ml⁻¹ threshold established by WADA one subject produced a sample slightly higher (1071 ng.ml⁻¹) (Figure 4.5).

4.3.4 Anthropometric Measurements

There were no significant differences in any of the anthropometric measurements assessed at either the 3 week or the 6 week assessment stages (Figure 4.6).

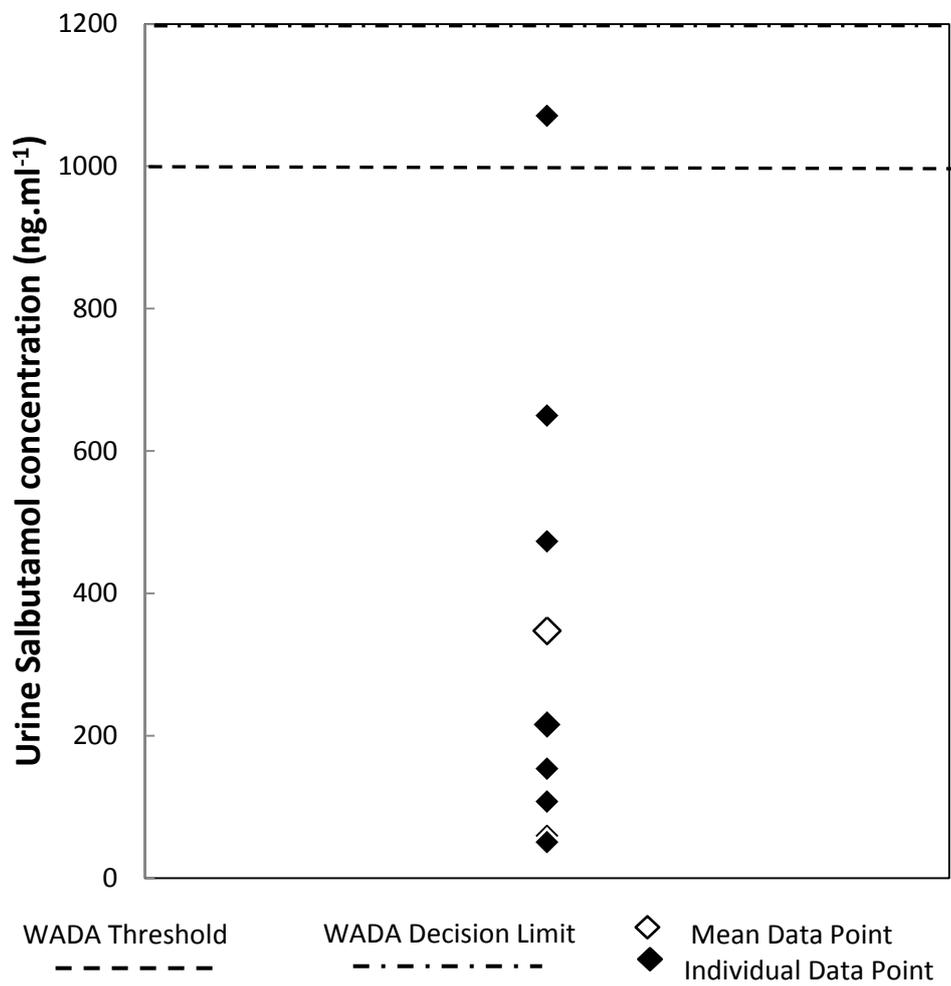


Figure 4.5: Urinary Salbutamol concentrations for the SAL group showing individual concentrations and the group mean.

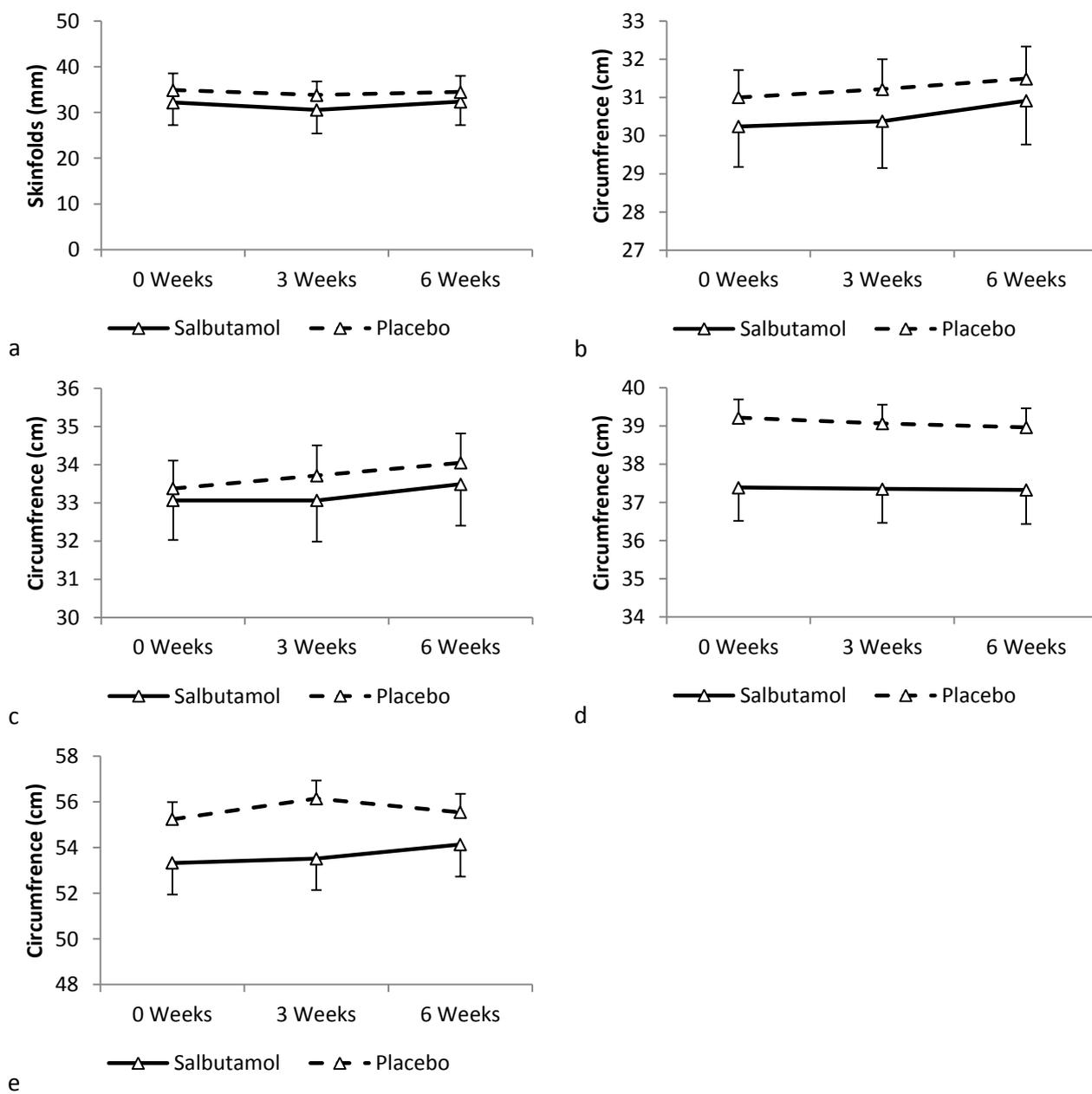


Figure 4.6: Anthropometric measurements for a) skinfolds b) relaxed arm circumference c) tensed arm circumference d) calf circumference e) thigh circumference.

4.4 Discussion

This is the first study to examine the impact of chronic, daily accumulated doses of 1600 µg of inhaled salbutamol on endurance, strength and power. The results indicate no significant performance improvement in 3 km running time-trial, 1RM bench and leg press, vertical jump height or isokinetic dynamometry when compared with placebo.

This study supports to previous research that has demonstrated inhaled salbutamol up to 1600 µg taken in either acute or accumulative doses over a one-off or 24 hour period does not improve endurance or strength and power performance (Pluim *et al.*, 2011; Dickinson *et al.*, 2014a; Dickinson *et al.*, 2014b; Decorte *et al.*, 2008, Sporer *et al.*, 2008). Further to the data in the present study and previous research, Elers *et al.* (2012a) suggests inhaling an acute dose of up to 4000 µg of salbutamol results in no improvement in cycling time to exhaustion or oxygen kinetics. Accordingly, from a performance perspective the current WADA guidelines permitting athletes to inhale up to 1600 µg in a 24 hour period are appropriate as there does not appear to be a resultant improvement in performance in non-asthmatic athletes following acute or chronic inhalation.

The findings from the present study contradict findings from studies investigating oral salbutamol. In a meta-analysis of the acute performance

enhancing effects of oral salbutamol, Pluim *et al.* (2011) concluded there was weak evidence to suggest an acute dose of oral salbutamol would significantly improve athletes' anaerobic capacity and strength. A recent study by Sanchez *et al.* (2012) examined the impact of oral salbutamol on maximal power from either a single, acute dose (6 mg) or a daily dose (12 mg.day⁻¹) for three weeks. They reported that oral salbutamol resulted in significantly improved maximal power with the one-off dose resulting in greater gains than three weeks daily intake (14% vs. 8%). The authors concluded that acute doses lead to greater gains and that long-term use of oral salbutamol may lead to down regulation of muscle β_2 -adrenoreceptor function leading to a dampening of the effect of salbutamol on strength gains, these findings are supported by Hancox *et al.*, (2002) who assessed the use of salbutamol for one week between repeat EVH challenges, finding that response to therapy was diminished in the salbutamol group compared to placebo in the follow up EVH challenge. The potential down regulation of muscle β_2 -adrenoreceptors from daily doses of salbutamol may be why the data from our study demonstrated a reduction in 1 RM peak torque leg extension at 60°·s⁻¹ in the SAL group compared to an improvement in the PLA group.

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 leading to a reduced airway resistance and improvements in \dot{V}_E and exercise performance (Collomp *et al.*, 2010). One of the proposed ergogenic mechanisms for inhaled salbutamol is a

significant bronchodilation in non-asthmatic athletes resulting in an improved \dot{V}_E during exercise and increases in oxygen uptake. In the present study we observed significant increases in \dot{V}_E during the $\dot{V}O_{2peak}$ assessment from the SAL group when compared against PLA which may have been attributable to a slight familiarization effect in the SAL group, however; this increase in \dot{V}_E did not result in an improved $\dot{V}O_{2peak}$. Previous research has reported similar improvements in \dot{V}_E following acute doses of up to 1600 μg of salbutamol in the absence of an improvement in 5 km running time-trial performance in endurance athletes or repeated sprint performance in football players (Dickinson *et al.*, 2014a; Dickinson *et al.*, 2014b). Previous studies have demonstrated non-significant improvements in FEV₁ of 0.2 L following inhalation of 800 μg , which did not result in greater \dot{V}_E or improved endurance performance (Decorte *et al.*, 2008).

Previous studies that have focused on oral salbutamol have primarily demonstrated performance gains in strength and power variables (Pluim *et al.*, 2011; Sanchez *et al.*, 2012; Caruso *et al.*, 1995; Martineau *et al.*, 1992). In addition to strength and power this study focused on endurance performance. This had an impact on the prescribed training programme as participants completed two strength and power sessions and two endurance sessions per week. If the focus had been on strength and power assessments the participants could have received greater training gains from a greater training volume (i.e. 4 sessions/week vs. 2 sessions/week). Future

studies could employ a greater strength and power training load allowing results from oral salbutamol studies to be more comparable.

Whilst dosing to the recommended maximal levels according to WADA (WADA Prohibited List 2015) produced no benefit in terms of enhanced performance, urinalysis results demonstrated a distinct possibility that an individual may contravene the anti-doping regulations as a consequence of a positive drugs test. Whilst all but one subject produced a negative test result ($<1000 \text{ ng.ml}^{-1}$) there is a concern that a single subject produced a sample which was greater than the current threshold as a result of the study's dosing regimen. This positive test highlights the large inter-individual variability for urinary thresholds, however the difference between the WADA threshold and the WADA decision limit appears to be sufficient as the positive responder did not exceed the decision limit of 1200 ng.ml^{-1} . Nevertheless, this does suggest that administration of an acute, high dose of salbutamol may lead to a breach of the threshold and thus result in an adverse analytical finding (AAF), which is supported by Dickinson *et al.*, (2014d) who found AAF's following acute doses of $1600 \mu\text{g}$ salbutamol with varying levels of dehydration. Further research is clearly needed to establish the variability surrounding urinary salbutamol levels amongst individuals dosing up to and including $1600 \mu\text{g}$ in a single acute dose, the present study would have also benefitted from investigations into the hydration status of the participants.

In conclusion, there is no improvement in endurance, strength and power performance following the inhalation of 1600 µg of salbutamol per day for six weeks in non-asthmatic recreationally trained males. This would suggest that the current WADA list of banned substances (WADA List of Prohibited Substances, 2015), which allows athletes to inhale up to 1600 µg is sufficient given the findings from this and previous studies. Future research should focus on establishing the variability surrounding urinary salbutamol levels amongst individuals dosing up to and including 1600 µg in a single acute dose.

Salbutamol is the preferred choice of treatment for athletes, however athletes with a TUE are permitted to use the alternative short-acting β_2 -agonist terbutaline. Terbutaline currently has no established thresholds for use to distinguish between route of administration and is potentially more ergogenic than salbutamol in supratherapeutic dosages. It is important therefore that urinary thresholds for terbutaline are established in order for athletes with a TUE to be effectively monitored.

**5. Urinary
concentrations of single
and multiple
administration of inhaled
and oral terbutaline:
influence of gender and
race**

5.1 Background

Short-acting β_2 -agonists act to reverse bronchoconstriction of the airways through stimulation of the β_2 -receptors present on bronchial smooth muscle, allowing the muscle to relax and the airways to dilate restoring airway function (Anderson *et al.*, 1997; Hoffman, 2001; Driessen *et al.*, 2013). These short-acting β_2 -agonists are the preferred immediate/acute treatment for EIB, in athletes this is via salbutamol administration (Fitch, 2006). Sanchez *et al.* (2013) have indicated that terbutaline is a fast-acting β_2 -agonist that is active for a maximum of 12 hours whereas salbutamol is active for a maximum of 6 hours. Sanchez *et al.* (2013) also state that terbutaline, with fewer reported side-effects than other β_2 -agonists, may be of benefit to athletes for the relief of EIB.

The World Anti-Doping Authority (WADA) Prohibited List of Substances and Methods (WADA Prohibited List, 2010-2014) has permitted the use of the short-acting β_2 -agonist salbutamol and the long acting β_2 -agonist salmeterol for inhalation in therapeutic doses since the removal of the need for a therapeutic use exemption (TUE) in 2010. Furthermore, the inhaled long acting β_2 -agonist formoterol has been permitted in therapeutic doses since 2012. However, despite the absence of evidence that therapeutic doses of inhaled terbutaline have an ergogenic effect on athletic performance (Kalsen *et al.*, 2014), terbutaline remains on the prohibited substances list (WADA prohibited List 2015) (UEFA Guide to the WADA Prohibited List, 2015)

except for those athletes with a therapeutic use exemption (TUE). A threshold for terbutaline would be useful in the fight against doping in sport and also in maintaining standards of healthcare for the elite athlete, as it would allow for a broader range of inhaled β_2 -agonists to be used by athletes and also allow for successful identification of the prohibited use of oral terbutaline that could potentially be ergogenic.

The inclusion of terbutaline on the WADA List is primarily due to the inability to distinguish between oral and inhaled use (Elers *et al.*, 2012b). Studies have been performed for salbutamol investigating the differences between oral and inhaled use that have successfully established concentration limits in the urine, included in the WADA Prohibited List (Elers *et al.*, 2012a; Berges *et al.*, 2000; Elers *et al.*, 2010; Pichon *et al.*, 2006). There is a large disparity between the amount of inhaled salbutamol (200 μ g) and the amount of oral salbutamol (4 mg) that is used therapeutically with an inhaled-oral ratio of roughly 1:20, yet this is not as apparent for terbutaline where normal dosing regimens for individuals with a TUE permit up to 2 mg inhaled terbutaline prophylactically for the relief of symptoms (Elers *et al.*, 2012b) and the therapeutic oral dose is a 5 mg tablet where the inhaled-oral ratio is 1:2.5, this is potentially the reason that it is difficult to distinguish between route of administration.

Recent work has highlighted that in order to improve measures for doping control hydration status is a factor that can affect the outcome of a test

(Dickinson *et al.*, 2014c). Other factors such as ethnic background can influence urine concentrations of metabolites, Kim *et al.*, (2004) state that there is inter-ethnic variability between male Caucasians and male Asians, highlighting that potential genetic difference between different ethnic groups could result in differences in the urinary concentrations of metabolites. The differences in metabolism between ethnicities is supported by Deshmuck *et al.*, (2010) who highlight that there are varying degrees of gene deletion/insertion/substitution polymorphisms between individuals of different ethnic backgrounds, in particular they highlight the reduced glucuronidation of steroids through a gene deletion polymorphism in uridine diphosphate glucuronosyl transferase 2B17 (UGT2B17) in individuals of Asian origin. Guo *et al.*, (2010) indicate also that variability in urinary concentrations of metabolites, occurring between males and females, are largely due to weight differences between individuals, in the study by Guo *et al.*, (2010) values in females were roughly 20% higher than that of males, equivalent to the weight differences between the two groups.

The purpose of the present study was to measure the urine concentrations of terbutaline following single and repeated doses of oral and inhaled terbutaline in male Caucasians, female Caucasians, male Afro-Caribbeans and male Asians to allow for comparisons between gender and race to better inform WADA with regard to doping policy and procedure relating to terbutaline use.

It is hypothesized that an acute therapeutic dose (2 mg) of inhaled terbutaline will lead to significantly lower urine concentrations than an acute therapeutic dose (5 mg) of oral terbutaline.

It is also hypothesized that a repeated therapeutic dose (8 x 1 mg over 36 hours) inhaled terbutaline will lead to significantly lower urine concentrations than repeated therapeutic dose (4 x 5 mg over 36 hours) oral terbutaline.

5.2 Methods

Prior to the initiation of the study ethical approval was obtained from Liverpool John Moores University Local Research Ethics Committee (Ethics no. P11SPS044). Twenty-two male and eight female subjects (8 male Caucasian, 8 female Caucasian, 6 male afro-Caribbean, 6 male Asian) provided written informed consent and were recruited for the study. All participants were free from asthma, EIB and AHR confirmed by no history of disease and a negative eucapnic voluntary hyperpnoea (EVH) challenge (Anderson *et al.*, 2001). All participants were non-smokers, were free from chest infection for 4 weeks prior to assessment and had no history of any pulmonary, cardiovascular or metabolic disease.

5.2.1 Oral and Inhaled Doses of Terbutaline

To enable close monitoring of participants responses to increasing doses of terbutaline it was deemed necessary to systematically order the trials, a schematic of study progression is shown in figure 5.1. During visit 1 all participants received a single therapeutic dose of 5 mg oral terbutaline (Bricanyl, AstraZeneca, UK), single oral administration (SOA). During visit 2 all participants received a therapeutic dose of 4 inhalations of 0.5 mg terbutaline (Bricanyl Turbohaler, AstraZeneca, UK) totalling 2mg inhaled, single inhaled administration (SIA). During visit 3 all participants inhaled the

therapeutic dosing regimen of 1 mg (2 x 0.5 mg inhalations) of terbutaline (Bricanyl Turbohaler, AstraZeneca, UK) at 08:00h, 12:00h, 16:00h and 20:00h for 2 days, repeated inhaled administration (RIA). During visit 4 all participants received the therapeutic dosing regimen of 5 mg oral terbutaline (Bricanyl, AstraZeneca, UK) at 10:00h and 18:00h for 2 days, repeated oral administration (ROA). There was a minimum of 7 days between the cessation of one trial and the commencement of the next trial to ensure complete washout of terbutaline which has a terminal half-life of ~17h (Kalsen *et al.*, 2014).

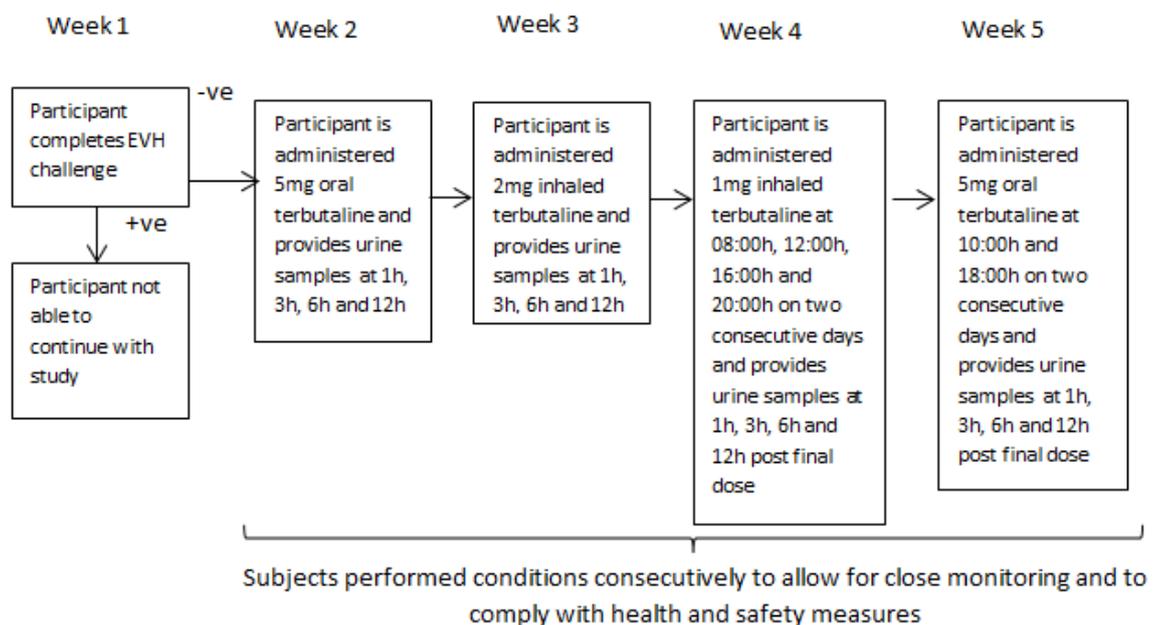


Figure 5.1: Timeline schematic of study progression

5.2.2 Urine Sample Collection

Following the final dose of terbutaline during each trial, participants were required to provide urine samples at 1h, 3h, 6h and 12h time-points. Participants were asked to record the volume and time of each sample provided. Each urine-sample was 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 °C until urinalysis (see below). Where samples were provided off-site participants were required to freeze immediately at -20°C until the next day when they were returned to the laboratories for pH and osmolality assessment prior to freezing at -80 °C.

5.2.3 Terbutaline Urinalysis

As described in general methods section 3.13.

5.2.4 Sample Correction

All urine concentrations of terbutaline were corrected to urine specific gravity of 1.020 prior to analysis using the following equation (Elers *et al.*, 2012b):

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

5.2.5 Statistical Analysis

Statistical analyses were performed using the statistical package for the social sciences SPSS (SPSS v20.0, IBM, New York, USA). All data were normally distributed and presented as mean and standard deviation unless otherwise stated. A mixed-model analysis of variance (ANOVA) was performed to compare between groups within each trial and to compare between trials within each group, 2-way ANOVA was used to compare between groups and between trials at all time-points. A post-hoc Bonferroni correction was applied to adjust for multiple comparisons. Statistical significance was set at $p < 0.05$ for all analyses.

5.3 Results

Twenty-eight participants successfully completed all trials, subject demographics and lung function screening values are shown in Table 5.1, two subjects reported fine tremor following both oral administration trials, which diminished within four hours of ingestion. No other adverse side-effects were reported.

Comparisons were performed examining differences between oral and inhaled terbutaline for single-dose administration (Figure 5.2) and for repeated administration (Figure 5.3). Comparisons between groups were performed examining differences between male and female Caucasians for gender related differences and differences between male Caucasian, Afro-Caribbean and Asian for ethnic differences (Figure 5.4). Finally, comparisons were performed between the highest individual peak values obtained within each group for each trial condition (Figure 5.5).

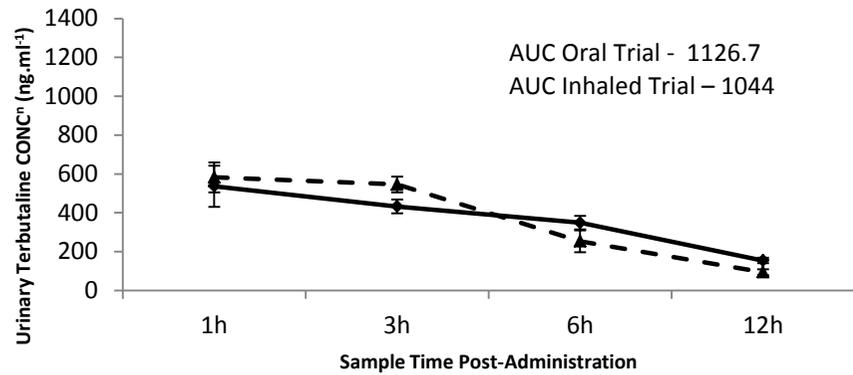
Table 5.1: Mean (\pm SD) Subject Demographics and Lung Function Values Pre- and Post EVH Challenge for: Male Caucasians (MC), Female Caucasians (FC), Male Afro-Caribbean's (MAC) and Male Asians (MA)

Group	Height	Weight	Age	Baseline FEV₁	% Predicted FEV₁	Post EVH FEV₁
MC (n=8)	181.05 (4.5)	78.48 (7.9)	23.38 (2.1)	5.03 (0.4)	110.63 (6.9)	4.81 (0.4)
MAC (n=6)	183.72 (6.5)	81.36 (9.3)	21.5 (3.5)	4.24 (0.7)	100.67 (12.7)	4.1 (0.8)
FC (n=8)	167.59 (5.7)	58.12 (6)	21.63 (1.9)	3.50125 (0.4)	100.63 (9.6)	3.31 (0.4)
MA (n=6)	170.83 (4.9)	69.35 (2.9)	31.5 (4)	4.08 (0.6)	111.34 (11.8)	4.01 (0.6)

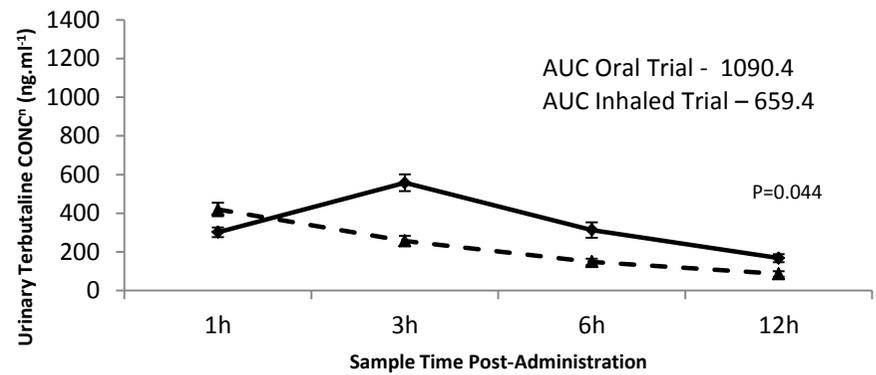
FEV₁ - Forced Expiratory Volume in 1 Second; EVH - Eucapnic Voluntary Hyperpnoea; MC – Male Caucasian; MAC – Male Afro-Caribbean; FC – Female Caucasian; MA – Male Asian

5.3.1 Single Administration

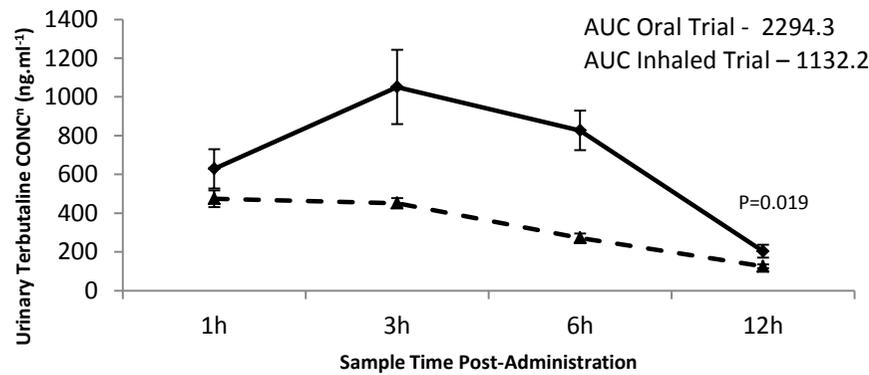
Following the single dose (5 mg) of oral terbutaline the urinary terbutaline concentration was significantly greater when compared to the single dose (2 mg) of inhaled terbutaline in the female Caucasian group (670.1 ± 128.3 vs. 361.8 ± 43.8 ng·ml⁻¹; P=0.019), the male afro-Caribbean group (343.18 ± 45 vs. 231.3 ± 32.95 ng·ml⁻¹; P=0.044) and the male Asian group (266.4 ± 23.7 vs. 143.3 ± 22 ng·ml⁻¹; P=0.004), respectively with no difference in the male Caucasian group (Figure 5.2). Of note, the peak concentrations following inhaled doses occurred at the 1 hour time-point and the peak concentrations following the oral doses occurred at the 3 hour time-point in all groups except the male Caucasians, where the peak for oral terbutaline was also at the 1 hour time-point.



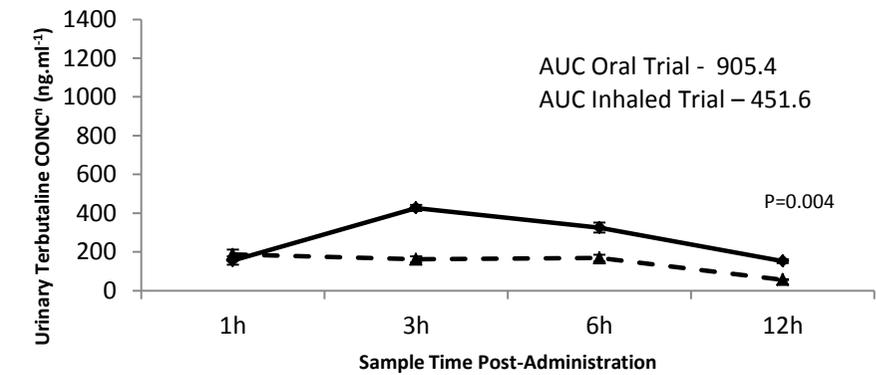
a Male Caucasian Oral Administration Male Caucasian Inhaled Administration



b Male Afro-Caribbean Oral Administration Male Afro-Caribbean Inhaled Administration



c Female Caucasian Oral Administration Female Caucasian Inhaled Administration

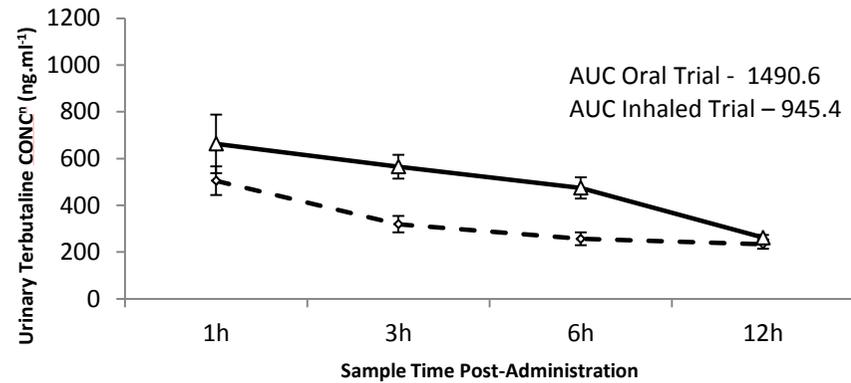


d Male Asian Oral Administration Male Asian Inhaled Administration

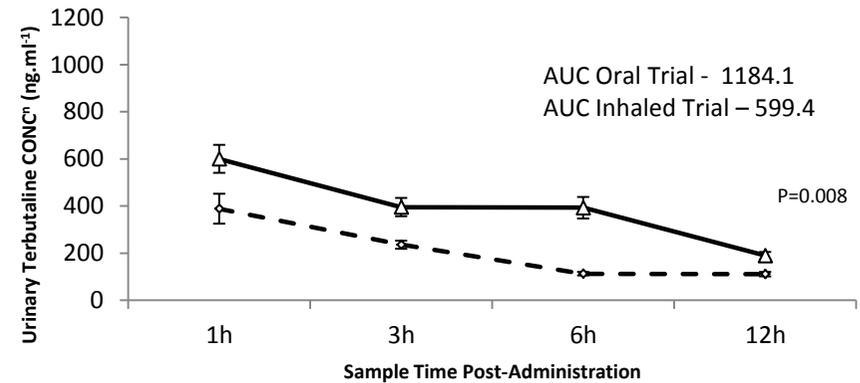
Figure 5.2: Mean \pm SE urine concentrations between single inhaled administration and single oral administration of terbutaline for a) male Caucasians; b) male Afro-Caribbeans; c) female Caucasians; d) male Asians. The area under the curve (AUC) for oral and inhaled trials has also been presented.

5.3.2 Repeated Administration

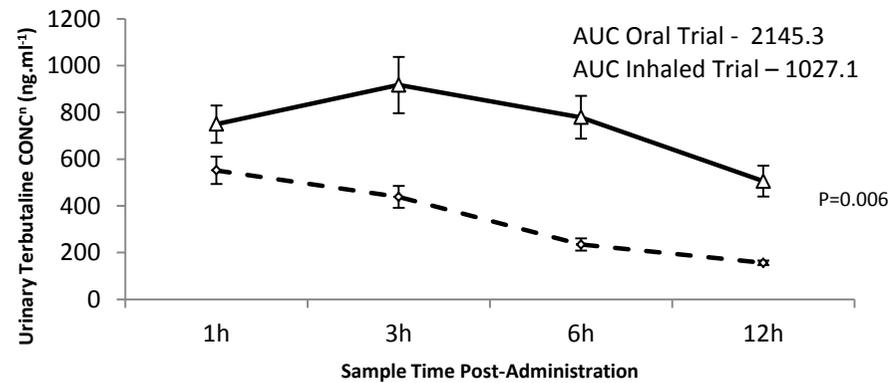
There were significant differences between urinary terbutaline concentrations for repeated dose oral vs. repeated dose inhaled terbutaline in the female Caucasian group (680.8 ± 91 vs. 369.9 ± 41.9 ng·ml⁻¹; $P=0.006$) the male afro-Caribbean group (389.73 ± 67.4 vs. 212.4 ± 50.3 ng·ml⁻¹; $P=0.008$) and the male Asian group (379.5 ± 50.4 vs. 197.5 ± 38.6 ng·ml⁻¹; $P<0.005$) for oral vs. inhaled terbutaline, respectively (Figure 5.3). There were no differences between trials for either single or repeated dose terbutaline in the male Caucasian group. Following repeated administration peak levels of urinary terbutaline occurred at the 1 hour time-point as expected in all groups except for the female Caucasian group.



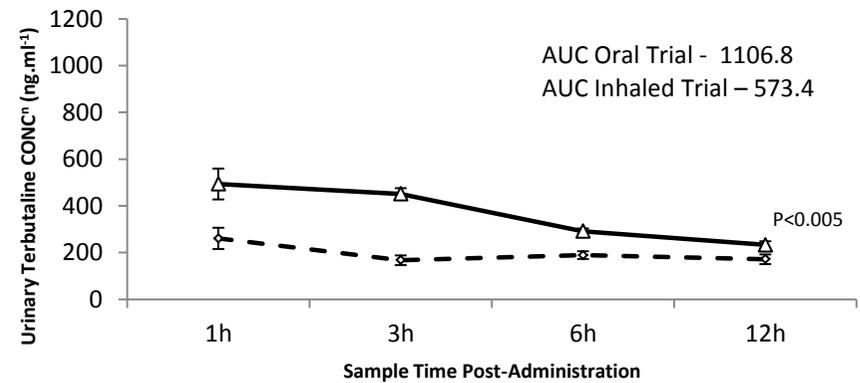
a —◆— Male Caucasian Inhaled Administration —▲— Male Caucasian Oral Administration



b —◆— Male Afro-Caribbean Inhaled Administration —▲— Male Afro-Caribbean Oral Administration



c —◆— Female Caucasian Inhaled Administration —▲— Female Caucasian Oral Administration



d —◆— Male Asian Inhaled Administration —▲— Male Asian Oral Administration

Figure 5.3: Mean (\pm SE) urine concentrations between repeated inhaled administration and repeated oral administration of terbutaline for a) male Caucasians; b) male Afro-Caribbean; c) female Caucasians; d) male Asians. The area under the curve (AUC) has also been presented

5.3.3 Gender Differences

There was a significant difference between male and female Caucasians for the repeated dose oral administration trial (406.9 ± 45.4 vs. 678.8 ± 94.8 ng·ml⁻¹; P=0.018), respectively. There were no gender differences between trials in any other conditions (Figure 5.4).

5.3.4 Ethnicity Differences

There was a significant difference between male Caucasians and male Asians for the single dose inhaled administration trial (372.14 ± 69.7 vs. 131.8 ± 19.7 ng·ml⁻¹; P=0.005), respectively.

5.3.5 Peak Values

There was a large inter-individual variation in the urinary concentrations of terbutaline. Figure 5.5 demonstrates the highest individual peak values obtained within each group for each trial condition. Values were generally higher following oral administration however there was a large crossover in values whereby a definitive cut-off value was not identified. Peak values after inhaled use barring one exception were all below 1284.3 ng·ml⁻¹ and apart from one exception peak values after oral use were all below 2376.3 ng·ml⁻¹.

5.3.6 *Upper limits following inhaled and oral use*

To calculate the upper limits the means for all participants in each trial were calculated. The upper limit was established as being two standard deviations above the mean. This was done for both the inhaled trial condition (1284.3 ng·ml⁻¹) and the oral trial condition (2376.3 ng·ml⁻¹). These values were then included in figure 5.5 to ascertain the sensitivity and specificity of the upper limits. These upper thresholds proved to have a specificity of 98.44% for both conditions but a sensitivity of only 14.1% to distinguish between oral and inhaled use.

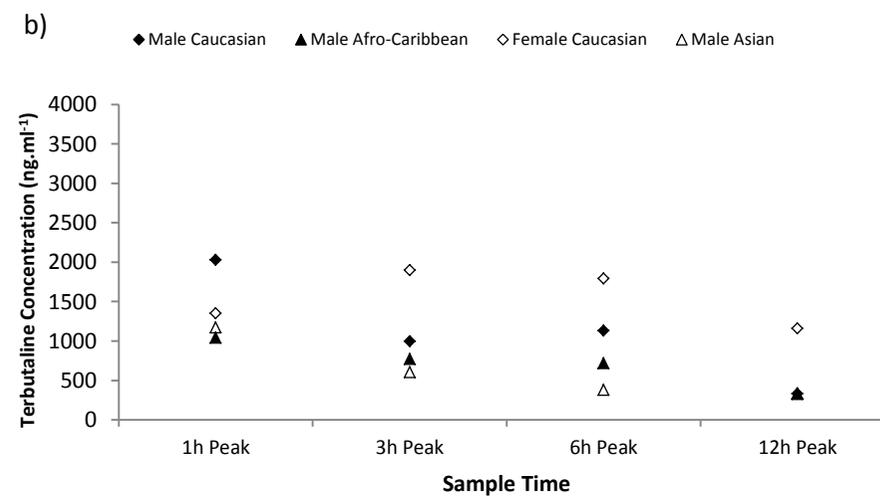
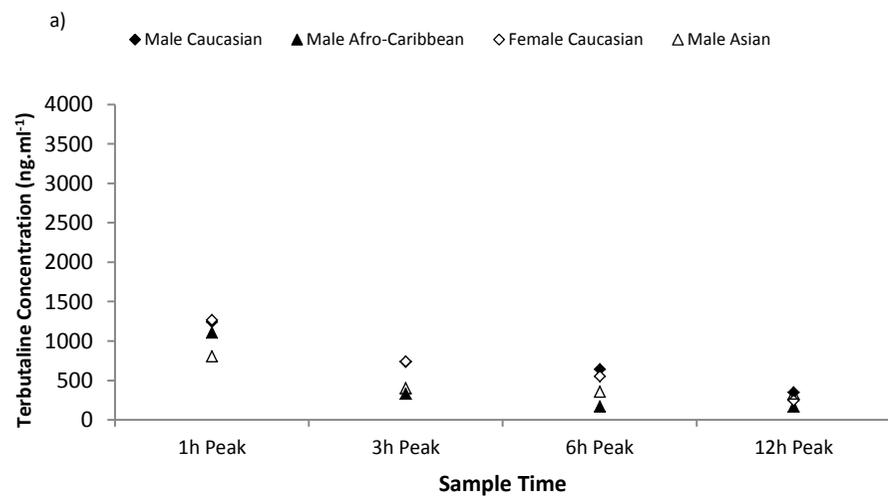
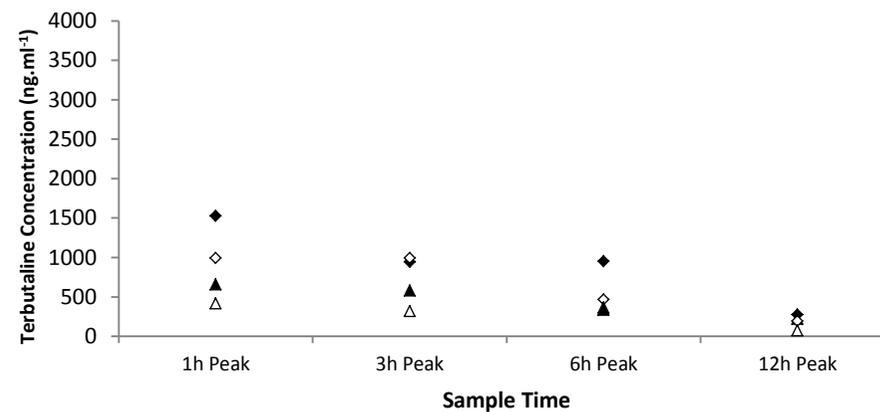
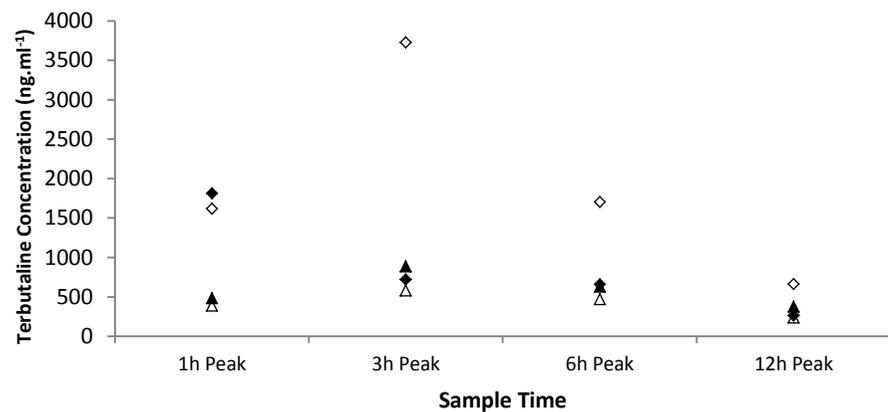


Figure 5.4: Highest individual peak urine concentration between groups for: a) single oral administration; b) single inhaled administration; c) repeated inhaled administration; d) repeated oral administration.

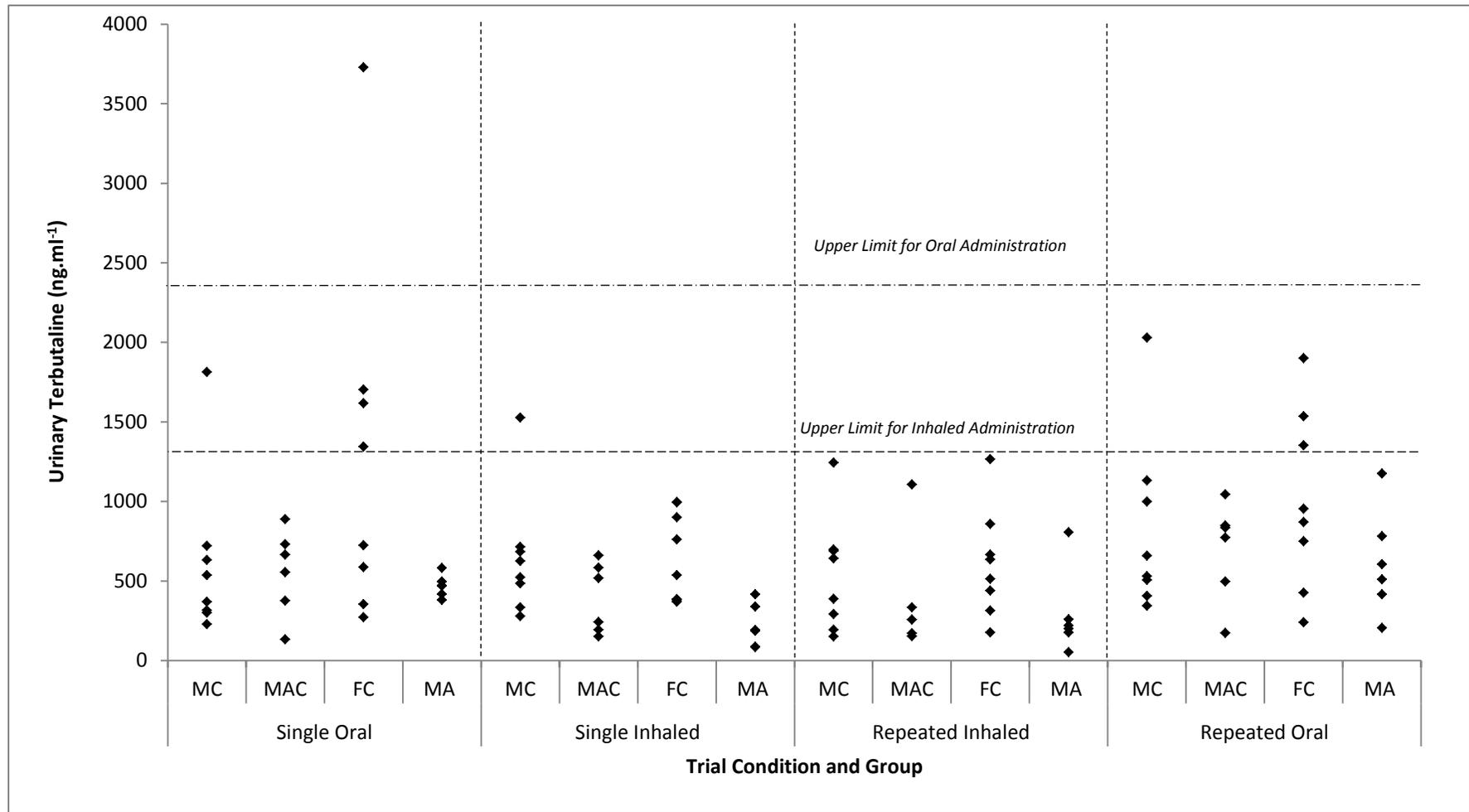


Figure 5.5: Peak values obtained during trials for each group with a proposed upper threshold for inhaled use and upper threshold for oral use; MC – Male Caucasian; MAC – Male Afro-Caribbean; FC – Female Caucasian; MA – Male Asian

5.4 Discussion

The findings of the present study demonstrate that there is no clear distinction between urinary concentrations of inhaled and oral terbutaline. The study did highlight, however, an upper threshold following therapeutic (2 mg) inhaled use of $1284.3 \text{ ng}\cdot\text{ml}^{-1}$ and an upper threshold following therapeutic (5 mg) oral use of $2376.3 \text{ ng}\cdot\text{ml}^{-1}$.

There are a number of asthmatic athletes currently using terbutaline through the therapeutic use exemption (TUE) process (Elers *et al.*, 2012b), therefore it is important to be able to distinguish between legitimate inhaled use and prohibited oral use in these athletes. The findings of the present study indicate that the urinary concentrations of terbutaline via oral and inhaled administration cannot be easily distinguished. The current status of terbutaline on the WADA List is appropriate until a cut-off threshold can be established, care is warranted for athletes using terbutaline through the TUE process in order to prevent athletes from illegally using supra-therapeutic doses of inhaled/oral terbutaline in the hope of gaining a competitive advantage (Kalsen *et al.*, 2014; Hostrup *et al.*, 2014).

The results from the present study show significant inter-individual variation in the urinary levels of terbutaline, dependent upon mode of administration, gender and race. In the female Caucasian group, the male Afro-Caribbean

group and the male Asian group there were significantly higher urinary concentrations of terbutaline in oral administration trials vs. inhaled trials but this was not apparent in the male Caucasian group. A recent study by Elers *et al.*, (2012b) reported similar findings when examining the levels of terbutaline in the plasma and urine following oral and inhaled administration (Elers *et al.*, 2012b). The study was able to find significant differences between the mode of administration of terbutaline, however they were unable to establish any thresholds due to the high variability between samples. In line with Elers *et al.*, (2012b) the present study was also able to highlight significant differences between dose and mode of administration, yet due to the large range in concentrations observed it has not been possible to distinguish between inhaled and oral use. However, our data suggest an upper threshold of 1284.3 ng.ml⁻¹ for inhaled use and 2376.3 ng.ml⁻¹ for oral use, however these results were not sensitive enough to distinguish fully between inhaled and oral use of terbutaline.

Recently Hostrup *et al.*, (2014) observed that a supra-therapeutic inhaled dose of terbutaline resulted in an improvement in muscle strength and maximal sprint performance. This was, however, an extremely high supra-therapeutic dose of 15mg inhaled terbutaline (30 x 0.5mg inhalations) which resulted in serum levels of terbutaline (23.6 ± 1.1 ng.ml⁻¹) roughly four times higher than that recorded by Elers *et al.*, (2012b) after a 10 mg oral dose (~6 ng.ml⁻¹). Such high dose inhalations of terbutaline would be easily distinguishable from the maximal therapeutic dose of 2 mg inhaled terbutaline which is permitted for athletes with a TUE (WADA 2015). In

contrast to the findings of Hostrup *et al.*, (2014), Sanchez *et al.*, (2013) reported 8 mg of oral terbutaline had no ergogenic effect during force-velocity, sprint and endurance cycling tests. This finding may be associated with a lower systemic availability of terbutaline (Sanchez *et al.*, 2013). Indeed, the oral dose that was administered by Sanchez *et al.*, (2013) would probably have elicited serum levels similar to those attained by Elers *et al.*, (2012b), which were around four times lower than that attained by Hostrup *et al.*, (2014). The present study would have benefitted from the analysis of blood plasma levels of terbutaline, yet the emphasis of the study was to replicate the procedures utilised during doping control tests, which currently only test urinary levels of β_2 -agonists.

The dose required for effective protection against EIB can be as little as one inhalation (0.5 mg) of terbutaline (Simpson *et al.*, 2013), which is the dose recommended by the manufacturer. The present study investigated a therapeutic dose at the upper recommended limit (Prior *et al.*, 1982), even though the present study was unable to detect a difference between the highest therapeutic inhaled dose and the therapeutic oral (5 mg) dose, it is possible that a difference in urinary concentration could exist between the minimum therapeutic (0.5 mg) inhaled dose and the 5 mg oral tablets. Future research that could investigate a cut-off threshold based upon the minimum dose required for a therapeutic effect (0.5 mg inhaled terbutaline) would provide useful information regarding the urinary levels attained following low dose inhaled terbutaline, these urinary levels may then be distinguishable from those of oral terbutaline.

Care is warranted when assessing differences between gender due to weight differences between males and females as found by Guo *et al.*, (2010) who noted that females exhibited urinary levels of fluconazole roughly 20% higher than males, which correlated with the average weight of the females being ~20% lighter, resulting in a higher volume distribution of the drug. Also of note is the possible difference in the metabolism of different substances, which can vary highly between individuals. Kim *et al.* (2004) suggested that an individual can be either an extensive metaboliser or a poor metaboliser. Variation in metabolism may also be due to inter-ethnic variation associated with genetic variations (Kim *et al.*, 2004; Deshmuck *et al.*, 2010)). Such a variation in the metabolism of terbutaline between ethnic groups could be a possible explanation as to why both Asian and Afro-Caribbean urinary concentrations appear lower than Caucasian values, conclusive evidence of this could only be obtained by identifying the enzyme responsible for metabolizing terbutaline and comparing gene variants across ethnicities. It cannot be discounted that these variations could simply be due to variations between inhalation technique, despite every effort to standardize this it may still have been a factor that allowed for variability between results. Further research examining gender and ethnic variations in urinary concentrations of metabolites, standardizing for weight differences and investigation enantiomers along with genetic variants in metabolizing enzymes, is needed in order to better inform anti-doping policy and procedure.

As previously mentioned, a limitation to the current study was the absence of an investigation of the ratio of enantiomers in the urine and calculation of the metabolic ratio (parent drug/metabolite) (Roig *et al.*, 2002), which would have further evidenced any possible gender or ethnic differences with regard to the metabolism of terbutaline and its excretion in the urine. The establishment of a correction equation which could standardise for body weight would also have been an advantage to allow for direct comparisons across the board. A further limitation was the absence of a direct comparison between a standardised inhaled dose and a standardised oral dose of equal proportion, however it would have been unethical to administer an inhaled dose equivalent to the minimum available oral dose of 5 mg.

In conclusion, the present study identified significant differences in the urine concentration of terbutaline following inhaled and oral administration, however due to high inter-individual variability a cut-off value was not identified. The study was able to identify upper thresholds following oral use and inhaled use, which could be used to identify supra-therapeutic use of terbutaline. Gender differences were identified between male and female Caucasians during the multiple oral administration trial. Ethnic differences were identified between male Caucasians and male Asians during the single inhaled administration trial. Further research incorporating both female Asians and female Afro-Caribbeans is required to fully elucidate inter-ethnic gender differences. Future research should also examine urine concentrations following a minimum therapeutic dose of inhaled terbutaline

versus oral terbutaline and also establish differences in enantiomers in the urine to provide further support for anti-doping cut-off limits.

Athletes that use terbutaline therapeutically through the TUE process may experience ergogenic effects during endurance performance. Following on from this study we wanted to test the ergogenic effect of therapeutic dosing of terbutaline (2 mg; 4 mg) on endurance exercise performance through 3 km running time-trials.

6. The impact of inhaled terbutaline on 3 km running time-trial performance in males and females

6.1 Background

As an alternative to salbutamol another short-acting β_2 -agonist, terbutaline, is available which is purported to have fewer adverse side-effects (Sanchez *et al.*, 2013) and is protective against bronchoconstriction following single-dose administration (Simpson *et al.*, 2013). Accordingly, terbutaline may provide a more desirable treatment option for the athletic population (Sanchez *et al.*, 2013). The findings from Chapter 5 indicate that therapeutic inhaled doses of terbutaline can lead to urinary terbutaline concentrations that do not exceed $1500 \text{ ng}\cdot\text{ml}^{-1}$ which may be useful in establishing between oral and inhaled use. It is still unclear, however, as to whether a therapeutic inhaled dose of terbutaline can lead to performance enhancement.

Unlike the permitted β_2 -agonists salbutamol, formoterol and salmeterol, terbutaline is prohibited during competition except for those athletes who provide demonstrable evidence of EIB sufficient for the issuing of a therapeutic use exemption (TUE) certificate (WADA Prohibited List 2015). This prohibited status is largely due to the inability to distinguish between inhaled and oral use, with oral use being banned for all β_2 -agonists (WADA Prohibited List 2015). Whilst investigations into threshold limits that can distinguish between inhaled and oral use of terbutaline are ongoing (including results from Chapter 5 of this thesis), it is important to evaluate whether there are any performance enhancing properties when taken at the therapeutic dose. Recent investigations examining the performance enhancing effects of supra-therapeutic doses of terbutaline have highlighted

an ergogenic potential during sprint cycling performance (Hostrup *et al.*, 2014) but not in endurance cycling performance (Kalsen *et al.*, 2014). A possible mechanism for the improved sprint cycling performance may be due to enhanced systemic availability of terbutaline following such high-dose inhalation, which may have had a more potent effect on skeletal muscle receptors than would occur following a therapeutic dose. However, athletes with a TUE that require the use of terbutaline should only be taking a therapeutic dose for the relief of symptoms, therefore it is this dose that warrants investigation for any possible ergogenic effect.

The highest acute therapeutic dose of inhaled terbutaline in the literature has been as high as 4 mg (8 x 0.5 mg inhalations) (Prior *et al.*, 1982) however, the dose required for therapeutic effects can be as low as a single inhalation of 0.5mg (Simpson *et al.*, 2013). With a large variation in what could be considered the ideal therapeutic dose, athletes with a TUE may feel the need to take doses towards the higher end of the spectrum in order to obtain adequate protection from the symptoms of EIB. With no standardised therapeutic dose outlined for the use of inhaled terbutaline in athletes with a TUE it is reasonable to assume that athletes would use single inhaled doses ranging from 0.5 mg to 4 mg.

Accordingly, the aim of the present study was to examine the effects of 2 mg and 4 mg inhaled terbutaline on 3km running time-trial performance.

6.2 Methods

6.2.1 Participants

Following ethical approval from the Liverpool John Moores University research ethics committee (Ethics No. P11SPS044), 8 males (age: 24.3 ± 2.4 years; weight: 77.6 ± 8 kg; height: 179.5 ± 4.3 cm) and 8 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 recreational 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. Subjects were informed about the nature and the risks of the experimental procedures before giving their informed consent.

6.2.2 EVH Challenge

As described in general methods sections 3.3 and 3.4. A negative EVH challenge was required in order to participate in the study. Criteria for progression into the study are outlined in figure 6.1.

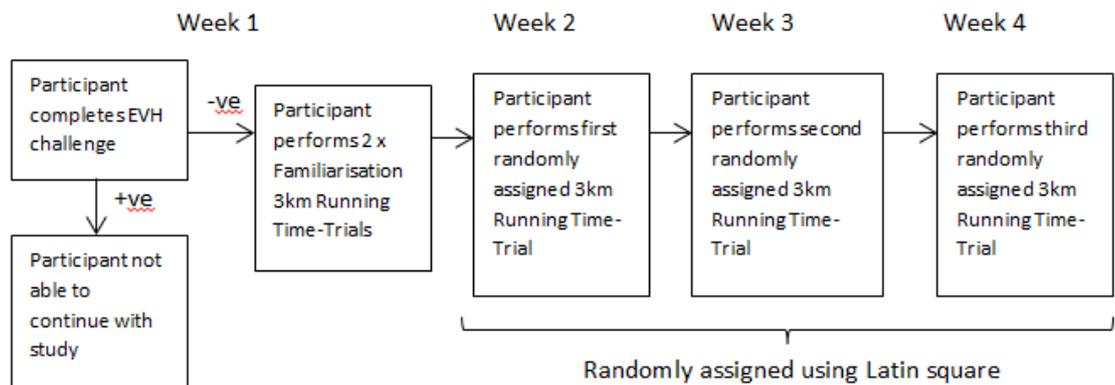


Figure 6.1: Schematic diagram of study progression

6.2.3 Three km Time-Trial

As described in general methods section 3.6.

Following familiarisation 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. Participants were assigned to one of the following groups:

- (1) Eight inhalations (via pocket chamber) of a non-active inhalant (placebo);
- (2) Four inhalations of non-active inhalant plus four inhalations of 0.5 mg terbutaline (2mg);
- (3) Eight inhalations of 0.5 mg terbutaline (4mg)

All inhalers looked identical and were kept out of view of the participants during dosing to ensure sufficient blinding. The 3km time-trials were performed under controlled laboratory conditions of 18°C and 40% humidity.

Following baseline spirometry, subjects were administered their treatment dependent upon trial condition, 10 minutes post-inhalation spirometry was repeated, before the completion of a standardised warm-up (5 minutes on a motorized treadmill at 10 km.h⁻¹); subjects then began the performance time-trial on the curve treadmill (Woodway, Wisconsin). 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 final spirometry and the provision of a urine sample.

During the 3 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. Time-trial progression is shown in figure 6.2.

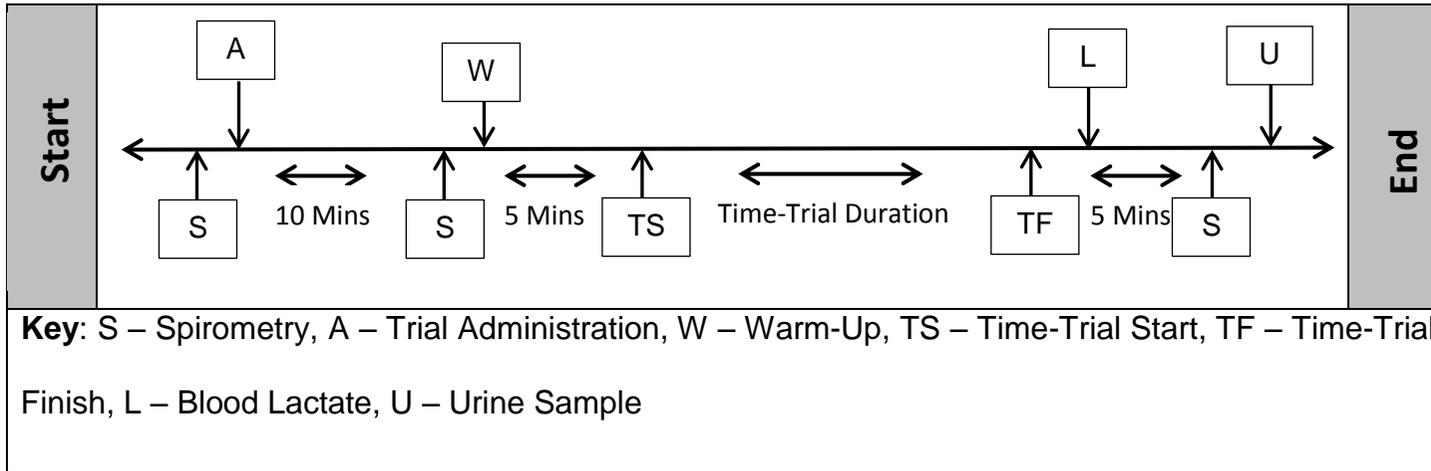


Figure 6.2. Schematic of the protocol used for each laboratory visit

6.2.4 Terbutaline Urinalysis

As described in general methods section 3.13.

6.2.5 Sample Correction

All urine concentrations were corrected to urine specific gravity of 1.020 prior to analysis using the following equation (Elers *et al.*, 2012b):

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

6.2.6 Statistical Analysis

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

6.3 Results

All sixteen participants successfully completed all trials. Participant demographics and lung function screening values are shown in Table 6.1. No side-effects were reported following terbutaline administration in any of the participants.

Table 6.1: Mean (\pm SD) Subject Demographics and Lung Function Values Pre- and Post EVH Challenge for Males and Females

Group	Height (cm)	Weight (kg)	Age (yrs.)	Baseline FEV ₁ (L)	% Predicted FEV ₁	Post EVH FEV ₁ % Fall
Males (n=8)	179.5 (4.3)	77.6 (8)	24.3 (2.4)	5.2 (0.2)	114 (4.6)	5.1 (6.1)
Females (n=8)	163 (9.2)	58.6 (6)	22.4 (3)	3.6 (0.5)	108.9 (13.4)	2.8 (11.4)

FEV₁ - Forced Expiratory Volume in 1 Second; EVH - Eucapnic Voluntary Hyperpnoea;

6.3.1 *Urinalysis*

There was no difference in urine concentration of terbutaline following either 2 mg inhalation or 4 mg inhalation post time-trial in males or females (Figure 6.3). The failure to record a difference between trials may largely be due to a high individual variation in urine concentration within each group. The highest individual peak value measured was 1244.4 ng.ml⁻¹ in the female group and 1244.4 ng.ml⁻¹ in the male group, with both occurring following the 4 mg inhaled dose (Figure 6.3).

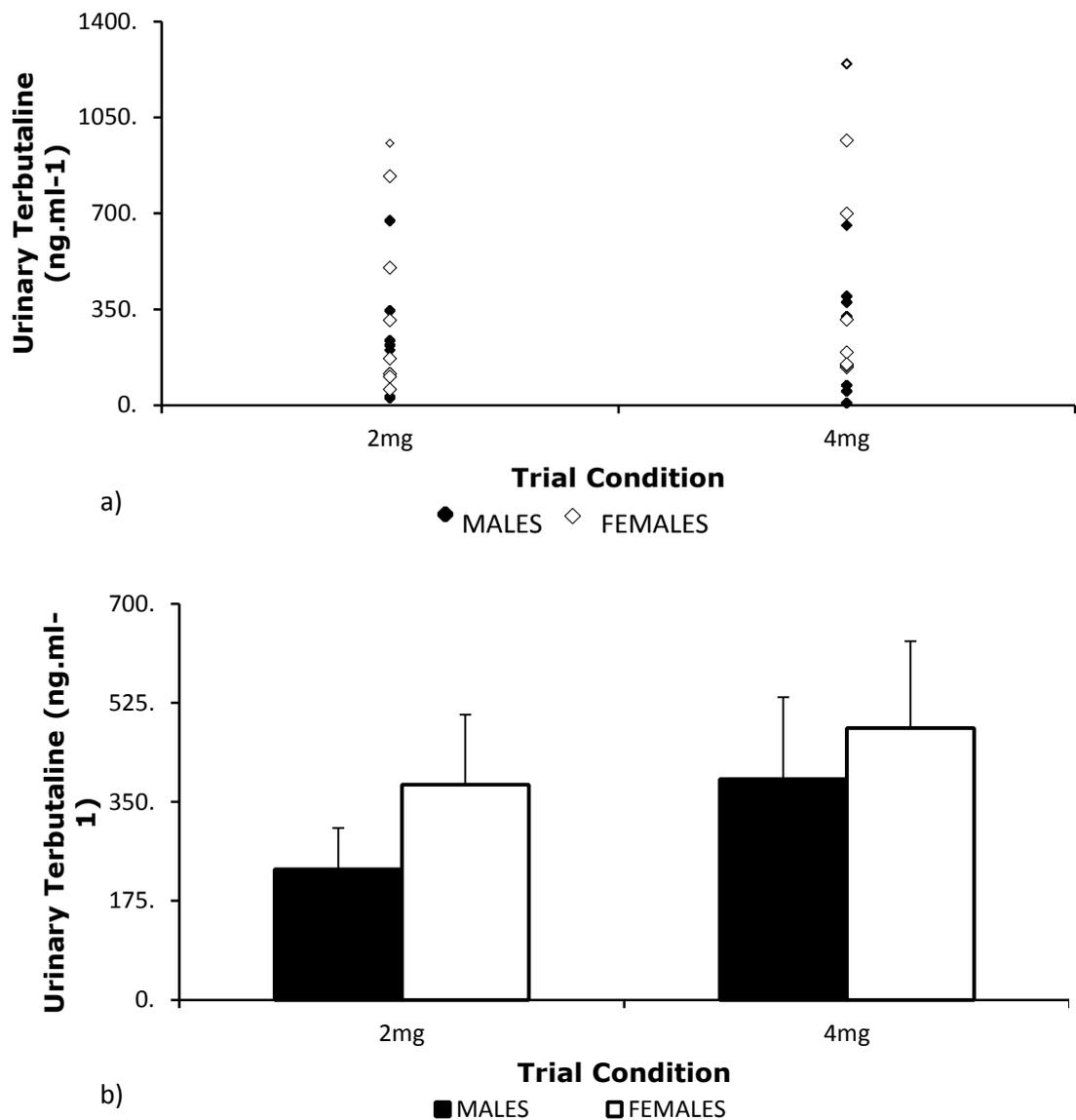


Figure 6.3: a) Individual peak and b) Mean \pm SD urinary concentrations of terbutaline between trials in males and females.

6.3.2 Three km Running Time-Trial Performance

There was no significant difference in completion time between trials in either males or females (Figure 6.5). Heart rate values were not significantly different between trial conditions yet did significantly increase over time during the 3km time-trial performances. Rating of perceived exertion values were also not significantly different between trials at any time-point during performance, indicating that all trials were performed with equal effort (Figure 6.4). In the female group there was a significant difference in lactate values between the placebo trial ($8.6 \pm 0.5 \text{ mmol}\cdot\text{L}^{-1}$) compared to the 4 mg inhaled terbutaline trial ($11.4 \pm 0.8 \text{ mmol}\cdot\text{L}^{-1}$) ($P=0.02$), yet this difference did not translate to a change in performance or perceived exertion, there were no significant differences in lactate values in males, no differences were observed between VO_2 , VCO_2 and RER in either group (Figure 6.6).

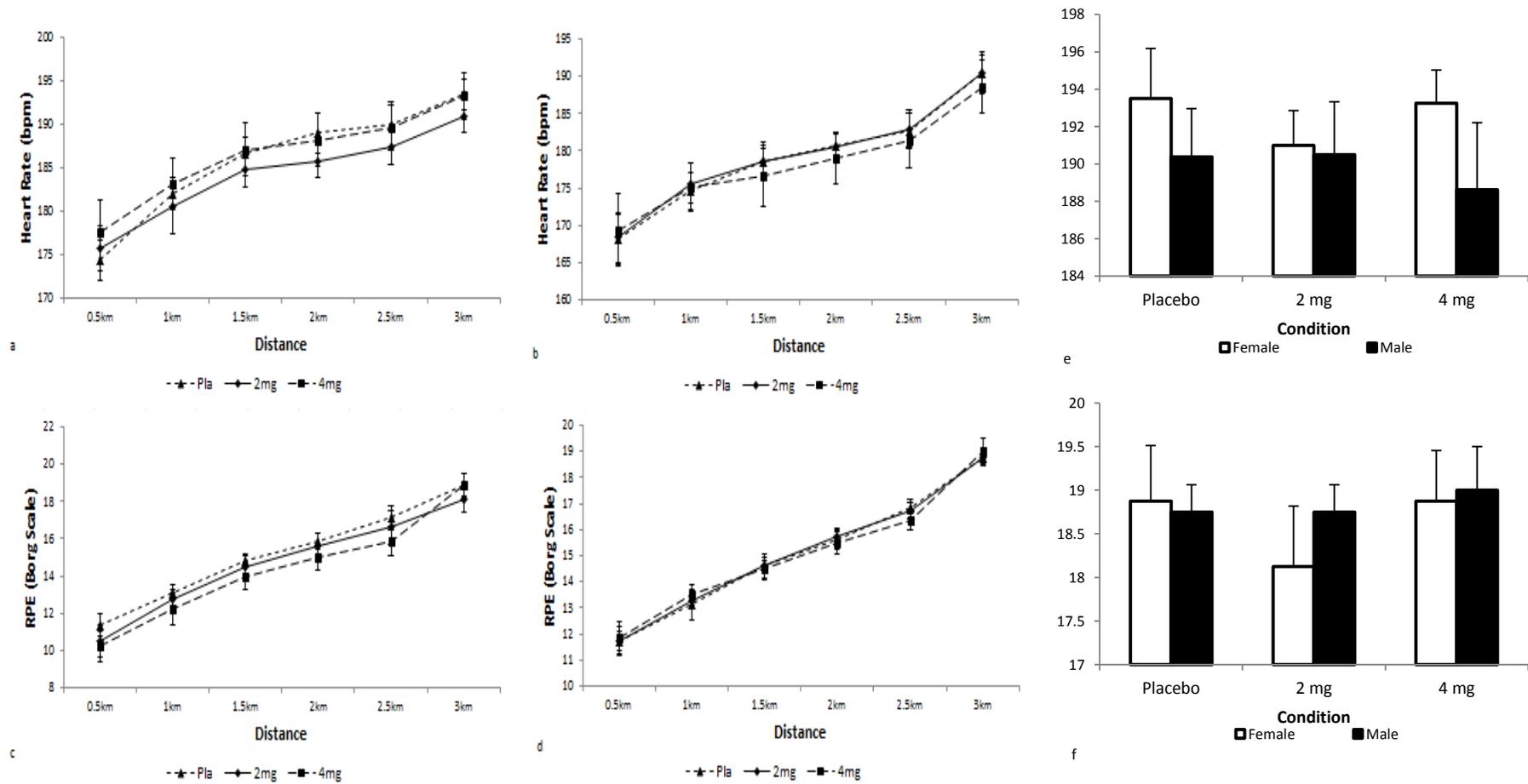


Figure 6.4: Heart rate during each of the three trials for a) Females and b) Males alongside Ratings of Perceived Exertion during each of the three trials for c) Females and d) Males including mean values for e) Heart rate and f) RPE.

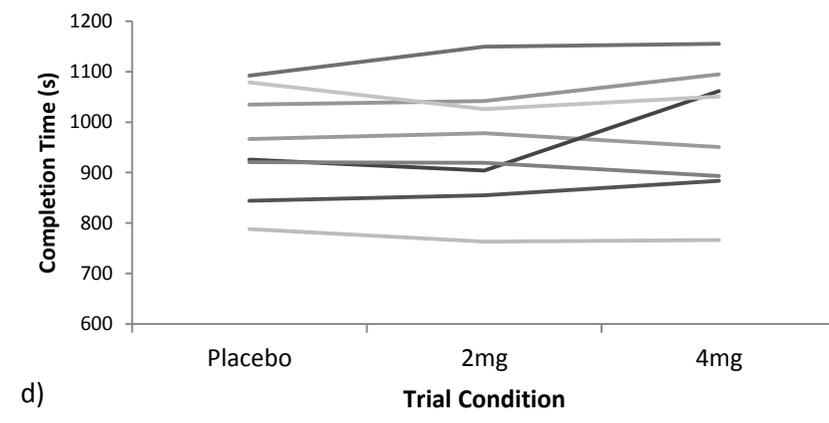
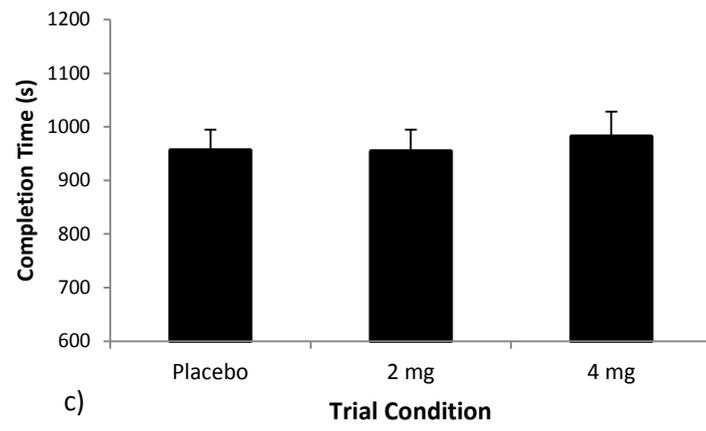
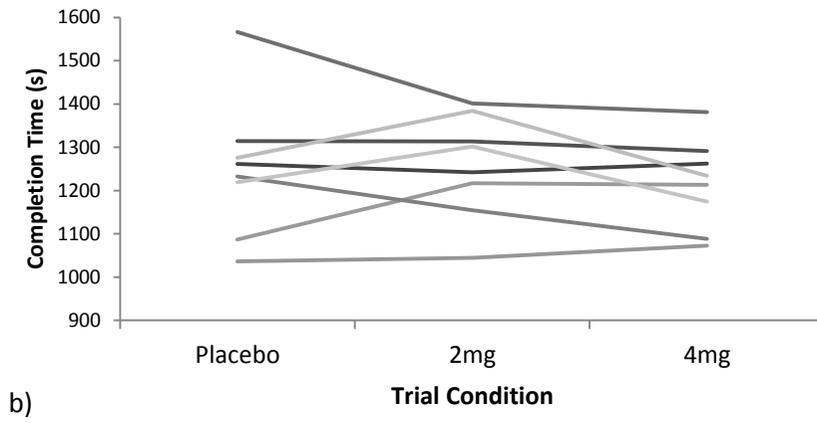
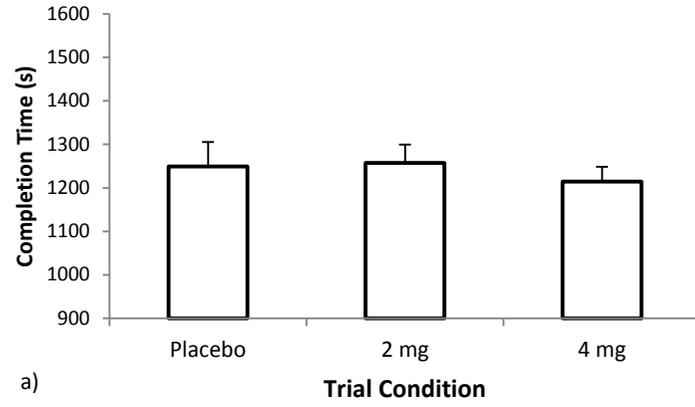


Figure 6.5: Mean (\pm SD) and individual 3km time-trial completion times for a) Female mean completion b) Female individual completion c) Male mean completion d) Male individual completion

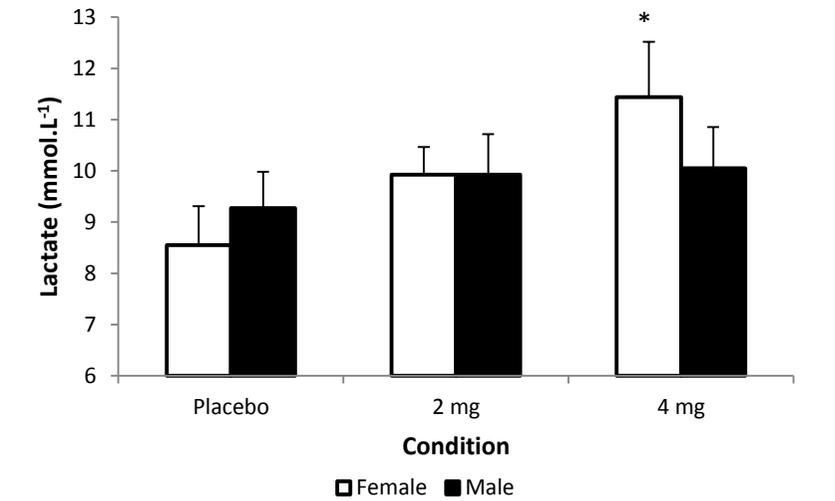
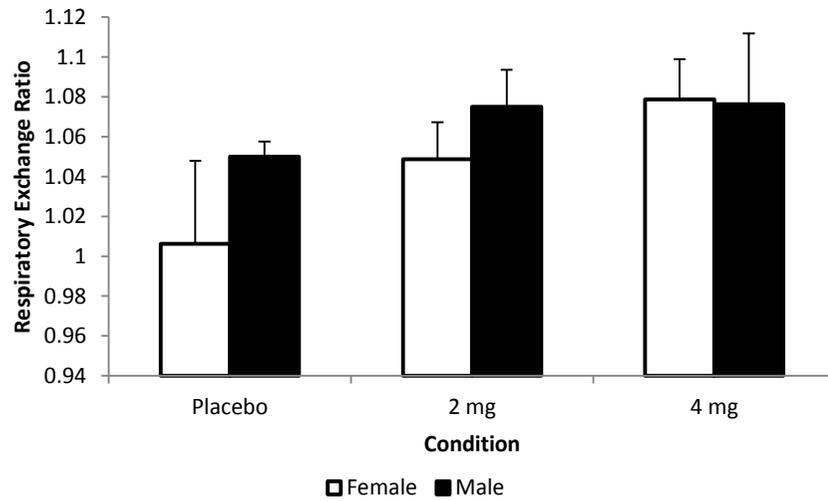
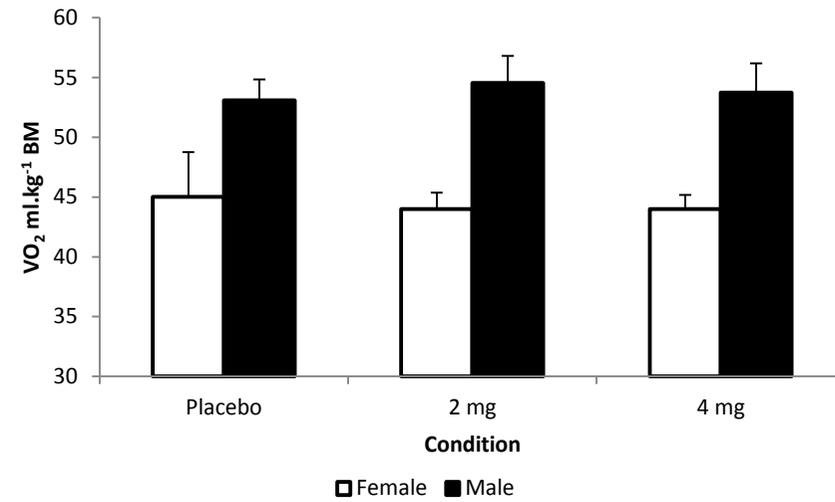
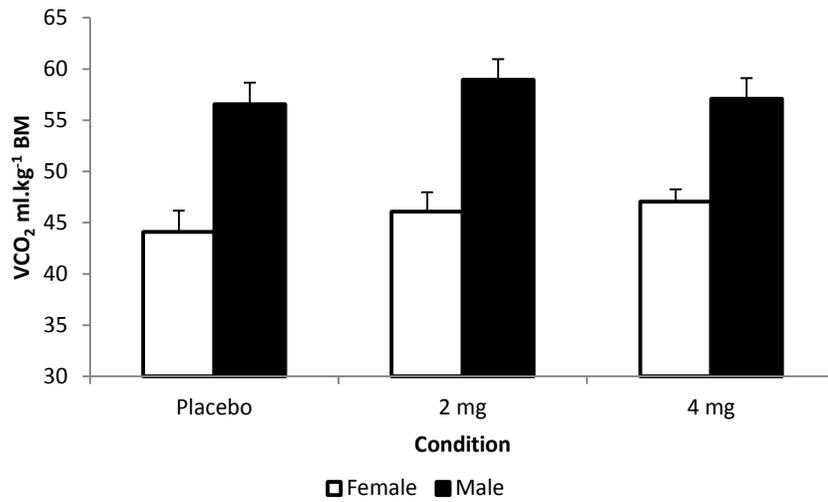


Figure 6.6: VCO₂, VO₂, RER and Lactate values post 3km running time-trial between conditions in males and females.

* - Significantly different to Placebo

6.3.3 Respiratory Measurements

No differences were seen between any parameters in females. In males there was a significant difference in FEV₁ between conditions (P=0.028). There were no differences between FEV₁ values in the placebo trial at any time point. There was a significant difference between baseline and both post inhalation (P=0.003) and post time-trial (P=0.014) FEV₁ values in the 2 mg inhaled trial (4.84 ± 0.2 L; 5.08 ± 0.2 L; 5.07 ± 0.2 L), respectively. There was a significant difference between baseline and both post inhalation (P<0.001) and post time-trial (P=0.028) FEV₁ values in the 4 mg inhaled trial (4.8 ± 0.2 L; 5.07 ± 0.2 L; 5.04 ± 0.2 L), respectively (Figure 6.7). There was no difference in baseline values between conditions. There was a significant difference in post inhalation FEV₁ values between placebo and both 2 mg (P=0.011) and 4 mg (P=0.026) inhalation trials (4.83 ± 0.2 L; 5.08 ± 0.2 L; 5.07 ± 0.2 L), respectively. There was a significant difference in post time-trial FEV₁ values between placebo and the 2 (P=0.04) mg inhalation trial but not the 4 mg inhalation trial (4.87 ± 0.2 L; 5.07 ± 0.2 L; 5.04 ± 0.2 L), respectively (Figure 6.7).

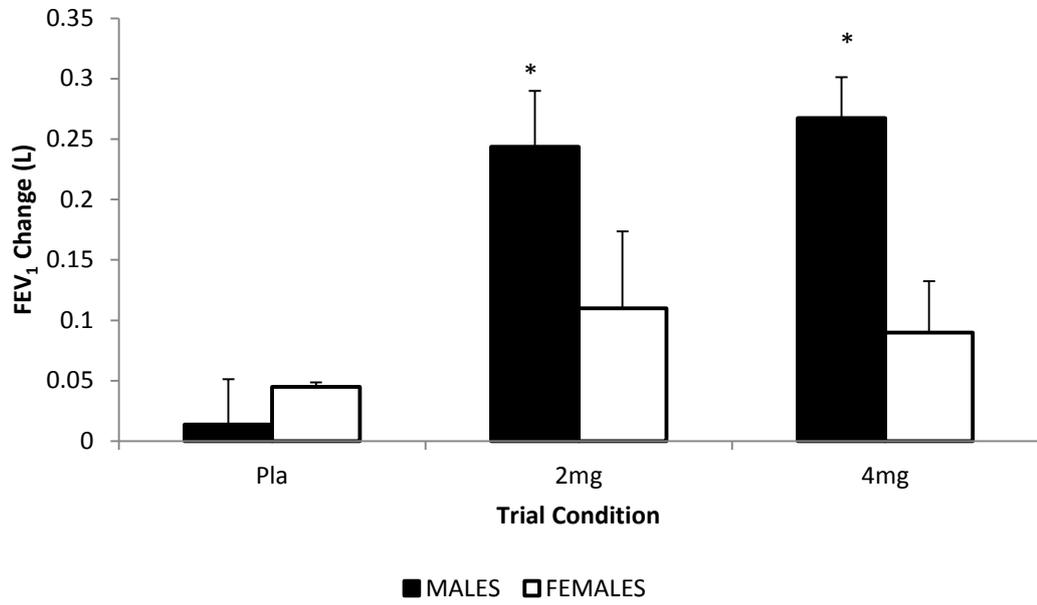


Figure 6.7: FEV₁ following inhaler administration for all trials for change in FEV₁ from baseline levels

* - Significantly different from baseline

6.4 Discussion

The aim of the present study was to investigate the effects of 2 and 4 mg inhaled terbutaline on 3 km running time-trial performance in males and females. This study demonstrated that inhaled terbutaline does not improve 3 km running time-trial performance in males or females. Terbutaline did result in a significantly increased resting lung function in healthy males and females similar to that observed with other short-acting β_2 -agonists *i.e.* salbutamol. The maximum observed urinary concentration of terbutaline was 1244.4 ng/ml⁻¹ following the inhalation of 4 mg terbutaline. This data is able to support the therapeutic use of terbutaline in those individuals with a TUE.

In contrast to the findings of this study Hostrup *et al.*, (2014) reported 15 mg inhaled terbutaline significantly improved muscle strength and sprint cycling performance but not 300 kcal cycling time-trial performance in trained males. Conversely, Kalsen *et al.*, (2014) also investigated the effects of 15 mg inhaled terbutaline on performance, during a 300 kcal cycling time-trial, finding no significant difference in performance compared to placebo. Hostrup *et al.*, (2014) in agreement with Sanchez *et al.*, (2012) explain that a possible mechanism for improved strength and power performance could be due to improved Ca²⁺ handling from the sarcoplasmic reticulum of skeletal muscles, mediated by cAMP-dependent phosphorylation of proteins associated with the sarcoplasmic reticulum. Of note, Kalsen *et al.*, (2014) suggested that inhaled terbutaline promotes a shift towards carbohydrate

metabolism during exercise. This conclusion could support the higher lactate values observed in the female participants in the present study following 4 mg of inhaled terbutaline despite no differences in performance or perceived exertion during the trial.

In the present study, therapeutic inhaled doses of terbutaline were investigated in order to ascertain their effects on endurance performance. The findings indicate no ergogenic effect of inhaled terbutaline and support the use of inhaled terbutaline for those athletes with a TUE. Currently a TUE is needed for the use of inhaled terbutaline during competition, largely due to the inability to distinguish between an inhaled dose and a prohibited oral dose (Elers *et al.*, 2012b). In a recent study by Elers *et al.*, (2012b) investigating the blood and urinary concentrations of terbutaline following either an inhaled or an oral dose, it was highlighted that although significant differences were found between the doses, no cut-off value could be established between the two modes of administration. 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 adverse analytical finding would indicate possible supra-therapeutic use or oral administration which may have ergogenic potential (Hostrup *et al.*, 2014). Therefore providing an accessible alternative to salbutamol in athletes who suffer with adverse side-effects or are unresponsive to salbutamol treatment.

In addition to the paucity of data at present examining the impact of inhaled terbutaline on performance there is a paucity of data examining the ergogenic impact of oral terbutaline. A recent study by Sanchez *et al.*, (2013) investigating the effects of a supra-therapeutic (8mg) oral dose of terbutaline on aerobic performance, found no significant difference versus placebo, highlighting the lack of ergogenic potential of terbutaline even at higher doses. Accordingly, further research examining the ergogenic impact of inhaled and oral terbutaline on strength and power performance is warranted.

In conclusion, inhaled doses of up to 4 mg of terbutaline do not improve 3 km running time-trial performance in males or females. The finding that the highest individual peak value measured was $1244.4 \text{ ng.ml}^{-1}$, is in agreement with the findings from Chapter 5 suggesting that terbutaline concentrations following inhaled use do not exceed 1284 ng.ml^{-1} . Further research is needed to investigate upper cut-off limits of terbutaline in the urine and the ergogenic effect of terbutaline on strength and power performance following therapeutic inhaled doses and supra-therapeutic oral doses.

With salbutamol being the most widely used medication for athletes with respiratory symptoms the next chapter will investigate the use of salbutamol in individuals exercising in a bronchoprovocative environment to assess the protective effects of salbutamol and also the potential to offset a decrement in performance in EVH+ve individuals compared to healthy controls.

7. The effects of inhaled salbutamol on 3 km running time-trial performance at low humidity in eucapnic voluntary hyperpnoea positive and negative males

7.1 Background

The majority of previous research investigating the effects of salbutamol use, with regard to possible performance enhancement, has used participants who are free from asthma or EIB (Meeuwisse *et al.*, 1992; Van Baak *et al.*, 2000; Sporer *et al.*, 2008). As such, it is still relatively unclear what effect this medication has at offsetting the potential decrement in performance or fall in lung function, that may be experienced in individuals who exhibit with EIB, who also regularly exercise in bronchoprovocative environments. No performance enhancing effects have been shown for either short-term or chronic administration of inhaled salbutamol in healthy individuals (see study 1 from the present thesis; Dickinson *et al.*, 2014d; Koch *et al.*, 2013).

With salbutamol now the sole permitted β_2 -agonist for use during competition for the treatment of asthma symptoms it is important to investigate the effects of this medication on performance in asthmatic individuals. Whilst historically, the majority of research investigating salbutamol has used healthy male participants, more recently, there have been investigations into the effects of salbutamol in both healthy and asthmatic individuals during cycling time-trial performance (Koch *et al.*, 2013; Koch *et al.*, 2014; Koch *et al.*, 2015), finding no difference between groups in either males (Koch *et al.*, 2013) or females (Koch *et al.*, 2014) following 400 μg salbutamol, and males following 1600 μg salbutamol (Koch *et al.*, 2015)

Dickinson *et al.* (2011) reported a large number of athletes presented with a positive EVH challenge with no previous history of asthma or EIB. A positive EVH challenge is indicative that the individual has some form of EIB and would benefit from inhaler therapy (e.g. salbutamol) to protect against bronchoconstriction during or post-exercise, especially in environmental conditions which could be potentially more provocative to the respiratory system (Sue-Chu *et al.*, 2012). It is hypothesized that exercise without salbutamol would be detrimental to performance and/or lung function in asthmatic athletes during performance, justifying the use of short-acting β_2 -agonists for maintaining standards of performance and for the prevention/relief of the symptoms of EIB in susceptible individuals.

Previous work investigating acute doses of salbutamol in athletes with EIB has indicated it does not improve cycling time-trial performance compared to placebo (Koch *et al.*, 2013; Koch *et al.*, 2014; Koch *et al.*, 2015). However the environments that these time-trials took place in were relatively unprovocative (Hum ~60% temp ~ 18°C). Therefore the impact of an acute dose of salbutamol on exercise performance in athletes with EIB in bronchoprovocative environments is unknown. The present study investigated the effect of using salbutamol in both healthy individuals and eucapnic voluntary hyperpnoea (EVH) positive individuals on 3 km running time-trial performance in a low humidity environment.

7.2 Methods

7.2.1 Participants

Following ethical approval from the Liverpool John Moores University research ethics committee (Ethics No. P13SPS041), 7 mild ($\Delta FEV_1 > -10\%$ $< -25\%$) EVH+ve males (age: 22.7 ± 1.9 years; weight: 71.7 ± 6.6 kg; height: 175.0 ± 6.0 cm) and 7 EVH-ve males (age: 22.1 ± 1.1 years; weight: 81.1 ± 8.1 kg; height: 184.3 ± 4.0 cm) volunteered to participate in the study providing their written informed consent. All participants were in good health, non-smokers and took part in recreational 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 were informed about the nature and the risks of the experimental procedures before their informed consent was obtained.

All participants undertook maximal flow-volume manoeuvres using a spirometer (Microlab ML3500, Cardinal Health, Basingstoke, UK). Flow-volume measures recorded from each maximal flow-volume loop were; Forced Expiratory Volume in one second (FEV_1), forced vital capacity (FVC), $FEV_1:FVC$ ratio ($FEV_1/FVC\%$), peak expiratory flow (PEF) and forced expiratory flow between 25% and 75% of FVC (FEF_{25–75}). Individual

maximal flow-volume loops were accepted in accordance with European Respiratory Society/ American thoracic society criteria (Miller *et al.*, 2005).

7.2.2 EVH Challenge

As described in general methods sections 3.3 and 3.4.

7.2.3 $\dot{V}O_{2\text{ peak}}$ Test

As described in general methods section 3.5.

7.2.4 Three km Time Trial

Once recruited participants were required to familiarise themselves with the 3 km running time-trial on the Woodway Curve non-motorised treadmill (Woodway, Wisconsin) on a minimum of two occasions. Each participant was required to perform a 3km time-trial on a further three occasions in a randomised, double blind, repeated measures design with a minimum of 7 days between trials. Participants were required to inhale (via pocket chamber) either 4 inhalations of non-active inhalant (placebo), Salbutamol (400 μ g) or control (nothing inhaled) prior to each 3km time-trial. The 3km time-trials were performed in an environmental chamber (Training with

Altitude, Sporting Edge UK, England) at 18°C and 21% O₂, 20%-25% humidity.

Following baseline spirometry, participants were administered their treatment dependent upon trial condition, 10 minutes post-inhalation spirometry was repeated, before the completion of a standardised warm-up (5 minutes on a motorized treadmill at 10 kph); subjects then began the performance time-trial on the curve treadmill (Woodway, Wisconsin). 3 km time-trials were performed as described in general methods section 3.6. Time-trial progression is shown in figure 7.1.

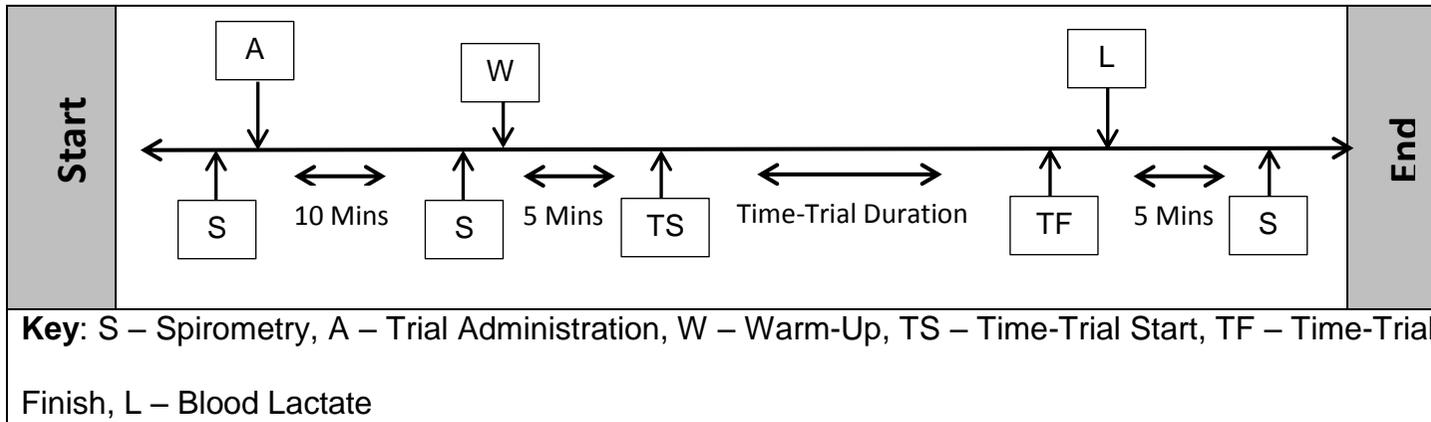


Figure 7.1: Schematic of the protocol used for each laboratory visit

7.2.5 Statistical Analysis

Two-way repeated measures analysis of variance (ANOVA) was used to compare time-trial performance between groups and trial conditions, a bonferroni correction was applied to correct for multiple comparisons. Spirometry measurements were also analysed to compare between groups, between condition and between measurements using a mixed model repeated measures ANOVA. Significance was assumed at $P < 0.05$ for all analyses. All data were reported as mean (\pm SD) unless otherwise stated. Statistical analysis was performed using the statistical package for the social sciences (SPSS v21, IBM, New York).

7.3 Results

7.3.1 Participant Characteristics

Fourteen participants (7 EVH+ve; 7 EVH-ve) successfully completed all trials, participant demographics and lung function screening values are shown in Table 7.1.

Table 7.1: Mean (\pm SD) Subject Demographics and Lung Function Values Pre- and Post-EVH Challenge for: EVH Positive Individuals (EVH+ve) and EVH Negative Individuals (EVH-ve)

Group	Height (cm)	Weight (kg)	Age (yrs)	Baseline FEV ₁	% Predicted FEV ₁	Post EVH % Δ FEV ₁
EVH+ve (n=7)	175 (6)	71.7 (6.6)	22.7 (1.9)	4.13 (0.8)	92.9 (13.1)	-14.4 (1.5)
EVH-ve (n=7)	184.3 (4)	81.1 (8.1)	22.1 (1.1)	4.94 (0.5)	102.6 (6.3)	-6.02 (0.9)

FEV₁ - Forced Expiratory Volume in 1 Second; EVH - Eucapnic Voluntary Hyperpnoea

7.3.2 Lung Function Values

Overall there was a significant difference in post-administration FEV₁ between the Salbutamol trial (4.66 ± 0.60 L) and both the Placebo trial (4.46 ± 0.60 L) and Control trial (4.44 ± 0.60 L) ($P < 0.01$) (Figure 7.2). During the Salbutamol trial there was a significant increase in FEV₁ from baseline to post-salbutamol administration (Δ FEV₁ 0.25 ± 0.07 L) ($P < 0.01$) and post time-trial (Δ FEV₁ 0.24 ± 0.12 L) ($P = 0.016$) (Figure 7.3). There was a significant difference in FEV₁ between groups at baseline with mean (\pm SD) values of 4.1 ± 0.8 L and 4.8 ± 0.4 L for the EVH+ve group and the EVH-ve group, respectively ($P = 0.032$). There was no significant difference between groups for the change in FEV₁ during the Salbutamol trial (Figure 7.3).

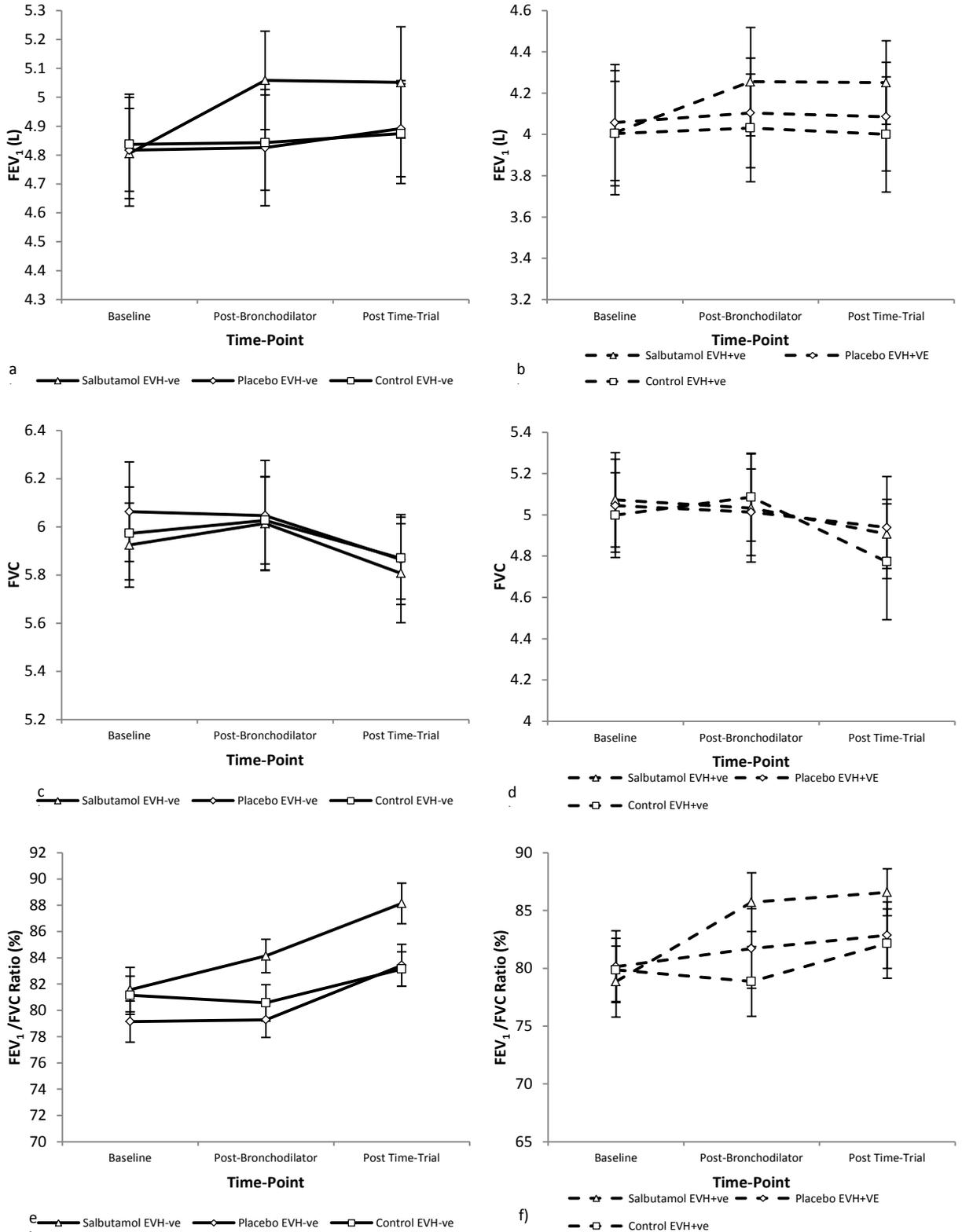


Figure 7.2: Lung Function Values for: a) EVH-ve FEV₁ values for each condition b) EVH+ve FEV₁ values for each condition c) EVH -ve FVC values d) EVH+ve FVC values e) EVH-ve ratio values f) EVH+ve ratio values

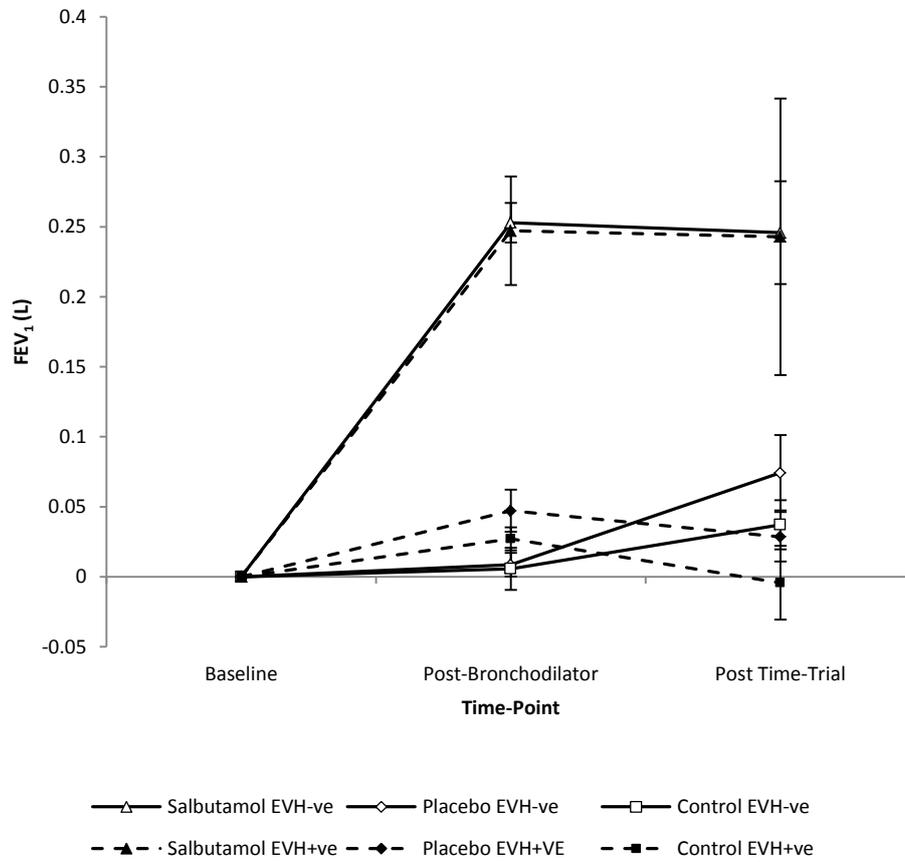
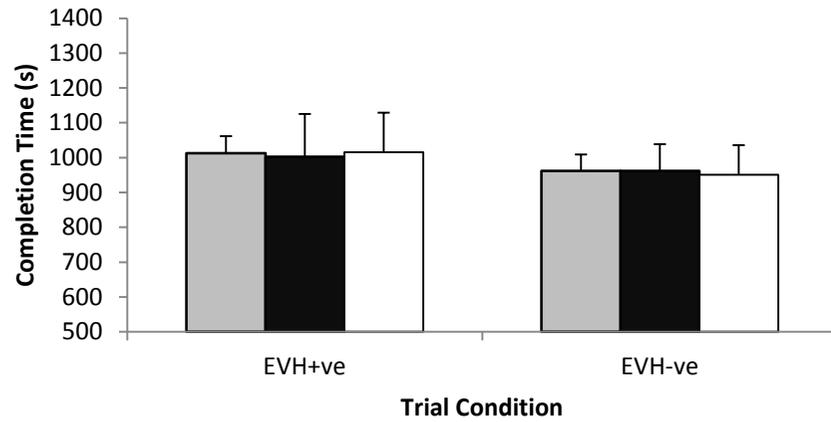


Figure 7.3: Change in FEV₁ from baseline in both groups during the three conditions

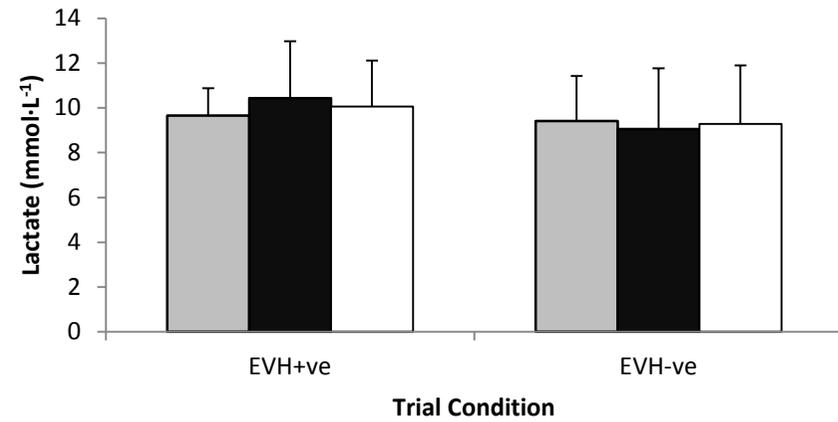
7.3.3 Performance Variables

There were no significant differences between completion times either between groups or between trials (Figure 7.4). There were no significant differences between post-exercise lactate concentrations or RPE highlighting that individual effort for each trial was the same. There were no differences between groups for minute ventilation (\dot{V}_E) and this was also not significantly higher in the Salbutamol trial despite higher values for FEV₁ post-Salbutamol inhalation. There was no difference between groups or between conditions for $\dot{V}O_2$.



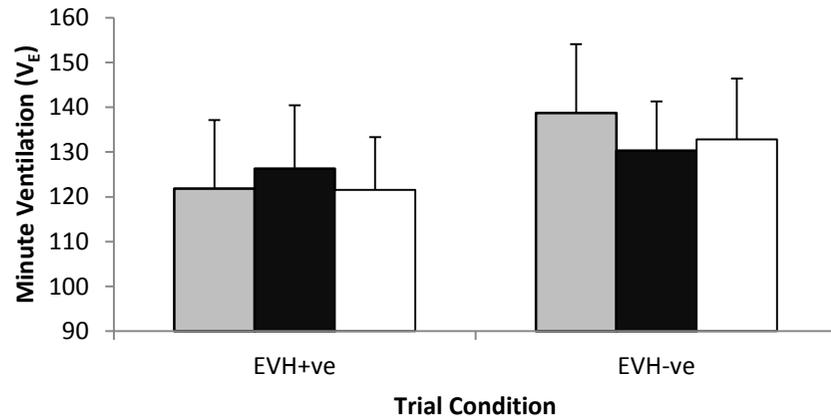
a

Salbutamol Placebo Control



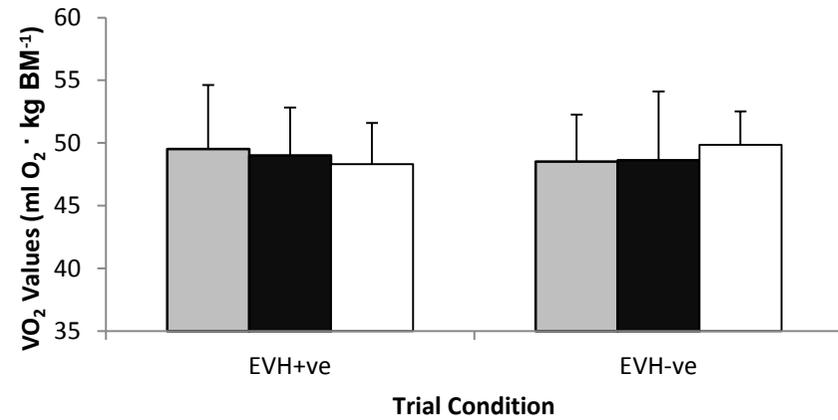
b

Salbutamol Placebo Control



c

Salbutamol Placebo Control



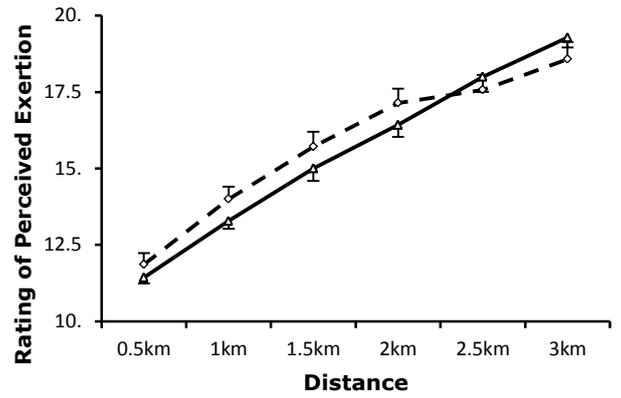
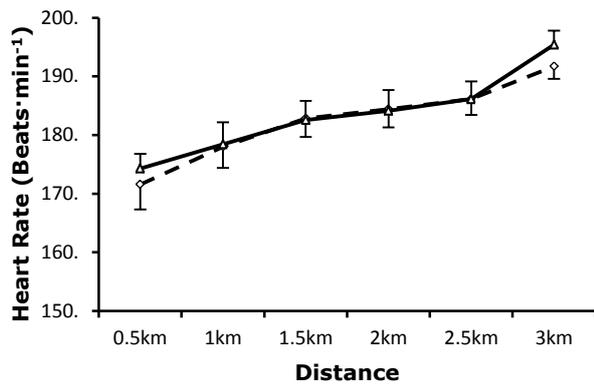
d

Salbutamol Placebo Control

Figure 7.4: Performance Variables for a) Completion Time; b) Post Time-Trial Lactate Values; c) Peak Minute Ventilation; d) Mean $\dot{V}O_2$ Values

7.3.4 Heart Rate

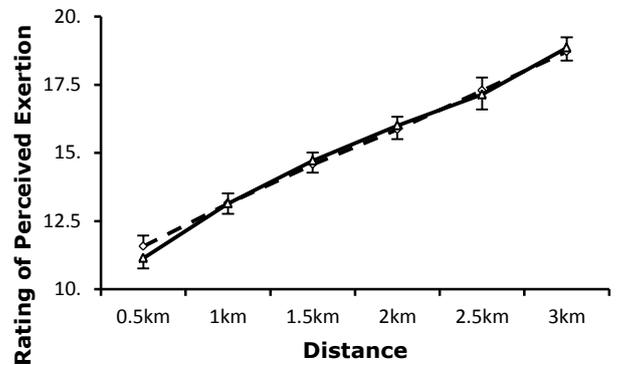
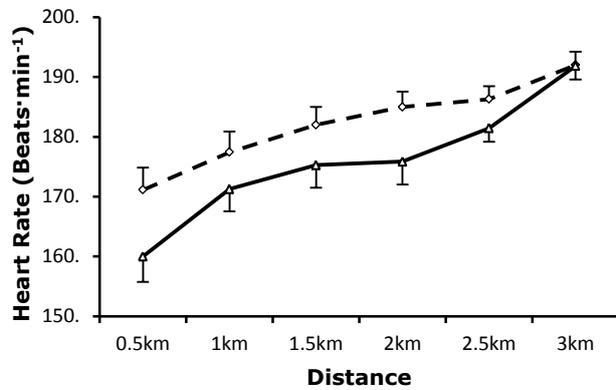
There was a significant difference in heart rate (HR) between the Salbutamol trial and the control trial, HR was higher in both groups during the Salbutamol trial at the start of exercise (171.5 ± 11.3 and 174.3 ± 6.6 bpm) compared to the control trial at the start of exercise (169.5 ± 11 and 166.3 ± 8.9 bpm) for the EVH+ve group and the EVH-ve group, respectively ($P=0.047$). There was also a significant difference in average HR throughout the trials in the Salbutamol trial (182.5 ± 8.4 and 183.5 ± 8.2 bpm) compared to the control trial (179.7 ± 9.8 and 179.5 ± 8.3 bpm) for the EVH+ve group and the EVH-ve group, respectively ($P=0.05$) as can be seen in figure 7.5. There was a significant difference in HR between groups during the placebo trial ($P<0.01$) yet there was no difference in either lactate, RPE or completion time between groups during this trial indicating that the lower heart rates did not affect performance and were not due to lesser effort in those trials.



a)

--◇-- EVH Positive
 —△— EVH Negative

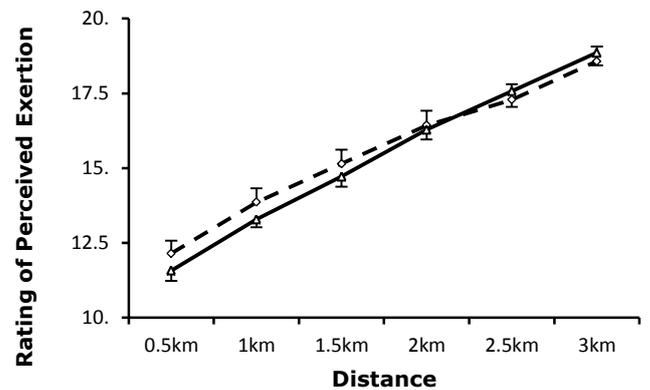
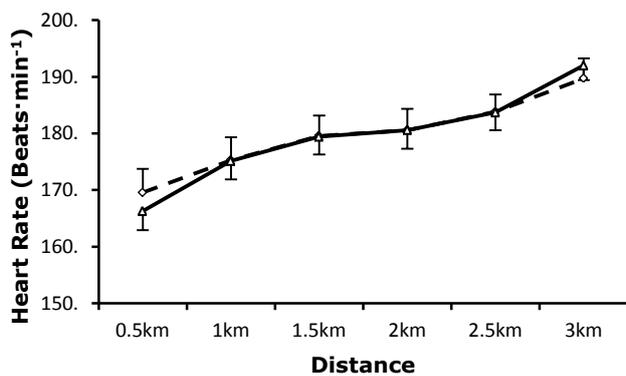
--◇-- EVH Positive
 —△— EVH Negative



b)

--◇-- EVH Positive
 —△— EVH Negative

--◇-- EVH Positive
 —△— EVH Negative



c)

--◇-- EVH Positive
 —△— EVH Negative

--◇-- EVH Positive
 —△— EVH Negative

Figure 7.5: Heart Rate and Ratings of Perceived Exertion for: a) Salbutamol Trial; b) Placebo Trial; c) Control Trial.

7.4 Discussion

The aim of the present study was to investigate the effect of salbutamol use on performance in EVH+ve and EVH-ve individuals in a bronchoprovocative environment. The study found no significant improvements in 3 km running time-trial performance following 400 µg inhaled salbutamol in mild EVH+ve individuals and also EVH-ve controls.

Despite the exercise environment being more provocative to induce AHR no significant falls in FEV₁ were seen post time-trial in the EVH+ve group, nor were there any differences in exercise performance. The results are in agreement with the findings of Koch *et al.* (2014) who examined the effects of Salbutamol inhalation on cycling performance in both asthmatic and non-asthmatic males. The results highlighted a significant increase in FEV₁ in both groups post-bronchodilator in the absence of any improvement in performance. Koch *et al.* (2014) found the same effects in female athletes where FEV₁ was significantly increased in both asthmatic and non-asthmatic individuals. Of note, their findings also highlighted that there was a significant decrease in cycling mean power output during the salbutamol time-trials in this female cohort (Koch *et al.*, 2014). The results of chapter 7 add to the findings of Koch *et al.*, (2013) by investigating the role of salbutamol in a low humidity environment, finding no differences in performance or lung function post performance in asthmatic individuals compared to healthy controls.

Lung-function values post-exercise in the EVH+ve group did not significantly fall as expected through the broncho-provocation which occurred during exercise at low humidity. This may highlight that although some individuals were mild EVH+ve they may have been negative to EIB due to the high sensitivity of the EVH challenge, a factor that is highlighted by Rundell *et al.* (2004) where 7 out of 38 (18%) athletes exhibited with mild to moderate falls in FEV₁ following EVH challenge, these falls were not apparent following an exercise challenge. Further research (Price *et al.*, 2014a) has also highlighted that participants with mild falls (-10% to -20%) following EVH challenge did not show reproducibility during follow-up EVH challenge tests. Therefore mild EVH+ve athletes may have transient AHR that does not occur following every exercise effort, alternatively the EVH challenge may be too sensitive and therefore individuals who are mild EVH+ve may not exhibit with EIB at all. The participants in the present study may also have been negative to any form of asthma due to the lack of full reversibility (>12% increase in FEV₁) to salbutamol. The post-exercise changes in lung function would have benefitted from flow-volume loops at 10 and 15 minutes post-exercise to fully elucidate this finding.

The present study found no effects on exercise performance in line with previous research (Koch *et al.*, 2013; Koch *et al.*, 2014). This is in contrast to the findings of Kalsen *et al.* (2014) and Decorte *et al.* (2013) who used higher doses of inhaled β_2 -agonists and also looked at muscle contractility

performance variables, possible reasons for the differences are due to higher systemic availability of the drug.

In addition, the hypothesis that exercising in a broncho-provocative environment (low humidity in this study) would adversely affect performance in individuals who were mild EVH+ve was also investigated. Previous studies examining the effects of exercise in broncho-provocative environments have found that dry, cold air adversely affects lung function values during high-intensity exercise (Rundell et al., 2004). It is not yet known how much of an effect this can have on performance, many elite athletes that experience EIB would be reluctant to exercise without the broncho-protective effects of β_2 -agonists. In the interests of safety the current study was only able to recruit participants with no previous history of asthma and/or AHR, who exercised regularly without the use of β_2 -agonists yet exhibited a mild positive response ($\Delta FEV_1 <10\% >25\%$) to the EVH challenge. However, given that an estimated 13% of individuals are exercising with previously unrecognized AHR/EIB, with some individuals experiencing moderate-severe falls in FEV_1 post-challenge (Molphy *et al.*, 2014), it is important to highlight the potential adverse effects of exercise in different environments to help inform these individuals of the need for the protective use of bronchodilators.

7.4.1 Conclusions

The findings of the present study highlight that there is a significant increase in resting and exercise FEV₁ with inhaled salbutamol in both healthy and mild EVH+ve individuals. However, this increase in FEV₁ does not translate to improved performance during a 3 km running time-trial. Finally, the low humidity environment (20-25%) did not induce a fall in FEV₁ in mild EVH+ve individuals.

It is possible that asthmatic athletes who are using salbutamol alongside a corticosteroid may get more beneficial effects from the short-acting β 2-agonist medication, a recent study by Spiteri *et al.* (2014) was able to show a non-significant but greater improvement in rugby fitness within a subset of EVH+ve rugby players whilst using a combination of salbutamol and corticosteroid continuously for 8 weeks. Haverkamp *et al.* (2007) were able to successfully identify improved performance and alveolar ventilation in steroid naïve asthma patients following 6 weeks ICS use, it would be useful to see the effects of similar studies conducted on athletes with EIB.

There is not sufficient evidence for either an ergogenic effect of salbutamol on performance or a decline in performance without salbutamol in asthmatic individuals, this does not mean that an athlete should avoid salbutamol use however, due to its bronchoprotective effects. The long-term effects of using

inhalers is still relatively unknown (Price *et al.*, 2014b) and further work is warranted looking into chronic use of inhaled asthma medications.

8. Synthesis of Findings

8.1 Reflection of Aims and Objectives

Currently only one short-acting β_2 -agonist (salbutamol) is available for use by athletes (WADA Prohibited List, 2015), and until recently it has been unclear whether prolonged (chronic) use has any ergogenic potential. The findings from the current thesis indicates that the WADA regulations stating a maximum of 1600 μg inhaled salbutamol per day (24 hour period) appear to be sufficient given that this dose failed to induce performance enhancement following long-term (6 weeks) use.

It has been previously suggested that terbutaline offers an alternative short-acting β_2 -agonist for athletes suffering adverse side-effects from salbutamol (Sanchez *et al.*, 2013). This thesis confirms the findings of a small number of previous studies demonstrating that terbutaline, when taken at high therapeutic doses of 2 mg and 4 mg does not have any ergogenic effect on endurance performance. Of note, the present thesis is the first to demonstrate the potential for an upper urinary concentration threshold for terbutaline following therapeutic inhaled administration which could be used to distinguish between therapeutic inhaled and oral use.

It is unknown whether the ergogenic potential of salbutamol would be greater in asthmatic athletes compared to placebo. Previous work investigating salbutamol on cycling performance in mild EVH+ve individuals has found no ergogenic effect (Koch *et al.*, 2013; Koch *et al.*, 2014; Koch *et al.*, 2015). To

date there has been no research into the ergogenic potential of salbutamol in asthmatic athletes exercising in a bronchoprovocative environment (low humidity), compared to placebo. This thesis found that in mild EVH+ve individuals there was no ergogenic potential of salbutamol during 3 km running time-trial performance at ~20-30% humidity, compared to placebo.

8.2 General Discussion

8.2.1 Do short-acting β_2 -agonists have ergogenic effects?

In Chapter 4 the impact of chronic (6 week), daily accumulated doses of 1600 μg of inhaled salbutamol on endurance, strength and power performance was examined. The results indicate there is no significant performance improvement in any marker of endurance, strength or power performance compared with placebo. This unique study is the first training study to investigate prolonged salbutamol use, at the maximum permitted therapeutic dose (1600 μg in a 24 hour period), on athletic performance.

The findings from Chapter 4 add to the current body of literature investigating the maximum therapeutic dose of inhaled salbutamol. Previous work has highlighted that inhaled salbutamol up to the WADA recommended daily limit of 1600 μg , either acutely or cumulatively, does not lead to

improvements in endurance, strength or power performance (Pluim *et al.*, 2011; Dickinson *et al.*, 2014a; Dickinson *et al.*, 2014b; Decorte *et al.*, 2008, Sporer *et al.*, 2008). Work investigating the supra-therapeutic use of inhaled salbutamol (up to 4000 µg in a single-dose; Elers *et al.*, 2012a) demonstrated no improvements in either cycling time to exhaustion or cycling oxygen kinetics at a sub-maximal work-rate equivalent to 75% of maximal exertion. In accordance with these findings the WADA guidelines, which permit athletes to inhale up to 1600 µg over a 24 hour period, are sufficient to avoid any performance improvements in non-asthmatic athletes.

The findings from Chapter 4 indicating no greater improvement in any performance variable in the salbutamol group compared to placebo are in contrast to the findings from studies investigating oral salbutamol. In a meta-analysis of the effects of β_2 -agonists Pluim *et al.* (2011) concluded that the performance enhancing effects of an acute dose of oral salbutamol showed weak evidence to suggest an improvement in anaerobic capacity and strength. Le Panse *et al.* (2007) demonstrated an increased cycling peak power following an acute dose of 4 mg oral salbutamol and earlier work by the same group (Le Panse *et al.*, 2006) reported that the chronic ingestion of supra-therapeutic oral salbutamol (12 mg.day⁻¹ for 4 weeks) resulted in significantly increased peak power and decreased time to peak power. Further support for the ergogenic effect of oral salbutamol was provided in a recent study by Sanchez *et al.* (2012) who examined the impact of oral salbutamol on maximal sprint cycling power from either a single, acute dose (6 mg) or a daily dose (12 mg.day⁻¹) for three weeks. They reported that oral

salbutamol resulted in significantly improved maximal power with the one-off dose resulting in greater gains than three weeks daily intake (14% vs. 8%). Of note, the authors concluded that an acute oral dose led to greater gains and that long-term use of oral salbutamol may lead to a down regulation of muscle β_2 -adrenoreceptors leading to a dampening of the effect of salbutamol on strength gains. The potential down regulation of muscle β_2 -adrenoreceptors from daily doses of salbutamol may offer an explanation for the reduction in 1 RM peak torque leg extension at $60^\circ \cdot s^{-1}$ in the SAL group compared to an improvement in the PLA group in Chapter 4 of this thesis. Overall, the findings from Chapter 4 and previous studies support the WADA upper daily limit of 1600 μg inhaled salbutamol whilst previous studies examining oral salbutamol supports the maintenance of oral salbutamol on the WADA restricted list.

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 leading to a reduced airway resistance and improvements in \dot{V}_E and exercise performance (Haverkamp *et al.*, 2007; Anderson & Kippelen., 2008). One of the proposed ergogenic mechanisms for inhaled salbutamol is a bronchodilation in non-asthmatic athletes resulting in an improved \dot{V}_E and an increased oxygen uptake during exercise. Findings in Chapter 4 provide, in part, support for this hypothesis with significant increases in \dot{V}_E during the $\dot{V}O_{2peak}$ assessment in the SAL group when compared to PLA, however; this increase in \dot{V}_E did not result in an

improved $\dot{V}O_{2\text{peak}}$. Previous research has reported similar improvements in \dot{V}_E following acute doses of up to 1600 μg of salbutamol in the absence of an improvement in 5 km running time-trial performance in endurance athletes or repeated sprint performance in football players (Dickinson *et al.*, 2014a; Dickinson *et al.*, 2014b). Furthermore, similar studies have demonstrated non-significant improvements in FEV₁ of 0.2 L following inhalation of 800 μg , which did not result in greater \dot{V}_E or improved endurance performance (Decorte *et al.*, 2008).

Previous oral salbutamol studies have demonstrated performance gains in strength and power variables (Pluim *et al.*, 2011; Sanchez *et al.*, 2012; Caruso *et al.*, 1995; Martineau *et al.*, 1992; Le Panse *et al.*, 2006; Le Panse *et al.*, 2007). In addition to strength and power Chapter 4 focused on endurance performance. This had an impact on the prescribed training programme as participants completed two strength and power sessions and two endurance sessions per week. Research supporting the efficacy of combined strength and endurance training has been presented by Hakkinen *et al.* (2003) investigating the impact of combined strength and endurance training (2 days strength, 2 days endurance per week) compared to strength training alone (2 days per week). Their findings demonstrated no differences between variables in either group. However, if the present study had focused solely on strength and power assessments the participants may have experienced greater strength gains from four days a week of strength training compared with two days per week. Future studies could employ a

greater strength and power training load allowing greater comparisons with oral salbutamol studies.

The findings of Chapter 6 demonstrate that terbutaline, when taken in therapeutic doses (2 mg and 4 mg), does not improve 3 km running time-trial performance in either males or females. Terbutaline did result in a significantly increased resting lung function in healthy males and females similar to that observed with other short-acting β_2 -agonists *i.e.* salbutamol.

Recently the ergogenic potential of inhaled terbutaline has been examined. Hostrup *et al.* (2014) observed that a supra-therapeutic inhaled dose of terbutaline allowed for an improvement in muscle strength (8.4 ± 3.0 %) during maximal voluntary contractions, as well as maximal sprint peak power (2.2 ± 0.8 %) and mean power (3.3 ± 1.0 %) during the Wingate test on a cycle ergometer. Interestingly, these power improvements did not translate to any improvements in time-trial performance. Of note, the power improvements in this study may be attributed to the increased systemic availability of the drug with peak plasma concentrations of 23.6 ± 1.1 ng·ml⁻¹. Additionally, Kalsen *et al.* (2014) investigated the effects of 15 mg inhaled terbutaline on endurance performance during a 300 kcal cycling time-trial finding no significant difference in performance compared to placebo. The findings reported in Chapter 6 are in line with Kalsen *et al.* (2014) and Hostrup *et al.* (2014) suggesting no improvement in endurance time-trial performance following terbutaline inhalation in both males and females. Of

note, Kalsen *et al.* (2014) suggested that inhaled terbutaline promotes a shift towards carbohydrate metabolism during exercise. This conclusion could support the higher lactate values observed in the female participants in the present study following 4 mg of inhaled terbutaline, despite no differences in performance or perceived exertion during the trial.

The findings of Chapter 6 and others (Kalsen *et al.*, 2014; Hostrup *et al.*, 2014; Larsson *et al.*, 1997; Sanchez *et al.*, 2013) have concluded that terbutaline administration has no significant effect on endurance performance however, it is important to note that the effects of a supra-therapeutic dose of terbutaline on increased strength and power performance has been clearly indicated (Hostrup *et al.*, 2014). There have been very few studies on terbutaline and exercise performance, according to a review by Kindermann, (2007) and supported by Pluim *et al.*, (2011) only two studies investigating terbutaline on exercise performance (Unnithan *et al.*, 1994; Larsson *et al.*, 1997) had been performed prior to 2011. Since 2011, there have been three studies investigating terbutaline on exercise performance (Sanchez *et al.*, 2013; Hostrup *et al.*, 2014; Kalsen *et al.*, 2014), none of which have investigated any ergogenic potential of a therapeutic dose. Future work should focus on the effects of therapeutic doses of inhaled terbutaline on strength and power performance.

Chapter 7 investigated the effects of either 400 µg or 800 µg inhaled salbutamol during 3 km running time-trial performance on the Woodway

curve non-motorised treadmill in a low-humidity environment, finding an increased FEV₁ with no significant improvements in time-trial performance. A recent study by Koch *et al.* (2013) examined the effects of salbutamol inhalation (400 µg) on 10 km time-trial cycling performance in both asthmatic and non-asthmatic males. The same research group (Koch *et al.*, 2014) went on to examine the effects of inhaled salbutamol (400 µg) in female athletes finding that FEV₁ was also significantly increased in both asthmatic and non-asthmatic individuals. Of note, Koch *et al.* (2014) reported a significant decrease in cycling mean power output during the salbutamol time-trials which the group explained could have been due to a possible over-stimulation of the β₂-adrenergic system impairing athletic performance, yet the exact mechanism for this was not provided.

The administration of a single acute dose of inhaled β₂-agonist does not appear to affect exercise performance in either healthy individuals or individuals with mild EIB in a low humidity environment. Recently, however, a study performed by Kalsen *et al.* (2013) examined the acute administration of multiple inhaled β₂-agonists simultaneously at the WADA maximum permitted daily limit, in healthy and airway hyper-responsive (AHR) individuals. The findings from this study demonstrated a significant increase in FEV₁ post-inhalation in both groups and significantly greater swim ergometer sprint performance and maximal voluntary contraction (MVC) with β₂-agonists, where no improvement in performance was seen in time to exhaustion during swimming. The findings that force of muscular contraction is improved during exercise is in contrast to the findings of Decorte *et al.*

(2013) who found that there was an increased time to fatigue during isokinetic dynamometry contractions of the quadriceps following salbutamol inhalation with no improvement in MVC. These differences could be explained due to the administration of multiple β_2 -agonists in the Kalsen *et al.* (2013) study which may have had a greater effect on the β_2 adrenergic receptors due to greater systemic availability of the drugs. There may have also been more potent stimulation due to structural differences between the different β_2 -agonists, leading to greater force of contraction and higher muscular fatigue resistance (Hoffman 2001).

In addition, Chapter 7 hypothesized that exercise in a broncho-provocative environment would adversely affect performance in individuals who were mild EVH+ve. Previous studies examining the effects of exercise in broncho-provocative environments have found that dry, cold air adversely affects lung function values during high-intensity exercise (Dickinson *et al.*, 2006). It is not yet known how much of an effect this can have on performance as many elite athletes that experience EIB would be reluctant to exercise without the broncho-protective effects of β_2 -agonists. In the interests of safety the current study was only able to recruit participants with no previous history of asthma and/or AHR, who exercised regularly without the use of β_2 -agonists yet exhibited a mild positive response (Δ FEV₁ >10% <25%) to an EVH challenge. However, around 13% of individuals that are exercising without any previous identification of AHR/EIB, can experience moderate to severe falls in FEV₁ post-challenge (Molphy *et al.*, 2014). Therefore, it is important to highlight the potential adverse effects of exercise in different environments

to help better inform these individuals of the need for the protective use of bronchodilators.

The findings in Chapter 7 highlight that salbutamol significantly increases FEV₁ in both healthy and mild EVH+ve individuals however, this difference has no impact upon either minute ventilation or exercise performance. This finding is in line with Koch *et al.* (2013) who reported a significant increase in FEV₁ in both groups post-bronchodilator in the absence of an improved performance. Lung-function values post-exercise in the EVH+ve group did not significantly fall as expected through the broncho-provocation which occurred during exercise at low humidity. This suggests that some EVH+ve individuals may be negative for EIB due to the highly aggressive nature of the EVH challenge. Furthermore, they may also have been negative for any form of asthma due to the lack of full reversibility (>12% increase in FEV₁) to salbutamol. The post-exercise changes in lung function would have benefitted from flow-volume loops at 10 and 15 minutes post-exercise to fully elucidate this finding.

8.2.2 Urinary concentrations of β_2 -agonists following therapeutic and supra-therapeutic use.

Dosing to the WADA recommended maximal daily levels of salbutamol produced no benefit in terms of enhanced performance; however urinalysis results demonstrate the possibility that an individual may contravene anti-doping regulations. Whilst all but one subject produced a negative test result ($<1000 \text{ ng}\cdot\text{ml}^{-1}$) there is a concern that a single subject produced a sample which was greater than the current threshold as a result of the study's dosing regimen. This positive test result highlights the large inter-individual variability for urinary thresholds and supports previous findings reporting adverse analytical findings (AAF) following the legitimate use of inhaled salbutamol (McKenzie, 2004; Schweizer *et al.*, 2004).

The difference between the WADA threshold and the WADA decision limit however, appears to be sufficient to differentiate legitimate and illegitimate use as the positive responder in Chapter 4 did not exceed the decision limit of $1200 \text{ ng}\cdot\text{ml}^{-1}$. Nevertheless, Chapter 4 does suggest the administration of the WADA upper daily limit of salbutamol may lead to a breach of the threshold and thus result in an AAF, which is supported by Dickinson *et al.*, (2014d) who found AAF's following acute doses of $1600 \mu\text{g}$ salbutamol with varying (2 – 5%) levels of dehydration. Dickinson *et al.* (2014b) also highlight the instance of an athlete presenting with an AAF following $1600 \mu\text{g}$ salbutamol prior to 5 km running time-trial performance. Further research is

clearly needed to establish the variability surrounding urinary salbutamol levels amongst individuals dosing up to and including 1600 µg in either a daily accumulated or single dose.

There is ambiguity regarding the optimal therapeutic dose of salbutamol, with guidelines that promote its use *pro re nata* (when required) with a maximum of 1600 µg over 24 h. Dickinson *et al.* (2014b) state that dosing over and above this guideline may happen either intentionally or inadvertently, indeed a case of such inadvertent misuse has been described by Chester *et al.* (2015) in a professional rugby league player, which led to a subsequent AAF. Dickinson *et al.* (2014b) indicate that athletes feeling the need to dose up to and above the 1600 µg limit are clearly experiencing uncontrolled asthma which, combined with high-dose β_2 -agonist use, may lead to desensitisation and tolerance to the medication, increasing the likelihood of unsuccessful treatment in an emergency and further overdosing in an attempt to control EIB. In line with current anti-doping practice Chapter 4 did not normalise drug concentrations for urine specific gravity. Elers *et al.* (2012a) highlight that urine samples corrected for specific gravity showed no urine samples breaching the WADA threshold of 1000 ng·ml⁻¹ following inhalation of 800 µg salbutamol. Normalising urine samples for specific gravity may be a potential doping control measure for WADA in the future.

The purpose of Chapter 5 was to examine differences in urine concentrations of terbutaline dependent upon dose (single/multiple), mode of administration (inhaled/oral), according to gender and race, within

therapeutic limits. The therapeutic use exemption (TUE) process (WADA, 2015; UEFA, 2015) permits athletes to use inhaled terbutaline (Elers *et al.*, 2012b), therefore it is important to be able to distinguish between legitimate inhaled use and prohibited oral use in athletes. Furthermore, it is important to distinguish between oral and inhaled terbutaline to allow a more informed approach to the inclusion of terbutaline on the WADA prohibited List of substances and methods.

WADA have established thresholds for the use of salbutamol and the long-acting β_2 -agonist formoterol, allowing for the legitimate inhaled use of these substances to be successfully monitored via an upper-limit and a decision limit (WADA Prohibited List 2014). Such threshold levels for the alternative long-acting β_2 -agonist salmeterol are not required as there is no oral equivalent. A threshold for terbutaline would be useful in the fight against doping in sport and also in maintaining standards of healthcare for the elite athlete as it would allow for a broader range of inhaled β_2 -agonists to be used by athletes and also allow for successful identification for the use of oral terbutaline, which, as with every orally administered β_2 -agonist, is prohibited due to the potential for ergogenic effects.

The findings of Chapter 5 indicate that there is no clear distinction between urinary concentrations of inhaled terbutaline and oral terbutaline which could be used to categorize the different routes of administration. The current status of terbutaline on the WADA List is appropriate until a clear cut-off

threshold can be established and prevent athletes from illegally using supra-therapeutic doses of inhaled/oral terbutaline to gain a competitive advantage (Kalsen *et al.*, 2014; Hostrup *et al.*, 2014). Care is warranted for athletes using terbutaline through the TUE process (Elers *et al.*, 2012b) as it is still difficult to identify between legitimate inhaled and prohibited oral use of terbutaline. Chapter 5 did identify ceiling urinary thresholds that did not exceed 1284 ng·ml⁻¹ following inhaled administration and that did not exceed 2376 ng·ml⁻¹ following oral administration. Such upper thresholds could identify possible anti-doping limits that would be useful in highlighting possible prohibited oral use that would then require further investigation through a controlled administration trial.

A study by Elers *et al.* (2012b) reports similar findings when examining the levels of terbutaline in the plasma and urine following oral and inhaled administration (Elers *et al.*, 2012b). Their study was able to find significant differences between inhaled (2mg) and oral (10mg) doses of terbutaline, however they were unable to establish any clear thresholds due to the high variability between samples. In line with Elers *et al.* (2012b) Chapter 5 was also able to highlight significant differences between dose and mode of administration, yet due to the large range in observed concentrations, the present study was unable to clearly distinguish between inhaled and oral use. With reference to the study by Elers *et al.* (2012b) it is necessary to note that following corrections for urine specific gravity, upper thresholds for urinary terbutaline concentrations were lower than 1500 ng·ml⁻¹ following 2

mg inhaled terbutaline. No differences were apparent in systemic availability of terbutaline in plasma serum following the different routes of administration (median concentration $6 \text{ ng}\cdot\text{ml}^{-1}$), yet differences were apparent in the time to peak concentration, which was delayed following oral administration.

Results from Chapter 5 highlight the high variability between urinary values following terbutaline administration, yet also highlight the potential for oral terbutaline to exceed the $1284 \text{ ng}\cdot\text{ml}^{-1}$ threshold seen following inhaled administration.

Recently Hostrup *et al.* (2014) examined an extremely high supra-therapeutic dose of 15mg inhaled terbutaline (30 x 0.5mg inhalations) which resulted in serum levels of terbutaline roughly four times higher ($23.6 \pm 1.1 \text{ ng}\cdot\text{ml}^{-1}$) than that recorded by Elers *et al.* (2012b) after a 10 mg oral dose ($\sim 6 \text{ ng}\cdot\text{ml}^{-1}$). It can be presumed that such high dose inhalations of terbutaline would be easily distinguishable from the maximal suggested therapeutic dose of 2 mg inhaled terbutaline which is permitted for athletes with a TUE. The dose required for effective protection against EIB can be as little as one inhalation (0.5 mg) of terbutaline (Simpson *et al.*, 2013), which is the dose recommended by many of the manufacturers. The present study investigated a maximum therapeutic dose (Prior *et al.*, 1982), therefore it is possible that a difference in urinary concentration could exist between 0.5 mg inhaled and 5 mg oral tablets that could allow for a threshold to be established. Future research that could distinguish a cut-off threshold based

upon the minimum dose required for a therapeutic effect (0.5 mg inhaled Terbutaline) is needed.

The results from Chapter 5 also demonstrated significant inter-individual variation in the urinary levels of terbutaline, dependent upon mode of administration, gender and race. In the female Caucasian group, the male afro-Caribbean group and also the male Asian group there were significantly higher urinary concentrations of terbutaline in the oral administration trials vs. the inhaled trials but this difference was not apparent in the male Caucasian group. Care is warranted when assessing differences between gender due to weight differences between males and females. A study by Guo *et al.*, (2010) highlighted that the plasma concentrations of the medication fluconazole were roughly 20% higher in females compared to males, which is in direct correlation to the average weight difference (~20%) between each group (Guo *et al.*, 2010). These findings were attributed to a higher volume distribution of the drug in lighter individuals. Also of note is the difference in the rate at which different substances can be metabolised, which can vary highly between individuals. Kim *et al.*, (2004) hypothesised that a person can be categorized as either an extensive metaboliser or a poor metaboliser through inter-individual variation. They went on to state that variations in the rate of metabolism can also be due to inter-ethnic differences and can be caused by genetic variations between individuals from different ethnic backgrounds (e.g. gene deletion/insertion/substitution polymorphisms).

Such variations in the metabolism of terbutaline between ethnic groups could provide a possible explanation as to why both Asian and Afro-Caribbean urinary concentrations appear lower than Caucasian values, however it cannot be discounted that variations between inhaler technique could also have been a factor that allowed for variability between results. Further research examining gender and ethnic variations in urinary concentrations of short-acting β_2 -agonist metabolites is needed in order to better inform WADA with regard to doping policy and procedure. In addition, Chapter 6 demonstrated that the highest urinary concentration of terbutaline following the inhalation of 4 mg terbutaline was 1244 ng·ml⁻¹. This finding provides further justification for the proposed upper urinary threshold discussed in Chapter 5.

8.3 Limitations of thesis

Following data collection and analysis some limitations of the present research were highlighted. Firstly, the long-term use of salbutamol at the WADA daily limit would have benefitted from an emphasis on strength and power based performance in which participants were able to train four sessions of strength and power per week instead of two. This would have led to potentially better gains in strength and power performance and the possibility of greater adaptations in either the SAL group and/or the PLA

group. Secondly, when analysing the urinary concentrations of terbutaline it would have been valuable to add a further arm to the study to examine the impact of a lower therapeutic dose of inhaled terbutaline to try to distinguish between inhaled and oral use. This may have led to lower urinary values of terbutaline in the inhaled trials which may have then identified clear cut-off thresholds to determine routes of administration.

A further limitation to chapter 5 was the absence of an investigation into the ratio of enantiomers in the urine and calculation of the metabolic ratio (parent drug/metabolite) (Roig *et al.*, 2002), which would have further evidenced any possible route of administration, gender or ethnic differences with regard to the metabolism of terbutaline and its excretion in the urine. The establishment of a correction equation which could standardise for body weight and volume distribution of the dose administered would also have been an advantage to allow for direct comparisons. Thirdly, when all subjects were performing time-trials every effort was made to highlight the importance of following the same dietary intake during the 24 hours prior to performance, however, it was not possible or feasible to fully track dietary intake, and therefore variations in time-trial performance could have been attributed to slight variations in nutritional intake.

With regard to chapter 7 the findings suggest that high-intensity exercise does not significantly affect FEV₁ in the absence of salbutamol in mild EVH+ve individuals. This finding may have been associated with: 1) The

EVH challenge may be too sensitive and could have highlighted mild positive results in individuals who do not exhibit EIB (Rundell *et al.*, 2004; Price *et al.*, 2014a); 2) The timing of spirometry 5-minutes post-exercise may have been too soon to detect changes as individuals may have still been experiencing the broncho-protective effects of exercise; 3) The low humidity of the exercise environment may have been offset by the use of the mouthpiece during online gas analysis as the humidification of the dry air may have occurred higher up the respiratory tract due to the micro-climate of the mouth-piece, thus not sufficiently provoking the lower airways; 4) The 5 minute standardised warm-up could have induced a refractory period which may have protected against the effects of intense exercise on lung function (Anderson *et al.*, 2012).

Chapter 7 would have benefitted from more clinically well-defined asthmatic individuals who were positive to the EVH challenge and also showed EVH challenge reproducibility in line with the findings of Price *et al.*, (2014a) who highlight that with regard to a mild positive EVH challenge test result, more than one EVH test is recommended to fully confirm or exclude diagnosis. It is possible, therefore, that during exercise in moderate to severe asthmatics there will be a reduction in lung function leading to a performance decrement during exercise in a low humidity environment that would be offset via salbutamol use.

8.4 Practical Applications

The findings of this thesis can be applied to current practice with a more informed approach to the WADA List of Prohibited Substances and Methods, the use of salbutamol at the WADA permitted daily limit does not appear to enhance performance following prolonged use, therefore the current guidelines appear to be sufficient. The inclusion of a TUE for the use of terbutaline would appear to be correct given the inability to distinguish between legitimate therapeutic inhaled use and prohibited supra-therapeutic or prohibited oral use. Data from this thesis supports the lack of ergogenic potential of high therapeutic dosages of terbutaline on 3 km running time-trial performance, however investigations into strength and power performance are warranted. The use of the EVH challenge to diagnose EIB in individuals who exhibit with falls lower than 20% should only be considered an accurate diagnosis alongside a comparable alternative test result in association with the findings of Price *et al.* (2014a).

8.5 Suggestions for future studies

Following on from the findings of chapter 4 a suggestion for future research would be to establish the variability surrounding urinary salbutamol levels

amongst individuals dosing up to and including 1600 µg in either a 24 hour accumulated dose or a single acute dose.

With regard to the findings of chapter 5 it would be useful that future research should also examine urine concentrations following a minimum therapeutic dose of inhaled terbutaline (0.5 mg) *versus* oral Terbutaline and also establish differences in enantiomers in the urine associated with mode of administration, the establishment of the urinary concentrations following a directly comparable dose would also be extremely useful alongside both urinary and plasma measurement of terbutaline. Further research incorporating both female Asians and female Afro-Caribbean's is required to fully elucidate any ethnic differences.

Following on from the findings of chapter 6 future research is warranted to investigate the effects of therapeutic inhaled terbutaline on strength and power performance which has recently been highlighted to be ergogenic following a suprathreshold inhaled dose of terbutaline in studies by both Kalsen *et al.* (2014) and Hostrup *et al.* (2014). This investigation will also benefit from urinary measurements of terbutaline in support of the establishment of upper urinary cut-off limits for anti-doping purposes.

Finally with the findings highlighted in chapter 7 future research should examine exercise performance in individuals who are mild-moderate

EZH+ve and also have a previous diagnosis of physician diagnosed asthma, who regularly exercise without the protective effects of inhaled salbutamol. Furthermore, symptoms experienced during exercise must also be considered when assessing for possible EIB. Spirometry should also be performed at more intervals post-exercise (up to 15 minutes post-exercise) to determine if a drop in FEV₁ may occur at a later stage in these individuals.

8.6 Conclusions

The present thesis investigated the legitimacy of the current WADA daily limits for salbutamol inhalation finding no improvement in endurance, strength and power performance following the inhalation of 1600 µg of salbutamol per day for six weeks in non-asthmatic males. This would suggest that the current WADA list of banned substances (WADA Prohibited List 2014), which allows athletes to inhale up to 1600 µg over a 24 hour period, is sufficient given the findings from this and previous studies (Pluim *et al.*, 2011; Dickinson *et al.*, 2014a; Dickinson *et al.*, 2014b; Decorte *et al.*, 2008, Sporer *et al.*, 2008).

Following on from these findings this thesis investigated the possibility of distinguishing between routes of administration of terbutaline identifying significant differences in urine concentration following inhaled and oral administration with high inter- and intra-individual variability between samples such that a clear cut-off value could not be identified. However, the

study was able to identify upper thresholds following oral use and inhaled use which could possibly be used to identify supra-therapeutic oral use of terbutaline in anti-doping tests. Gender differences were identified and occurred between male and female Caucasians during the multiple oral administration trial, and ethnic differences were identified between male Caucasians and male Asians during the single inhaled administration trial.

In addition the present thesis investigated the ergogenic impact of inhaled terbutaline. The findings highlight that terbutaline, when taken in therapeutic doses (up to 4 mg), does not improve 3 km running time-trial performance in males or females. Furthermore, urinary concentrations that were measured following 4 mg inhaled terbutaline peaked at $1244.4 \text{ ng}\cdot\text{mL}^{-1}$ in both males and females, these levels are lower than the upper threshold established in chapter 5.

Finally this thesis investigated the impact of salbutamol on lung function following 3 km running time-trials in a low humidity (RH: 20-25%) environment. The findings highlight that there is a significant increase in FEV_1 with inhaled salbutamol in both healthy and mild EVH+ve individuals however; this did not translate to improved performance during 3km running time-trials in either group. Care is warranted in the interpretation of this finding as the failure to induce a fall in FEV_1 in mild EVH+ve individuals may be due to the aggressive nature of the EVH challenge identifying positive individuals who may not necessarily exhibit with EIB.

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Appendices

Appendix A

HEALTH SCREENING QUESTIONNAIRE CARDIOVASCULAR/RESPIRATORY RISK ASSESSMENT

Personal Information

Name: _____ Gender: Male/Female
Date of Birth: ____/____/____ Height (cm): _____ Weight (kg): _____

Personal Medical History Assessment

1. Has your doctor ever said you have a heart condition? **Yes/No**
2. Have you ever been instructed to perform physical activity only recommended by a doctor? **Yes/No**
3. Have you ever had a real or suspected heart attack? **Yes/No**
4. Have you ever experience rapid heart beating or palpitations? **Yes/No**
5. Have you ever had angina or a sharp heavy pain in your chest as the result of physical activity? **Yes/no**
6. Do you lose your balance because of dizziness? **Yes/No**
7. Do you ever lose consciousness? **Yes/No**
8. Have you ever been severely breathless as a result of low/moderate exercise? **Yes/No**
9. Do you suffer from high or low blood pressure? **Yes/No**
If so which one? _____
10. Are you currently taking prescribed medication to control your blood pressure? **Yes/No**
11. Have you ever been told your cholesterol is too high? **Yes/No**
12. Are you currently taking prescribed medication to control your cholesterol? **Yes/No**
13. Do you suffer from diabetes? **Yes/No**
If yes, how is it controlled? (please tick)
Dietary Means Insulin Injection Oral Medication Uncontrolled
14. Have you to your knowledge had any adverse reaction/allergic reaction to any medication/drugs e.g. pseudoephedrine, aspirin, paracetamol, penicillin? If yes, please provide details. **Yes/No**

15. Do you suffer from asthma, or any respiratory disorders? If yes, please provide details. **Yes/No**

16. Do you have any musculo-skeletal problems that could be made worse by a change in physical activity? If yes, please provide details. **Yes/No**

17. Do you know of any other reason why you should not undertake physical activity? If yes, please provide details. **Yes/No**

Appendix B

EVI ATHLETE QUESTIONNAIRE

NAME							
SPORT							
DATE		D.O.B.		HEIGHT	cm	WEIGHT	KG

1) Have you ever suffered from asthma? Yes/No (Circle as appropriate)

2) Are you currently diagnosed asthmatic and use medication? Yes/No

3) Do you suffer from Exercised Induced Asthma and use medication? Yes/No

If you have answered **YES** to either or both of questions 2 and 3 please complete table below.

Asthma Medication Table: Example

Type of Drug	Drug Name	Dose	Dose Frequency	Year Started
Reliever	Ventolin	200 mcg	3 times a day	1998
Preventer	Formoterol	250mcg	Twice a day	1998
Other	Serenoar	150 mcg	2 times a week	1998

Type of Drug	Drug Name	Dose	Dose Frequency	Year Started
Reliever	1.			
	2.			
Preventer	1.			
	2.			
Other	1.			
	2.			

4) Have you taken any of the above in the past 72 hours? Yes/No If yes, how long ago? _____

5) Have you consumed any caffeine within the past 4 hours? Yes/No

6) Do you currently smoke? Yes/No If yes, how many cigarettes per day? _____

7) During or after training or competition do you experience any of the following?

Please tick as many as appropriate

Coughing _____ Wheezing _____ Chest Tightness _____
 Difficulty in Breathing (Dyspnoea) _____ Excess Mucus Production _____

8) During training or competition what environmental conditions seem to make your breathing worse?

Please tick as many as appropriate

Cold Climate _____ Dry Air _____ High Pollen Content _____
 High Pollution _____ Altitude _____ Other _____

9) In addition to medication do you use any other form of therapy/training to aid your breathing?

Appendix C

EVH - PROVOCATION CHALLENGE

Name: _____ Date: _____

Sex: _____ DOB: _____

Height (cm): _____ Weight (kg): _____

Current Medication: _____

Baseline Spirometry

Spirometry	1 st Flow Loop	% Predicted	2 nd Flow Loop	%Predicted	3 rd Flow Loop	% Predicted
FEV ₁ (litres)						
FVC (litres)						
FEV ₁ /FVC%						
PEF (l/min)						
FEF ₂₅₋₇₅ (l/sec)						

Best Baseline FEV₁ _____ *must be within 5% of second best FEV₁

Target Minute Ventilation (Best FEV₁x30) _____ Duration of EVH Challenge _____

Venue of EVH Challenge _____

EVH Challenge

Time	Total volume of air expired (litres)
1 min	
2 min	
3 min	
4 min	
5 min	
6 min	

Post EVH Spirometry

Post EVH	Effort 1			Effort 2			FEV ₁ fall from baseline (%)
	FEV ₁	FVC	FEV ₁ %	FEV ₁	FVC	FEV ₁ %	
3 mins							
5 mins							
7 mins							
10 mins							
15 mins							
20 mins							

Largest FEV₁ fall from baseline _____ %

2nd Largest FEV₁ fall from baseline _____ %

POST BRONCHODILATOR

Bronchodilator _____ Dose _____

Time	Effort 1 FEV ₁	Effort 2 FEV ₁	% from baseline FEV ₁
10 min			

COMMENTS:

Appendix D

PHYSIOLOGICAL SOCIETY BIOMEDICAL BASIS OF ELITE PERFORMANCE

The ergogenic impact of sustained high-dose short acting β 2-agonist use during a six week training programme in healthy individuals

J. Molphy¹, J. Dickinson¹, N. Chester¹, M. Loosemore² and G. Whyte¹

¹*Sport and Exercise Science, Liverpool John Moores University, Liverpool, Merseyside, UK* and ²*English Institute of Sport, London, UK*

There is little evidence available to demonstrate that inhaled short acting β 2-agonists provide an ergogenic effect. However, the majority of research in this area has focused on acute doses of inhaled β 2-agonists. At present there are no investigations examining the chronic use of short acting β 2-agonist use during training. Ten healthy well-trained males (mean \pm SD; age 20.4 \pm 2.1 years; height 178.1 \pm 8.8 cm; weight 71.2 \pm 11.3 kg) who had no history of asthma and presented with a negative indirect airway challenge, volunteered to participate in the study. Athletes were randomly assigned to one of two groups in a randomised double blind design; either placebo or 1600 μ g salbutamol (400 μ g (4x100 μ g inhalations) at 08:00h, 12:00h, 16:00h and 20:00h every day for 6 weeks). Baseline tests consisted of a VO₂ peak assessment and a 3km time-trial. Strength assessments consisted of isokinetic dynamometry assessment for peak torque during maximal knee extension and flexion at slow (60°s⁻¹) and fast (240°s⁻¹) contracting speeds, alongside one repetition maximum (1RM) lifts for bench press and leg press, power was assessed via a vertical jump test. Subjects then underwent a 6 week training programme, which consisted of two resistance sessions and two endurance sessions per week, whilst inhaling either 1600 μ g salbutamol per day or placebo.

Follow-up assessments for 3 km time-trial, 1RM bench and leg press, vertical jump heights, VO₂ peak and isokinetic dynamometry were undertaken following 6 weeks of training. Mixed-model repeated measures ANOVA were used to compare baseline and 6 week assessments of endurance, strength and power between the salbutamol and placebo groups. There was a significant decrease in 3km completion time post training programme (983.5 \pm 183.8 vs. 945.6 \pm 186 s, p=0.05) with no difference between groups (salbutamol mean change 23.4 \pm 16.5 vs. placebo 52.5 \pm 37.1 s, p>0.05). There was no significant effect of the training programme on maximal isokinetic strength or jump height (p>0.05), nor was there a difference between groups (p>0.05). There was a significant increase in VO₂ peak post-training (52.5 \pm 5.4 vs. 57.7 \pm 6.6 ml.kg.min⁻¹, p=0.01) with no difference between groups (p>0.05). There was a significant increase in 1RM leg strength post-training (218.3 \pm 45.5 vs. 272.8 \pm 48.9 kg, p<0.01) with a significant difference between groups (salbutamol mean change 35 \pm 24.7 vs. placebo 78.3 \pm 55.3 kg, p=0.04). In conclusion there were significant improvements in performance variables post-training, however these improvements were equal in both groups with no additive effect of inhaled salbutamol on any of the performance or physiological variables. The WADA guidelines that permit up to 1600 μ g inhaled salbutamol are appropriate as there appears to be no significant performance enhancing effect of taking this dose on a daily basis.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

Appendix E

Abstract for ACSM 2013

The Ergogenic Effect of Chronic High Dose Salbutamol

Molphy, J., Dickinson, J., Chester, N., Loosemoore, M., and Whyte, G., FACSM.

Liverpool John Moores University, Liverpool, UK, English Institute of Sport, Sheffield, UK,
University of Kent, Chatham Maritime, UK

There is limited evidence to suggest the acute inhalation of short acting β_2 -agonist have an ergogenic effect. To date, no study has examined the ergogenic impact following the chronic use of inhaled Salbutamol at the WADA upper daily limit (1600 μg). **PURPOSE:** To determine the effect of the WADA upper limit of 1600 μg per day Salbutamol every day for six weeks, on endurance, strength, power and body composition. **METHODS:** Sixteen trained male athletes provided written consent and agreed to take part in the study (mean \pm SD: age 20.1 ± 1.6 years; height 179.9 ± 8.2 cm; weight 74.6 ± 9.1 kg). Participants entered into a 6-week, 4 times per week training study having been assigned to one of two groups in a double blind design. Group 1 (n=8) inhaled 4 x100 μg of placebo, via pocket chamber, 4 times per day for 6 weeks (PLA). Group 2 (n=8) inhaled 4 x100 μg of Salbutamol, via pocket chamber, 4 times per day for 6 weeks (SAL). Pre- and post-training endurance, body composition, power and strength was assessed. **RESULTS:** In both groups there was an overall improvement in (Pre- Post-) $\dot{V}O_{2\text{peak}}$ (51.7 ± 4.7 – 56.8 ± 7.1 ml.min.kg⁻¹; 53.1 ± 6.1 – 55.0 ± 6.7 ml.min.kg⁻¹); 3 km running time-trial performance (988.6 ± 194.6 – 947.5 ± 155.5 s; 1040.4 ± 187.4 – 1004.2 ± 199.4 s); 1RM bench press (65.7 ± 15.4 – 70.3 ± 13.8 kg; 64.3 ± 14.0 – 72.5 ± 15.3 kg) and leg press (250.0 ± 76.4 – 282.5 ± 63.6 kg; 217.9 ± 54.0 – 282.8 ± 51.9 kg) between SAL and PLA, respectively. Peak extension and flexion torque, and body composition remained unchanged across the study period. Of note, no difference in any endurance; strength and power; or body composition measures were noted between SAL and PLA groups pre-, during, or post-intervention. **CONCLUSION:** There was no significant improvement in endurance, or strength and power performance following the inhalation of 1600 μg Salbutamol per day for six weeks in non-asthmatic males, compared to placebo. The current WADA recommendations, of up to 1600 μg inhaled Salbutamol per day, appear sufficient to avoid an ergogenic impact on endurance, strength and power performance. Data from this study will assist WADA in the implementation of regulations on the use of inhaled short acting β_2 -agonist and in the resolution of contested doping violations.

Appendix F

BASES ORAL PRESENTATION ABSTRACT 2013

The Ergogenic Effect of Chronic High Dose Salbutamol

Molphy, J.,¹ Dickinson, J.,² Chester, N.,¹ Loosemoore, M.,³ and Whyte, G.,
FBASES¹

1) Liverpool John Moores University, Liverpool, UK,

2) University of Kent, Chatham Maritime, UK

3) English Institute of Sport, London, UK,

1) Heart rate monitor Polar Manias heart rate monitor, Polar Electro Oy, Kempele, Finland; 2) Face mask Hans Rudolf, Kansas City, MO, USA; 3) Online gas analysis system Oxycon Pro portable gas analysis system, Jaeger, Carefusion, Kent, UK; 4) Pre-injection swab Medlock medical, Oldham, UK; 5) Safety lancet Sarstedt DS1588, Numbrecht, Germany; 6) Lactate pro Randox RX Daytona; 7) Hawksley micro haematocrit centrifuge Haemospin 1300, Lansing, Sussex; 8) Hemocue device Hb201+, Angleholm, Sweden; 9) Motorised treadmill Pulsar, H/P Cosmos, Germany; 10) Non-motorised treadmill Woodway Curve, Woodway, Wisconsin, USA; 11) Biodex System 3 Pro Isokinetic Dynamometer Biodex Medical Inc., Shirley, NY, USA; 12) CETL Gymnasium Centre for Excellence in Teaching and Learning (CETL) Building; 13) Skinfold Calipers Harpenden Skinfold Caliper, Baty International, Burgess Hill, West Sussex, UK; 14) Microlab ML3500 Spirometer Micromedical, Cardinal Health Ltd, UK.

BACKGROUND: There is limited evidence to suggest the acute inhalation of short acting β 2-agonist have an ergogenic effect. To date, no study has examined the ergogenic impact following the chronic use of inhaled Salbutamol at the WADA upper daily limit (1600 μ g).

PURPOSE: To determine the effect of the WADA upper limit of 1600 μ g per day Salbutamol every day for six weeks, on endurance, strength, power and body composition.

METHODS: Sixteen trained male athletes provided written consent and agreed to take part in the study (mean + SD: age 20.1 \pm 1.6 years; height 179.9 \pm 8.2 cm; weight 74.6 \pm 9.1 kg). Participants entered into a 6-week, 4 times per week training study having been assigned to one of two groups in a double blind design. Group 1 (n=8) inhaled 4 x100 μ g of placebo, via pocket chamber, 4 times per day for 6 weeks (PLA). Group 2 (n=8) inhaled 4 x100 μ g of Salbutamol, via pocket chamber, 4 times per day for 6 weeks (SAL). Pre- and post-training endurance, body composition, power and strength was assessed.

RESULTS: In both groups there was an overall improvement in (Pre- Post-) O₂peak (51.7 \pm 4.7 – 56.8 \pm 7.1 ml.min.kg⁻¹; 53.1 \pm 6.1 – 55.0 \pm 6.7 ml.min.kg⁻¹); 3 km running time-trial performance (988.6 \pm 194.6 – 947.5 \pm 155.5 s; 1040.4 \pm 187.4 – 1004.2 \pm 199.4 s); 1RM bench press (65.7 \pm 15.4 – 70.3 \pm 13.8 kg; 64.3 \pm 14.0 – 72.5 \pm 15.3 kg) and leg press (250.0 \pm 76.4 – 282.5 \pm 63.6 kg; 217.9 \pm 54.0 – 282.8 \pm 51.9 kg) between SAL and PLA, respectively. Peak extension and flexion torque, and body composition remained unchanged across the study period. Of note, no difference in any endurance; strength and power; or body composition measures were noted between SAL and PLA groups pre-, during, or post-intervention.

DISCUSSION: There was no significant improvement in endurance, or strength and power performance following the inhalation of 1600 μ g Salbutamol per day for six weeks in non-asthmatic males, compared to placebo.

CONCLUSION: The current WADA recommendations, of up to 1600 μ g inhaled Salbutamol per day, appear sufficient to avoid an ergogenic impact on endurance, strength and power performance. Data from this study will assist WADA in the implementation of regulations on the use of inhaled short acting β 2-agonist and in the resolution of contested doping violations.

Appendix G
ABSTRACT FOR ACSM 2014

The effect of 2 mg and 4 mg inhaled Terbutaline on 3 km running time-trial performance in males and females.

Molphy, J., Dickinson, J., Chester, N., Loosemoore, M., and Whyte, G., FACSM.

Liverpool John Moores University, Liverpool, UK, English Institute of Sport, Sheffield, UK, University of Kent, Chatham Maritime, UK

Limited research investigating the effects of inhaled Terbutaline on exercise performance has led to uncertainty regarding the inclusion of Terbutaline on the WADA List of Prohibited Substances and Methods. **PURPOSE:** Investigate the effect of therapeutic and suprathreshold doses of Terbutaline on 3 km running time-trial performance in males and females. **METHODS:** Six males (Mean \pm SD age 25 ± 1.5 years; height 178.3 ± 1.4 cm; weight 79.7 ± 6.3 kg) and six females (Mean \pm SD age 21.7 ± 3.1 years; height 162.4 ± 10.7 cm; weight 57.6 ± 6.6 kg) provided written consent and agreed to take part in the study. Participants had no history of asthma confirmed by a negative eucapnic voluntary hyperpnoea (EVH) challenge. All participants completed 3 km running time-trials under three separate conditions in a double blind randomised design; placebo, 2 mg and 4 mg inhaled Terbutaline. Measurements of time, heart rate, $\dot{V}CO_2$, $\dot{V}O_2$, respiratory exchange ratio (RER), ratings of perceived exertion (RPE) and blood lactate were taken during all trials, a 3-way mixed model analysis of variance was used to compare between groups, between conditions and between time-points, significance was set at $P < 0.05$ for all analyses. **RESULTS:** There were no significant differences in time taken to complete the 3 km time trial between conditions in either males (922.8 ± 104.7 s; 928.2 ± 118.7 s; 951.7 ± 138.5 s) or females (1289.5 ± 156.4 s; 1285.4 ± 97.8 s; 1245.3 ± 88.2 s) for placebo, 2mg inhaled and 4mg inhaled Terbutaline, respectively. Both males and females demonstrated significant increases in heart rate, $\dot{V}CO_2$, $\dot{V}O_2$ and RPE during each time trial ($p < 0.001$). Mean \pm SD increases in FEV₁ were 11 ± 117 ml; 200 ± 107 ml and 233 ± 81 ml following administration of Placebo, 2mg and 4mg inhaled Terbutaline respectively. Heart rate values were significantly higher in females than in males ($P = 0.49$) and completion times were also higher in females compared to males ($P < 0.001$). **CONCLUSION:** There was no significant improvement in 3km Time-Trial performance following the inhalation of either 2 mg or 4 mg inhaled Terbutaline. The current findings suggest that the use of inhaled Terbutaline during exercise provides no performance enhancement, however its position on the WADA list still remains unclear due to the difficulties in distinguishing between oral and inhaled use.

Appendix H

YIA ABSTRACT FOR ECSS 2015

URINARY CONCENTRATIONS OF SINGLE AND MULTIPLE ADMINISTRATION OF INHALED AND ORAL TERBUTALINE: INFLUENCE OF GENDER AND ETHNICITY

Molphy, J., Dickinson, J. W., Chester, N. J., Loosemore, M., Whyte, G.

Introduction

Elite athletes have a higher prevalence of Exercise-induced bronchoconstriction (EIB) than the general population. Treatment for asthma and EIB includes inhalation of short-acting β_2 -agonists (SABA). The World Anti-Doping Agency (WADA) has permitted the use of the SABA salbutamol for inhalation in therapeutic doses since 2010. In contrast, therapeutic doses of the inhaled SABA terbutaline still require a therapeutic use exemption. The purpose of the present study was to measure the urine concentrations of terbutaline following single and repeated doses of oral and inhaled terbutaline in Caucasian males, Caucasian females, Afro-Caribbean males and Asian males to distinguish between routes of administration and to allow for comparisons between gender and ethnicity.

Methods

Twenty-two male and eight female subjects (8 male & 8 female Caucasian, 6 male afro-Caribbean, 6 male Asian) were recruited for the study. All participants were free from asthma, EIB and AHR confirmed by no history of disease and a negative eucapnic voluntary hyperpnoea (EVH) challenge. Participants were assigned to one of four groups in a cross-over design:

1. Single dose of 5 mg oral terbutaline.
2. Single dose of 4 inhalations of 0.5 mg terbutaline totalling 2mg inhaled.
3. Repeated doses of 1 mg (2 x 0.5 mg inhalations) of terbutaline at 08:00h, 12:00h, 16:00h and 20:00h for 2 days.
4. Repeated doses of 5 mg oral terbutaline at 10:00h and 18:00h for 2 days.

Participants were required to provide urine samples at 1h, 3h, 6h and 12h time-points post-final dose.

Results

The study identified upper thresholds following inhaled ($1,500 \text{ ng}\cdot\text{ml}^{-1}$) and oral ($2,000 \text{ ng}\cdot\text{ml}^{-1}$) administration which could be used to identify the use of supra-therapeutic doses of terbutaline. Gender differences existed (406.9 ± 45.4 vs. $678.8 \pm 94.8 \text{ ng}\cdot\text{ml}^{-1}$; $P=0.018$) for male vs. female Caucasians, respectively following multiple oral administration. Ethnic differences (372.14 ± 69.7 vs. $131.8 \pm 19.7 \text{ ng}\cdot\text{ml}^{-1}$; $P=0.005$) were identified following single inhaled administration for male Caucasians and male Asians, respectively.

Discussion

All trials resulted in the presence of terbutaline in urine. Upper thresholds for urinary terbutaline following inhaled and oral administration were observed along with gender differences between male and female Caucasians, and ethnic differences between male Asians and Caucasians. These upper thresholds could be useful in establishing anti-doping limits that can distinguish between routes of administration. Further investigation is warranted in order to fully elucidate these findings. Future research should examine urine concentrations following a minimum therapeutic dose of inhaled terbutaline versus oral terbutaline to provide further distinction between routes of administration.

Appendix I

Statement of Contribution for Chapter 4

With regard to the ownership of the published work for chapter 4 of this thesis, I would like to state the following order of contributions:

1. **John Molphy** – Responsible for study design, data collection, participant recruitment, statistical analysis, interpretation of results, writing of the methods and results sections, jointly responsible for writing of the introduction and discussion sections.
2. **John Dickinson** – Responsible for securing grant funding, ethical approval, study design, participant recruitment, interpretation of results, reviewing the writing up of the methods and results sections, responsible for writing the introduction and discussion sections.
3. **Neil Chester** – Responsible for securing grant funding, ethical approval, study design, participant recruitment, proof-reading the writing up of the published paper.
4. **Mike Loosemore** – Responsible for securing grant funding, ethical approval, providing of all medications used in study, proof-reading the writing up of the published paper.
5. **Greg Whyte** – Responsible for securing grant funding, ethical approval, study design, participant recruitment, interpretation of results, proof-reading the writing up of the published paper.

I agree that the above statement with regard to the contributions of the aforementioned published authors, are correct:

Signed – John Molphy  Date25/08/15.....

Signed – Dr John Dickinson  Date03/09/15.....

Signed – Dr Neil Chester  Date27/08/15.....

Signed – Dr Mike Loosemore  Date03/09/15.....

Signed – Prof Greg Whyte  Date05/09/15.....

Appendix J

Published Material:

Dickinson, J., Molphy, J., Chester, N., Loosemore, M., Whyte, G. (2014) The ergogenic effects of long-term use of high dose salbutamol. **24(6)**:474-481.

http://journals.lww.com/cjsportsmed/Abstract/2014/11000/The_Ergogenic_Effect_of_Long_term_Use_of_High_Dose.7.aspx

Appendix K

Ventolin (Salbutamol) Manufacturer Guidelines

DATA SHEET

Ventolin® Inhaler (CFC-Free)

Salbutamol (as sulphate) Inhaler (CFC-free) 100mcg per actuation.

Qualitative and quantitative composition Ventolin Inhaler (CFC-Free) comprises a suspension of salbutamol sulphate in the non-CFC propellant HFA 134a. The suspension is contained in an aluminium alloy can, internally coated with fluoropolymer and sealed with a metering valve. Each canister is fitted with a plastic actuator incorporating an atomising nozzle and fitted with a dustcap.

Ventolin Inhaler (CFC-Free) is a pressurised metered-dose inhaler which delivers 100µg salbutamol (as sulphate) per actuation, into the mouthpiece of a specially designed actuator. The inhaler also contains the CFC-free propellant HFA134a. Each canister contains at least 200 actuations. Pharmaceutical form Pressurised metered-dose aerosol.

Clinical particulars

Therapeutic Indications Salbutamol is a selective β₂ adrenoceptor agonist indicated for the treatment or prevention of bronchospasm. It provides short acting (four hours) bronchodilation in reversible airways obstruction due to asthma, chronic bronchitis and emphysema. For patients with asthma salbutamol may be used to relieve symptoms when they occur and to prevent them prior to a known trigger. Bronchodilators should not be the only or main treatment in patients with persistent asthma. In patients with persistent asthma unresponsive to salbutamol, treatment with inhaled corticosteroids is recommended to achieve and maintain control. Failure to respond promptly or fully to such rescue medication signals a need for urgent medical advice and treatment.

2 Posology and Method of Administration

Ventolin Inhaler (CFC-Free) is administered by the oral inhaled route only, to be breathed in through the mouth. Salbutamol has a duration of action of 4 to 6 hours in most patients. Increasing use of β₂ agonists may be a sign of worsening asthma. Under these conditions a reassessment of the patient's therapy plan may be required and concomitant glucocorticosteroid therapy should be considered. In patients who find co-ordination of a pressurised metered-dose inhaler difficult a spacer device may be used with the Ventolin Inhaler (CFC-Free). Babies and young children may benefit from use of a spacer device with the Ventolin Inhaler (CFC-Free). As there may be adverse effects associated with excessive dosing, the dosage or frequency of administration should only be increased on medical advice.

Relief of acute bronchospasm:- Adults: 100 or 200µg. Children: 100µg, the dose may be increased to 200µg if required. **Prevention of allergen or exercise-induced bronchospasm:-** Adults: 200µg before challenge Children: 100µg before challenge, the dose may be increased to

200µg if required. Chronic therapy:- Adults: Up to 200µg four times daily Children: Up to 200µg four times daily

On demand use of Ventolin should not exceed four times daily. Reliance on such supplementary use or a sudden increase in dose indicates deteriorating asthma (see Special Warnings and Special Precautions for Use). Contra-indications Ventolin Inhaler (CFC-Free) is contra-indicated in patients with a history of hypersensitivity to any of its components (see List of excipients). Non-i.v. formulations of salbutamol must not be used to arrest uncomplicated premature labour or threatened abortion. Special Warnings and Special Precautions for Use 3 The management of asthma should normally follow a stepwise programme, and patient response should be monitored clinically and by lung function tests. Increasing use of short-acting inhaled β_2 agonists to control symptoms indicates deterioration of asthma control.

Under these conditions, the patient's therapy plan should be reassessed. Sudden and progressive deterioration in asthma control is potentially life-threatening and consideration should be given to starting or increasing corticosteroid therapy. In patients considered at risk, daily peak flow monitoring may be instituted. In the event of a previously effective dose of inhaled salbutamol failing to give relief for at least three hours, the patient should be advised to seek medical advice in order that any necessary additional steps may be taken. Patients' inhaler technique should be checked to make sure that aerosol actuation is synchronised with inspiration of breath for optimum delivery of the drug to the lungs. Salbutamol should be administered cautiously to patients with thyrotoxicosis. Potentially serious hypokalaemia may result from β_2 agonist therapy mainly from parenteral and nebulised administration. Particular caution is advised in acute severe asthma as this effect may be potentiated by concomitant treatment with xanthine derivatives, steroids, diuretics and by hypoxia. It is recommended that serum potassium levels are monitored in such situations.

As with other inhalation therapy, paradoxical bronchospasm may occur, resulting in an immediate increase in wheezing after dosing. This should be treated immediately with an alternative presentation or a different fast-acting inhaled bronchodilator, if immediately available. The specific salbutamol presentation should be discontinued, and if necessary a different fast-acting bronchodilator instituted for ongoing use.

Interaction with Other Medicaments and Other Forms of Interaction Salbutamol and non-selective β -blocking agents, such as propranolol, should not usually be prescribed together. Salbutamol is not contra-indicated in patients under treatment with monoamine oxidase inhibitors (MAOIs).

Pregnancy and Lactation There is no information on the effects of salbutamol on human fertility. There were no adverse effects on fertility in animals (see Preclinical safety data). Administration of medicines during pregnancy should only be considered if the expected benefit to the mother is greater than any possible risk to the foetus. 4

During worldwide marketing experience, rare cases of various congenital anomalies, including cleft palate and limb defects have been reported in the offspring of patients being treated with salbutamol. Some of the mothers were taking multiple medications during their pregnancies. Because no consistent pattern of defects can be discerned, and baseline rate for congenital anomalies is 2-3%, a relationship with salbutamol use cannot be established.

As salbutamol is probably secreted in breast milk its use in nursing mothers is not recommended unless the expected benefits outweigh any potential risk. It is not known whether salbutamol in breast milk has a harmful effect on the neonate.

Effects on ability to drive and use machines None reported.

Undesirable Effects Adverse events are listed below by system organ class and frequency. Frequencies are defined as: very common ($\geq 1/10$), common ($\geq 1/100$ and $< 1/10$), uncommon ($\geq 1/1000$ and $< 1/100$), rare ($\geq 1/10,000$ and $< 1/1000$) and very rare ($< 1/10,000$) including isolated reports.

Very common and common events were generally determined from clinical trial data. Rare and very rare events were generally determined from spontaneous data.

Immune system disorders Very rare: Hypersensitivity reactions including angioedema, urticaria, bronchospasm, hypotension and collapse.

Metabolism and nutrition disorders Rare: Hypokalaemia. Potentially serious hypokalaemia may result from beta2 agonist therapy.

Nervous system disorders Common: Tremor, headache. Very rare: Hyperactivity. Cardiac disorders Common: Tachycardia. Uncommon: Palpitations. Very rare: Cardiac arrhythmias including atrial fibrillation, supraventricular tachycardia and extrasystoles. 5

Vascular disorders Rare: Peripheral vasodilatation.

Respiratory, thoracic and mediastinal disorders Very rare: Paradoxical bronchospasm.

Gastrointestinal disorders Uncommon: Mouth and throat irritation.

Musculoskeletal and connective tissue disorders Uncommon: Muscle cramps. *Tachycardia may occur in some patients.

Overdose The most common signs and symptoms of overdose with salbutamol are transient beta agonist pharmacologically mediated events (see Special Warnings and Special Precautions for Use and Undesirable Effects). Hypokalaemia may occur following overdose with salbutamol. Serum potassium levels should be monitored. Lactic acidosis has been reported in association with high therapeutic doses as well as overdoses of short-acting beta-agonist therapy, therefore monitoring for elevated serum lactate and consequent metabolic acidosis (particularly if there is persistence or worsening of tachypnea despite resolution of other signs of bronchospasm such as wheezing) may be indicated in the setting of overdose.

Pharmacological properties

Pharmacodynamic properties Salbutamol is a selective β_2 adrenoceptor agonist. At therapeutic doses it acts on the β_2 adrenoceptors of bronchial muscle providing short acting (4 to 6 hour) bronchodilation with a fast onset (within 5 minutes) in reversible airways obstruction..

Pharmacokinetic properties Salbutamol administered intravenously has a half-life of 4 to 6 hours and is cleared partly renally and partly by metabolism to the inactive 4'-O- sulphate (phenolic sulphate) which is also excreted primarily in the urine. The faeces are a minor route

of excretion. The majority of a dose of salbutamol given 6 intravenously, orally or by inhalation is excreted within 72 hours. Salbutamol is bound to plasma proteins to the extent of 10%.

After administration by the inhaled route between 10 and 20% of the dose reaches the lower airways. The remainder is retained in the delivery system or is deposited in the oropharynx from where it is swallowed. The fraction deposited in the airways is absorbed into the pulmonary tissues and circulation but is not metabolised by the lung. On reaching the systemic circulation it becomes accessible to hepatic metabolism and is excreted, primarily in the urine, as unchanged salbutamol and as the phenolic sulphate. The swallowed portion of an inhaled dose is absorbed from the gastrointestinal tract and undergoes considerable first-pass metabolism to the phenolic sulphate. Both unchanged salbutamol and conjugate are excreted primarily in the urine.

Preclinical safety data

In common with other potent selective β_2 receptor agonists, salbutamol has been shown to be teratogenic in mice when given subcutaneously. In a reproductive study, 9.3% of foetuses were found to have cleft palate, at 2.5 mg/kg, 4 times the maximum human oral dose. In rats, treatment at the levels of 0.5, 2.32, 10.75 and 50mg/kg/day orally throughout pregnancy resulted in no significant foetal abnormalities. The only toxic effect was an increase in neonatal mortality at the highest dose level as the result of lack of maternal care. A reproductive study in rabbits revealed cranial malformations in 37% of foetuses at 50mg/kg/day, 78 times the maximum human oral dose.

In an oral fertility and general reproductive performance study in rats at doses of 2 and 50 mg/kg/day, with the exception of a reduction in number of weanlings surviving to day 21 post partum at 50 mg/kg/day, there were no adverse effects on fertility, embryofetal development, litter size, birth weight or growth rate.

HFA 134a has been shown to be non-toxic at very high vapour concentrations, far in excess of those likely to be experienced by patients, in a wide range of animal species exposed daily for periods of two years. Pharmaceutical particulars List of excipients 1,1,1,2-tetrafluoroethane (also known as HFA 134a or norflurane).

Incompatibilities

None reported. 7

Shelf Life 24 months Special precautions for storage Replace the mouthpiece cover firmly and snap it into position. Ventolin Inhaler (CFC-Free) should be stored below 30°C. Protect from frost and direct sunlight. As with most inhaled medications in aerosol canisters, the therapeutic effect of this medication may decrease when the canister is cold. The canister should not be broken, punctured or burnt, even when apparently empty. Nature and contents of container Ventolin Inhaler (CFC-Free) comprises a suspension of salbutamol sulphate in the non-CFC propellant HFA 134a. The suspension is contained in an aluminium alloy can, sealed with a metering valve. Each canister is fitted with a plastic actuator incorporating an atomising nozzle and fitted with a dustcap. Ventolin Inhaler (CFC-Free) delivers 100µg of salbutamol (as sulphate) per actuation. Each canister contains at least 200 actuations. Instructions for Use/Handling Testing your inhaler:- Before using for the first time remove the mouthpiece

cover by gently squeezing the sides of the cover, shake the inhaler well, and release two puffs into the air to make sure that it works. If it has not been used for 5 days or more, shake it well and release two puffs into the air to make sure that it works.

Using your inhaler:- 1. Remove the mouthpiece cover by gently squeezing the sides of the cover. 2. Check inside and outside of the inhaler including the mouthpiece for the presence of loose objects. 3. Shake the inhaler well to ensure that any loose objects are removed and that the contents of the inhaler are evenly mixed. 4. Hold the inhaler upright between fingers and thumb with your thumb on the base, below the mouthpiece. 5. Breathe out as far as is comfortable and then place the mouthpiece in your mouth between your teeth and close your lips around it but do not bite it. 6. Just after starting to breathe in through your mouth press down on the top of the inhaler to release salbutamol while still breathing in steadily and deeply. 7. While holding your breath, take the inhaler from your mouth and take your finger from the top of the inhaler. Continue holding your breath for as long as is comfortable. 8. If you are to take further puffs keep the inhaler upright and wait about half a minute before repeating steps 2 to 6. 9. Replace the mouthpiece cover by firmly pushing and snapping the cap into position.

IMPORTANT:- Do not rush Stages 5, 6 and 7. It is important that you start to breathe in as slowly as possible just before operating your Inhaler. Practise in front of a mirror for the first few times. If you see 'mist' coming from the top of the inhaler or the sides of your mouth you should start again from stage 2. If your doctor has been given you different instructions for using your inhaler, please follow them carefully. Tell your doctor if you have any difficulties.

Cleaning your inhaler:- Your inhaler should be cleaned at least once a week. 1. Remove the metal canister from the plastic casing of the inhaler and remove the mouthpiece cover. 2. Rinse the actuator thoroughly under warm running water. 3. Dry the actuator THOROUGHLY inside and out. 4. Replace the metal canister and mouthpiece cover.

DO NOT PUT THE METAL CANISTER INTO WATER.

Medicines classification Prescription Only Medicine 9 Name and address GlaxoSmithKline NZ Limited Private Bag 106600 Downtown Auckland NEW ZEALAND Phone: (09) 367 2900 Facsimile: (09) 367 2506 Date of preparation Issue date: 18 June 2014 Version: 4.0 VENTOLIN® is a registered trade mark of the GlaxoSmithKline group of companies

Appendix L

Bricanyl (Terbutaline) Manufacturer Guidelines

Bricanyl Product Information RITA.000-292-506.7.0 1(8)

BRICANYL® terbutaline sulfate

PRODUCT INFORMATION

NAME OF THE MEDICINE

BRICANYL is terbutaline sulfate, 2-(tert-butylamino)-1-(3,5-dihydroxyphenyl) ethanol sulfate, a sympathomimetic bronchodilator with a degree of selective β_2 -stimulant activity on the respiratory system.

The chemical structure of terbutaline sulfate is: Molecular formula: $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$ CAS number: 23031-32-5

DESCRIPTION

BRICANYL TURBUHALER® is a breath activated multiple dose powder inhaler free from propellant, lubricant, preservative, carrier substances or other additives. BRICANYL Elixir is a 0.3 mg/mL oral solution with sorbitol, glycerol, citric acid - monohydrate, sodium hydroxide, sodium benzoate, disodium edetate, ethanol, purified water and raspberry flavour as inactive ingredients. BRICANYL Injection solution for injection contains 0.5 mg/mL of terbutaline sulfate with sodium chloride, hydrochloric acid (for pH adjustment) and water for injections as the inactive ingredients.

PHARMACOLOGY

The tertiary butyl group attached to the terminal nitrogen of the terbutaline molecule is thought to confer selective stimulation of the pulmonary β_2 -receptors and only relatively minor stimulation of cardiac β_1 receptors. The presence of the two phenolic hydroxyl groups in the meta positions confers resistance to metabolism by the enzyme catechol-o-methyl transferase. The potent bronchospasmolytic effect is rapid in onset and reaches a maximum about 30 minutes after subcutaneous injection, 1 hour after aerosol and 2 - 3 hours after oral administration. The duration of action is between 4 and 5 hours. In addition to its bronchospasmolytic effect, terbutaline has also been shown to improve Bricanyl Product Information RITA.000-292-506.7.0 2(8) mucociliary clearance. Metabolism of terbutaline sulfate which is ingested orally or swallowed following inhalation is principally by conjugation in the gastrointestinal mucosa. The drug is absorbed unchanged from the respiratory tract and is excreted mainly as such in the urine. Practically all of an administered dose of terbutaline is eliminated after 72 hours.

INDICATIONS

For relief of bronchospasm in patients with asthma or chronic obstructive pulmonary disease, and for acute prophylaxis against exercise-induced asthma or in other situations known to induce bronchospasm.

BRICANYL TURBUHALER BRICANYL TURBUHALER is intended for short-term management of bronchospasm as well as maintenance therapy.

BRICANYL Injection BRICANYL injection solution is recommended for acute use only.

CONTRAINDICATIONS Hypersensitivity to sympathomimetic amines or any other ingredient.

PRECAUTIONS Cardiovascular diseases and hyperthyroidism Caution is advised when terbutaline is administered to patients with thyrotoxicosis and to patients with hypertension, coronary artery disease, arrhythmias and tachyarrhythmia. Cardiovascular effects may be seen with sympathomimetic drugs, including BRICANYL. There is some evidence from post-marketing data and published literature of rare occurrences of myocardial ischaemia associated with beta agonists. Patients with underlying severe heart disease (eg ischaemic heart disease, arrhythmia or severe heart failure) who are receiving BRICANYL, should be warned to seek medical advice if they experience chest pain or other symptoms of worsening heart disease. Attention should be paid to assessment of symptoms such as dyspnoea and chest pain, as they may be of either respiratory or cardiac origin. Arrhythmogenic potential β 2-stimulants have an arrhythmogenic potential which must be considered for each patient when receiving treatment for bronchospasm.

Bricanyl Product Information RITA.000-292-506.7.0 3(8) Diabetes Due to the blood-glucose increasing effects of β 2-stimulants, extra blood glucose controls are initially recommended when diabetic patients are commenced on terbutaline. Sensitivity to sympathomimetic amines Some patients may be unusually sensitive to β -adrenergic stimulants. Terbutaline should be used with caution when an increased susceptibility to sympathomimetic amines can be expected for instance in other patients with hyperthyroidism not yet adequately controlled. Lack of response If the usual dose does not provide the usual relief, a non-responsive state may be developing. If a previously effective dose lasts less than usual, patients should be instructed to consult a doctor.

Hypokalaemia Potentially serious hypokalaemia may result from β 2-agonist therapy. Particular caution is recommended in acute severe asthma as the associated risk may be augmented by hypoxia. The hypokalaemic effect may be potentiated by concomitant treatments (see Interactions with other medicines). It is recommended that serum potassium levels are monitored in such situations. Lactic acidosis Lactic acidosis has been reported in association with high therapeutic doses of parenteral and nebulised short-acting β -agonist therapy, mainly in patients being treated for an acute asthma exacerbation (see Adverse effects and Overdosage sections).

In patients not adequately responding to acute therapy with BRICANYL Injection, consideration should be given to the presence of lactic acidosis as a possible contributing factor to ongoing respiratory symptoms. Acute asthma If patients with an acute attack of asthma fail to respond to a dry powder inhaler of β 2-agonist they should be advised to follow their personal asthma action plan. Failure to respond to β 2-agonists in general can be due to various reasons related to drug administration or the disease itself. Particularly in children 5 years or younger, and

exceptionally in other cases, inspiratory flow through a dry powder inhaler may not be sufficient for optimal drug delivery. If a non-response occurs, medical help should be sought while a β_2 -agonist treatment is continued. In such a situation, and if available, a nebuliser or pressurised metered dose inhaler with spacer should be used. (see also Precautions - Lack of response).

Cardionecrosis Animal studies suggest that cardionecrotic lesions may occur with high doses of some sympathomimetic amines. On this evidence, it is not possible to exclude myocardial lesions as a possible hazard resulting from long-term treatment. Bricanyl Product Information RITA.000-292-506.7.0 4(8) Use in pregnancy - Category A Although no adverse effects in pregnant women or their foetuses have been reported, care with BRICANYL, as with all other drugs, is recommended during the first 3 months of pregnancy. Use in lactation Although terbutaline is secreted into breast milk, and milk concentrations are approximately those in maternal plasma, two individual case studies indicate that the infant is likely to receive 0.2-0.7% of the maternal dose (0.4 and 0.7 $\mu\text{g}/\text{kg}/\text{day}$ respectively), depending (for example) on the time of feeding in relation to administration of the drug. In the 4 infants studied this did not result in any signs of β -adrenoceptor stimulation. Transient hypoglycaemia has been reported in newborn preterm infants after maternal β_2 -agonist treatment.

INTERACTIONS WITH OTHER MEDICINES

Care is recommended if it is proposed to administer terbutaline in concomitant therapy with other sympathomimetic amines as excess sympathetic stimulation may occur. β -adrenergic blocking drugs, including eye drops, may inhibit the bronchodilating effect of sympathomimetic bronchodilators and may increase airways resistance in asthmatic patients. Hypokalaemia may result from β_2 -agonist therapy and may be potentiated by concomitant treatment with xanthine derivatives, steroids and diuretics (see Precautions - Hypokalaemia).

ADVERSE EFFECTS

Most of the side effects are characteristic of sympathomimetic amines. The incidence and severity of particular side effects depends on the dose and rate of administration. An initial dose-titration will often reduce side effects. At recommended therapeutic doses, the frequency of side-effects is minimal. More commonly observed side effects include tremor and headache. Commonly observed side effects include nervousness, tachycardia, palpitations, tonic muscle cramps and hypokalaemia.

Bricanyl Product Information RITA.000-292-506.7.0 5(8)

Less common reactions Cardiovascular Ectopic beats Gastrointestinal Nausea, vomiting, bad taste, diarrhoea General Sweating Musculoskeletal Muscle twitching, cramps Nervous system Drowsiness, dizziness, sleep disturbance, behavioural disturbances (such as agitation, hyperactivity, restlessness) Dermatological Rash, urticaria, exanthema Rare cases of lactic acidosis have been reported with high therapeutic doses of Bricanyl injection. Serious or life threatening reactions Cardiac arrhythmias (eg atrial fibrillation, supraventricular tachycardia and extrasystoles) and myocardial ischaemia have been rarely reported. Overdose of terbutaline preparations may produce significant tachycardia, arrhythmia and hypotension (see Overdosage).

In rare cases, through unknown mechanisms, drugs for inhalation may cause bronchospasm.

DOSAGE AND ADMINISTRATION

Inhaled bronchodilators should be used as required rather than regularly. Dosage should be individualised. If long-term use of terbutaline is proposed, particularly if the patient is asked to take terbutaline in conjunction with other medications, objective pulmonary function testing (for example, by peak flow meter or spirometer) may be useful as part of assessment of the efficacy or treatment.

Adults and children over 12 years

Oral - BRICANYL Elixir (0.3 mg/mL terbutaline) 10 to 15 mL up to 3 times daily.

Inhalational - BRICANYL TURBUHALER (1 inhalation = 500 µg terbutaline) 1 inhalation as required up to every 4 to 6 hours. In severe cases the single dose may be increased to 3 inhalations. The total daily dose should not exceed 12 inhalations per 24 hours.

Parenteral - BRICANYL Injection (0.5 mg/mL terbutaline) subcutaneous 0.5 mL. Repeat as required up to every 6 hours. Bricanyl Product Information RITA.000-292-506.7.0 6(8)

Paediatric

Oral - BRICANYL Elixir (0.3 mg/mL terbutaline) 0.075 mg (0.25 mL)/kg/dose. Repeat as required up to every 6 hours.

Inhalational - BRICANYL TURBUHALER (1 inhalation = 500 µg terbutaline) 1 inhalation as required up to every 4 to 6 hours. In severe cases the single dose may be increased to 2 inhalations. The total daily dose should not exceed 8 inhalations per 24 hours. Use in children Dosage schedules for children for oral formulations of terbutaline should be prescribed on a mg/kg basis. The larger safety margins with the dry powder formulation permit a less specific dosage schedule. Oral administration is indicated in children who are unable to inhale satisfactorily via a metered dose inhaler and who do not have access to a compressor/nebuliser unit. BRICANYL TURBUHALER is suitable for use by children since it is breath activated and does not require co ordination of dose release and inhalation as with use of aerosol inhalers. Impaired hepatic function Hepatic failure has not been shown to influence the metabolism of terbutaline. However, caution should be exercised in patients with impaired liver function. Impaired renal function As terbutaline sulfate is largely excreted in urine, caution should be exercised in patients with renal impairment.

OVERDOSAGE For information on the management of overdose, contact the Poison Information Centre on 131126 (Australia). There is a potential for progressive accumulation of dry powder in the mouthpiece of the BRICANYL TURBUHALER that could be released if dropped (for example, from a table) towards the end of inhaler life. To minimize unnecessary systemic exposure to terbutaline, the patients should be advised to, when possible, rinse their mouth after each use. Possible symptoms and signs Too frequent administration, as with other sympathomimetic agents, may cause nausea, headaches, changes in blood pressure, anxiety, tension, restlessness, insomnia, tremor, excitement, tonic muscle cramps, palpitations, tachycardia and cardiac arrhythmias. The symptoms and signs are those characteristic of excessive sympathetic stimulation. Bricanyl Product Information RITA.000-292-506.7.0 7(8)

Laboratory findings

Hyperglycaemia and lacticidosis (see Precautions section) sometimes occur. β_2 - agonists may cause hypokalemia as a result of redistribution of potassium. Treatment The specific antidote for accidental overdose with terbutaline sulfate is a cardioselective β -adrenergic blocking drug such as metoprolol (5-10 mg by slow intravenous injection, repeated if necessary after 5 minutes). β -blockers should be used with care because of the possibility of inducing bronchospasm in sensitive individuals.

PRESENTATION AND STORAGE CONDITIONS BRICANYL TURBUHALER: 500 μ g per inhalation, breath activated; propellant and additive free. 100 and 200 doses.

BRICANYL Elixir: 0.3 mg/mL in bottles of 300 mL BRICANYL Injection: 0.5 mg/mL of 5 x 1 mL ampoules.

Storage conditions BRICANYL TURBUHALER: Store below 30°C. Replace cap firmly after use. BRICANYL Elixir: Store below 30°C. BRICANYL Injection: Store below 25°C. Protect from light. Solutions containing terbutaline are sensitive to excessive heat and light. Solutions should not be used if discoloured.

NAME AND ADDRESS OF THE SPONSOR AstraZeneca Pty Ltd ABN 54 009 682 311 Alma Road NORTH RYDE NSW 2113 POISON SCHEDULE OF THE MEDICINE S3 - Pharmacist Only Medicine BRICANYL TURBUHALER S4 - Prescription Medicine BRICANYL elixir and injection Bricanyl Product Information RITA.000-292-506.7.0 8(8) DATE OF FIRST INCLUSION IN THE AUSTRALIAN REGISTER OF THERAPEUTIC GOODS (THE ARTG) 11th July 1991 DATE OF MOST RECENT AMENDMENT 11th November 2013 Bricanyl and Turbuhaler are registered trade marks of the AstraZeneca group of companies. © AstraZeneca Pty Ltd 2013