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EXERCISE INDUCED BRONCHOCONSTRICTION:

A COMPARISION OF TWO PROVOCATION TESTS FOR THE SCREENING OF ATHLETES.

A thesis submitted to the University of Gloucestershire for the degree of

MSc by Research in the Faculty of Sports Science

By

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ABSTRACT

BACKGROUND*:* Exercise induced bronchoconstriction (EIB) affects a large number of athletes, with rates within literature between 7% to 50% for elite athletes (Dickinson *et al.*, 2006; Falvey *et al.*, 2010). Two provocation methods for the diagnosis of EIB include an exercise challenge test (ECT) and a eucapnic voluntary hyperpnoea (EVH) test. Previous research has compared the airway response to ECT to EVH but has often failed to ensure that the ECT is conducted according to standardised guidelines (ATS., 1999; Trumper *et al.*, 2009; Stickland *et al.*, 2010). **PURPOSE:** Therefore, the aim of this study was to compare the airway responses to ECT and EVH following standardised guidelines. **METHODS:** In a randomised order and on separate days, seventeen participants completed an ECT and an EVH test. Participants were all University level athletes or professional rugby league player (Age 25 ± 2 yr.; height 1.81 ± 0.06 m; mass 85.4 ± 13.7 kg) recruited via open enquiry to the study. The ECT procedure followed the American Thoracic Society (ATS) protocol, whilst the EVH test followed the procedures recommended by Anderson *et al.* (2001). Spirometry was performed prior to and at 0, 3, 5, 10, 15, 20, and 30 min post challenge. A positive EIB diagnosis was regarded as a ≥10% decrease in forced expired ventilation over one second (FEV_1). **RESULTS:** Nine participants experienced a fall in FEV_1 ≥10%, with five having falls ≥10% in both provocation tests. Two participants experienced falls in the ECT alone and EVH alone respectively. Out of the nine participants, only two had a previous history of asthma. There was no significant difference in the peak ΔFEV_1 between the two provocation tests (p=0.143). **CONCLUSION:** In summation, athletes should be tested for EIB, with both the ECT and EVH being acceptable methods, although our results are inconclusive due to poor agreement and limitations. Future research should aim to have a greater number of participants.

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Gloucestershire and is original except where indicated by specific reference in the text. No part of the thesis has been submitted as part of any other academic award. The thesis has not been presented to any other education institution in the United Kingdom or overseas.

Any views expressed in the thesis are those of the authors and in no way represent those of the University.

Signed: Date: June 2015

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LIST OF SYMBOLS AND ABBREVIATIONS

≥	Greater Than or Equal To
Δ	Delta or Difference
˚C	Temperature Celsius
µ	Micro
ATS	American Thoracic Society
BUSC	British Universities and College Sport
ECT	Exercise Challenge Test
EIA	Exercise Induced Asthma
EIB	Exercise Induced Bronchoconstriction
ERS	European Respiratory Society
EVH	Eucapnic Voluntary Hyperpnoea
FCεRI	Immunoglobulin Receptors
FEF_{25-75}	Forced Expiratory Flow at 25 to 75%
FEV_1	Forced Expiratory Volume in One Second
FVC	Forced Vital Capacity
GxT	Gas Exchange Threshold
hrs	Hours
HR	Heart Rate
HR_{max}	Max Heart Rate
IgE	Immunoglobulin E
IL -	Interleukin 4, 5, 9, 13, 17A
IMC-OC	International Olympic Committee Medical Commission
kg	Kilograms
$[La^+]$	Lactate Concentration
$L \cdot min^{-1}$	Litres per Minute
LECT	Laboratory Exercise Challenge Test

m	Meters
min	Minutes
MVV	Maximum Voluntary Ventilation
PEF	Peak Expiratory Flow
PGE2	Prostaglandin E 2
RH	Relative Humidity
s	Seconds
SSECT	Sports Specific Exercise Challenge Test
T_H	T Helper Cells Type 2, 9, 17
WADA	World Anti-Doping Agency
$\dot{V}CO_2$	Volume of Carbon Dioxide Expired
$\dot{V}_E$	Minute Ventilation
$\dot{V}_{Emax}$	Maximum Minute Ventilation
$\dot{V}O_2$	Volume of Oxygen Consumed
$\dot{V}O_{2max}$	Maximum Oxygen Consumption
$\dot{V}O_{2peak}$	Peak Oxygen Consumption
yr	Years

CHAPTER ONE

INTRODUCTION

Asthma is regarded as a highly prevalent chronic inflammatory disease, characterised by intermittent airway obstruction, causing breathlessness, wheezing and coughing. (Curran *et al.*, 2010). Asthma is a common diagnosis in both adults and children, affecting 300 million people worldwide; with 5.4 million in the United Kingdom. Out of those affected, 4.3 million are adults and 1.1 million are children (Kudo *et al.*, 2013; UK, 2015). The mechanisms of asthma are triggered via an external stimulus, such as foreign bodies within inspired air, dry environmental conditions and exercise, stimulating T helper type 2 release cells (T_H2) (Kudo *et al.*, 2013). The release of T_H2 causes an immediate responsiveness, mediated via immunoglobulin E (IgE), which gets captured via eosinophils and mast cells within the airway, causing the release of inflammatory molecules and subsequently, airway obstruction (Barrios *et al.*, 2006).

The term exercise-induced asthma (EIA) describes the transient narrowing of the airways seen after the stimulus of exercise in those with clinically diagnosed asthma, causing abnormal coughing, wheezing, chest tightness and/or dyspnoea (ATS., 1999; Dryden *et al.*, 2010). Symptoms of EIA can also occur after exercise in individuals without a clinical diagnosis of asthma, and in these circumstances the term exercise-induced bronchoconstriction (EIB) is used (Holzer & Brukner, 2004; Dryden *et al.*, 2010). The term EIB will be used within this study.

The two hypothesised theories that cause these responses are the osmotic and the thermal hypotheses (Anderson & Daviskas, 2000; Rundell & Jenkinson, 2002; Stoloff *et al.*, 2011). The osmotic hypothesis considers that during exercise, inspired air gets conditioned within the upper airway. This conditioning process evaporates airway surface liquid, causing dehydration as osmosis draws water from the nearby epithelial cells, in turn, shifting the condition process into the smaller, lower airways, causing

airway constriction (Anderson & Daviskas, 2000; Anderson & Kippelen, 2012). The thermal hypothesis states that airway cooling during exercise followed by rapid rewarming post causes oedema within the airway walls, assisting to the constrictive response (Anderson & Daviskas, 2000).

Up to 80% of people with clinical asthma experience symptoms after exercise (Anderson & Holzer, 2000). It is also frequently reported within the elite athlete population; with 25% of European athletes at the Beijing Olympic Games, and 50% of winter sport athletes experiencing symptoms of EIB (Moreira *et al.*, 2011). The high prevalence in elite athletes in sporting events such as swimming, winter and endurance sports reflect the mechanisms which onset EIB due to the high rates of ventilation during these events, as well as the environmental conditions these events are competed in.

The athletic population, specifically those of an elite standard are more prevalent to asthmatic symptoms of EIB, however, the developed mechanisms theories do not fully explain the increase in susceptibility (Anderson & Holzer, 2000; Moreira *et al.*, 2011). During training and competition, athletes are exposed to varied environmental conditions, airborne allergens and irritants which enhance the magnitude of the bronchoconstriction, coinciding to the increased prevalence in elite swimmers, winter sports and endurance athletes (Wilber *et al.*, 2000; Knopfli *et al.*, 2007). Most athletes present a performance driving mentality and increased pain threshold which affect their perception of airway constriction and its subsequent performance limitations, which although desirable within sports, may hinder athletes' perception, education and treatment of EIB (Rundell *et al.*, 2001).

To control the severity of the bronchoconstriction, athletes commonly use inhaled short action and long action β_2-agonist before exercise to reduce respiratory symptoms (Dryden *et al.*, 2010). The current World Anti-Doping Agency (WADA) guidelines for β_2-agonist allow for salbutamol, salmeterol and formoterol to be used following pre-prescribed dosage, and to be monitored to ensure dosages and within therapeutic ranges, so a therapeutic usage exemption form (TUE) is not needed (WADA, 2015).

Two physiological bronchial provocation tests used for the diagnosis of EIB are; the exercise challenge test (ECT), and the eucapnic voluntary hyperpnoea (EVH) (ATS., 1999; Anderson *et al.*, 2001; Dickinson *et al.*, 2006). The ECT, recommended by the American Thoracic Society (ATS) aims to stimulate the airways via increased ventilation during high intensity exercise at 80% to 90% heart rate max (HR_{max}), whereas the EVH, designed as a surrogate for the ECT by the American military, substitute exercise with controlled hyperventilation of a dry gas mixture at 80% maximum voluntary ventilation (MVV) (Anderson *et al.*, 2001). For both challenges, a positive diagnosis occurs when forced expiratory ventilation (FEV_1) falls by ≥10% when compared to the baseline lung function (ATS., 1999).

Both provocation tests have been used for the diagnosis of EIB in athletes and are both accepted methods by the International Olympic Committee Medical Commission (IOC-MC). Out of the two provocation tests, previous research has suggested that the EVH is a more sensitive method of diagnosis (Dickinson *et al.*, 2006), although the methods used within the research have not followed current guidelines. A review by Stickland *et al.* (2010) found that previous research failed to complete the ECT following the current ATS guidelines, with lack of control of air water content, exercise intensity and duration, and follow up spirometry. These deviations would alter the severity of the ECT, leading

to an under or over diagnosis. In this case, the sensitivity of the EVH relative to the ECT is unclear.

The review by Stickland *et al.* (2010) brings into question both the need to standardise the testing procedure, specifically the ECT, but also brings into question the previous research comparing the ECTs and EVHs sensitivity. Also, with the high prevalence of EIB in the athletic population, it is important to tests those who compete on a regular basis to ensure those who may be unaware are educated and informed, specifically in high risk sports. Therefore, the aim of the present study was to determine whether there was a significant difference between the ECT and EVH provocation tests for screening athletes when adhering to current guidelines.

CHAPTER TWO

LITERATURE REVIEW

2.1 Asthma Pathology

Asthma effects more than 300 million people all over the world, with approximately 250,000 annual deaths caused by the disease (Kudo *et al.*, 2013). Within the United Kingdom alone, 5.4 million people are affected by asthma (UK, 2015).

The precise basis of the development of airway inflammation in patients with asthma is not fully defined (Barrios *et al.*, 2006). Allergic asthma is a disease characterised by intermittent airway obstruction that causes difficulty in breathing, ultimately mediated by hyper-responsive bronchial smooth muscle, separated airway glycoproteins and inflammatory debris produced by airway goblet cells (Barrios *et al.*, 2006; Kudo *et al.*, 2013). Two principal immune mechanisms lead to airway obstruction in allergic inflammation, both dependent on the presence of a terminally differentiated subset of T helper cells, the T_H2 cell in the lung (Barrios *et al.*, 2006). The T_H2 cell secretes a highly characteristic cytokine repertoire that includes interleukin-4 (IL-4), IL-5, IL-9, and IL-13, all of which contribute to the manifestation of inflammation (Yazdanbakhsh *et al.*, 2002; Kudo *et al.*, 2013). Common inhaled allergens which stimulate T_H2 cell are house dust mites, animal dander, fungi and pollen (Kudo *et al.*, 2013).

The immediate hypersensitivity response is mediated by IgE, an antibody isotype produced by B cells activated by IL-4 (Barrios *et al.*, 2006). Circulating IgE is captured by immunoglobulin receptors (FCεRI) present on immune effector cells such as mast cells, basophils and eosinophils (Barrios *et al.*, 2006). Subsequent encounters with antigen induce FCεRI cross linking, cellular activation, degranulation of mast cells, basophils and eosinophils, and the release of a variety of toxic inflammatory molecules, specifically leukotrienes, lipid mediators of inflammation derived from arachidonic acid.

Leukotrienes are short-lived molecules existing in a variety of isoforms which act through G protein-coupled receptors. They induce airway hyper-responsiveness, eosinophilia and airway glycoprotein hyper secretion, ultimately eliciting obstruction (Barrios *et al.*, 2006).

The type 4 hypersensitivity response is mediated through T_H2 cytokines IL-4 and predominantly IL-13, as it acts directly on airway smooth muscle and the epithelium to elicit airway hyper-reactivity, enhanced glycoprotein production, and eosinophilia. IL-4 and IL-13 are signalled through the α chain of the IL-4 receptors, members of the Janus family of tyrosine kinases. Both contribute indirectly by aiding immediate hypersensitivity reactions. IL-4 and IL-9 are required for mast cell maturation, IgE recreation, and signals through α chain of the IL-4 receptors mediate eosinophil recruitment. More recent developments within the pathophysiology has found that the T_H17 and T_H9 cells also modulate asthma through the production of IL-17A, which enhances smooth muscle contractibility, and IL-17F and IL-22, which induce airway inflammation (Kudo *et al.*, 2013).

Most pathologic patterns of asthma have been derived from autopsy studies. These patterns are based on individuals who died of asthmaticus over-inflation and mucus plugs obstructing the medium-sized bronchi, small bronchi and bronchioles. In asthmatics, over-inflation occurs when the lungs fill the chest cavity during status asthmaticus and do not collapse when the pleural space is opened, even when the lungs appear normal between attacks. Mucous plugs are made of heavily viscous inspired mucus produced by both sub-mucosal gland hypertrophy and goblet cell hyperplasia which fill both bronchi and bronchioles (Kudo *et al.*, 2013). In asthmatics, mucin and goblet cells in the epithelium can be three times higher when compared to a non-asthmatic. Patients dying of status asthmaticus have a two to three fold increase in the

amount of airway smooth muscle, especially in the medium sized bronchi, with thickness increased with age, due to myofibroblast, characterised as airway remodelling. Airway remodelling has a profound effect on airway narrowing mechanics, exacerbating the chronicity and progression of the disease (Kudo *et al.*, 2013). It is postulated that the injured airway epithelium acts as a continuous stimulus for airway remodelling.

2.2 Exercise Induced Bronchoconstriction Mechanisms

EIB is described as the drying and cooling of the intra-thoracic airways and mucosa, stimulating the release of inflammatory mediators causing airway narrowing during or following exercise (Rundell *et al.*, 2002; Tecklenburg *et al.*, 2007; Anderson, 2012; Mainardi *et al.*, 2012). The fundamental mechanics of EIB are believed to be part of a multifaceted approach, explaining why both healthy and asthmatic individuals can develop EIB (Rundell *et al.*, 2002; Parsons & Mastronarde, 2005; Stoloff *et al.*, 2011). The mechanism whereby airway narrowing occurs due to the response of exercise is subdivided into two key hypotheses; the thermal and the osmotic (Anderson & Daviskas, 2000; Rundell *et al.*, 2002; Stensrud *et al.*, 2006).

The thermal hypothesis considers that EIB is initiated by the thermal effects on the airways during and post exercise, that is, airways cooling during exercise following rapid rewarming of the airways after exercise. These thermal effects are proposed to cause a reactive hyperaemia of the bronchial microvascular, and oedema of the airway wall (McFadden *et al.*, 1986; Anderson & Daviskas, 2000). Stimulation during post exercise environmental conditions may affect severity of EIB through reducing the reactive hyperaemia. McFadden *et al.* (1986) aimed to observe changes in respiratory airways whilst controlling the temperature and humidity during both exercise and recovery. Data confirmed that when the respiratory tract is rapidly rewarmed post exercise, there is a significant decrease in FEV_1 compared to a controlled temperature and gradual

rewarming, even when exercise was performed in sub-freezing temperatures, concluding that thermal aspects do play a part in constriction.

The osmotic hypothesis puts forward that the increase in ventilation during exercise and subsequent conditioning of the inspired dry air causes dehydration within the respiratory cells, initiating the contraction of the bronchial smooth muscle (Anderson & Daviskas, 2000), and because EIB can occur without significant cooling of the airways, the osmotic effects are thought to be more important than the thermal effects (Rundell *et al.*, 2002). During activities with high ventilatory flow rates in dry conditions, the conditioning of inspired air becomes more intensive, requiring the smaller airways to aid in the conditioning process. It is theorised that water loss from the airways surface causes water to move out of nearby cells in response to increases in osmolality (Anderson & Daviskas, 1997; Rundell *et al.*, 2002).

2.2.1 Airway Inflammation

Airway inflammation during EIB plays a pivotal role in airway sensitivity during the osmotic stimulus (Dryden *et al.*, 2010). Inflammation response occurs via stimulation of the mast cells and eosinophils within the respiratory tract due to increased ventilation during exercise (Hallstrand *et al.*, 2007; Anderson, 2012). Mast cells are highly developed cells derived from bone marrow, associated with structures such as blood vessels and nerves, in proximity to surfaces that interface the external environment (Metcalfe *et al.*, 1997; Anderson, 2012). When activated, mast cells and eosinophils release mediators, including histamines, leukotrienes and eicosanoids, such as cysteinyl, leukotraines and prostaglandin D_2, inducing allergic inflammation (Metcalfe *et al.*, 1997; Hallstrand *et al.*, 2007; Philip *et al.*, 2007; Mainardi *et al.*, 2012).

Two principle immune mechanisms are believed to lead to airway obstruction, both dependent on T helper cells (Marsh *et al.*, 1994; Barrios *et al.*, 2006). Immediate responsiveness is mediated by IgE. IgE antibody is associated with atopic allergies and linked with T Cell-B interaction. Stimulation between the T helper cells and B-cells, and the release of IL-4 and IL-13 result in a specific IgE response (Marsh *et al.*, 1994). These IgE responses are bound to high affinity IgE receptors on mast cells and basophils (Walley & Cookson, 1996). The input IL-4 enhances the degranulation of mast cells by increasing the saturation of IgE receptors with allergen complexes (Walley & Cookson, 1996). IL-13 is a T_H2 cytokine which binds to the α chain of IL-4 (Wills-Karp *et al.*, 1998), playing a key role in airway hyper-responsiveness. Research by Wills-Karp *et al.* (1998) found a blockade of IL-13 via soluble IL-13α2-IgEFc fusion protein 24 hr before intra-tracheal allergen challenge resulted in significant decrease in airway hyper-responsiveness, concluding that IL-13 is the primary T cell derived factor responsible for allergen-induced airway inflammation and EIB.

2.2.2 Refractoriness

Refractoriness is the time post exercise, whereby if an asthmatic develops EIB and then spontaneously recovers within about an hour, a secondary exercise stimulus results in a marked reduction in EIB in about 50% of subjects (Larsson *et al.*, 2011). Research by Larsson *et al.* (2011) found that completing one ECT achieved a fall in FEV_1 of 26.9%, which reduced to a 13.3% after a second challenge. Because refractoriness disappears if an asthmatic is treated with a nonsteroidal anti-inflammatory medication, it may be mediated by a prostaglandin, such as prostaglandin E2 (PGE2). PGE2 is a known bronchodilator produced in the lungs (Suman *et al.*, 2000). Suman *et al.* (2000) study on PGE2 effects on guinea pigs found that prolonged hyperventilation caused a slow release of PGE2, due to airway cooling, drying or stretching, but could not fully conclude that PGE2 involvement caused refractoriness.

2.3 Affected Populations

EIB has become more common place in athletes competing at an Olympic level, with 607 athletes at the Sydney Olympics notifying the medical commission of the use of a β_2-agonist, a notable increase above the 383 athletes seen at the 1996 Atlanta games (Holzer *et al.*, 2002). However, a recent study at the Beijing Olympics reported that one in four European athletes experienced symptoms associated with EIB (Carlsen *et al.*, 2008). Overall, high performing athletes are at a higher risk of EIB compared to the general population (Knopfli *et al.*, 2007). Although EIB can affect any athlete, with up to 50% of athletes suffering from EIB (Wilber *et al.*, 2000; Holzer *et al.*, 2002; Parsons & Mastronarde, 2005), it is more prevalent in certain sports, specifically: endurance sports, swimming and winter sports, or sports that induce a high ventilatory component (Rundell *et al.*, 2002; Moreira *et al.*, 2011).

2.3.1 Winter Sports

Larsson *et al.* (1993) investigated elite cross country skiers found that due to the cold and dry environmental conditions experienced during both training and competition, asthmatic symptoms occurred in 55% of elite skiers. Similar research by Rundell *et al.* (2001) found that in a wide range of elite cold-weather athletes, EIB was present with 26% of subjects being classified with the condition.

Wilber *et al.* (2000) conducted a case study on the US winter Olympic team and aimed to determine whether differences occurred within winter sports, even those performed in similar environmental conditions, could affect possible EIB rates. 23% of the 170 subjects from 7 sports had incidences of EIB post testing. Although environmental conditions were similar, certain sports had greater incidences of EIB. 50% of cross country skiers were diagnosed with EIB, coinciding with previous research by Larsson *et al.* (1993). 6 out of

7 sports did show incidences of EIB, with biathlon being the exception. Wilber *et al.* (2000) concluded that due to an unseasonably warm day during competition (+11°C), the stimulus of cold weather was reduced. The similarity between biathlon and cross-country skiing should procure a different result when performed in a cold, dry condition. Dickinson *et al.* (2006) also researched the prevalence within winter sports, specifically British short track speed skaters and biathletes using the ECT, EVH and sports specific tests (SST). Out of the 14 athletes tested, 10 achieved peak falls in FEV_1 ≥10% of baseline, but results were dependent on the screening method used. The EVH had the highest percentage of falls ≥10%, with 10 out of the 14. For the ECT, none of the participants had falls ≥10%, and the SST had 3.

2.3.2 Summer and All Year Round Sports

Weiler *et al.* (1998) worked alongside the US summer Olympic team addressing EIB incidences in multiple sports for athletes who competed in the 1996 Atlanta Olympics. Out of the 699 athletes who took part in the study, 17% were diagnosed with having EIB. With high ventilatory rates seen in track, road and off road cycling, these sports had the greatest asthmatic response with 50% of athletes being diagnosed.

Research by Schoene *et al.* (1997) on US elite track and field athletes found that after their events, long distance athletes, such as 10km runners and 20km walkers had diagnosed cases of EIB, due to their larger vital capacities and high ventilation rates during events. Similarly, Helenius *et al.* (1997) research with 213 elite track and field athletes, found that event type relates to the amount of athletes who get diagnoses with asthma. In their results, like Schoene *et al.* (1997), long distance runners were much more susceptible to EIB symptoms compared to sprinters, field eventers and sedentary individuals, due to short events do not stimulate the ventilation system for a sufficient amount of time to cause the thermal or osmotic changes.

Studies in all year round sports like football have found varying results into the severity of EIB above the general population. Ventura *et al.* (2009) investigated young football players who play as part of a major team via self-reported questionnaire. These athletes had a slightly greater percentage of EIB than that seen in recreational players, but not enough to be statistically significant (5.1% = Players, 2.9% = Recreational). Although the study found no difference, the design was aimed more to allergies with airborne particles rather than a pre-existing asthmatic condition. Research by Rupp *et al.* (1992) analysed a range of middle and high school athletes with possible unrecognised EIB found that out of the 230 tested, 66 were diagnosed with EIB. Although participants were not selected on an asthmatic basis, those with a medical history criteria, or risk factors indicating EIB, such as abnormal spirometry results were chosen.

2.3.3 Athletes at High Risk

There is an overwhelming body of evidence which shows that athletes, specifically those of a high standard are at an increased risk of asthmatic symptoms and EIB (Larsson *et al.*, 1993; Wilber *et al.*, 2000; Knopfli *et al.*, 2007; Moreira *et al.*, 2011). However, the reasoning and mechanisms for this remain unclear, with possible theories relating to; increased exposure to environmental factors, genetic pre-disposition and physiological/psychological variations (Moreira *et al.*, 2011). An elite athlete goes above and beyond, both physiologically and psychologically, when training and competing, which can affect the diagnosis of asthma and EIB (Rundell *et al.*, 2002). Psychologically, elite athletes become more performance driven, and look upon asthma interventions as both a physical and psychological battle against their training and competing (Rundell *et al.*, 2002). During training and competition, athletes may become more exposed to triggers which influence the occurrence of EIB, such as airborne allergens and irritants for example, pollen and exhaust fumes, as well as environmental conditions with extreme temperatures and differing humidities (Rundell *et al.*, 2002). Intensive training can

increase susceptibility via effecting autonomic regulation and cardiac function, thus effecting airway smooth muscle (Parsons & Mastronarde, 2005; Moreira *et al.*, 2011).

2.4 Gender Variations

Although athletes have a high prevalence of EIB when compared to the general population, little is known about the differences between the genders (Langdeau *et al.*, 2009). Prior to puberty, asthma is greater in males, with females being greater post puberty. The possible shift may be due to the start of the menstrual cycle, caused by the release of progesterone and oestrogen (Balzano *et al.*, 2001; Postma, 2007). In conjunction with Balzano *et al.* (2001) research by Stanford *et al.* (2006) with females who recreationally train found a significant differences in forced vital capacity (FVC) and FEV_1 when comparing the early follicular (day 5) and mid-luteal (day 21) parts of the menstrual cycle.

In the athletic population, research by Langdeau *et al.* (2009) into gender variations for respiratory symptoms and asthma found that for exercise-induced respiratory symptoms, female athletes were significantly more susceptible when compared to males. The difference was possibly down to hormonal variations, following previous literature (Balzano *et al.*, 2001; Postma, 2007), although no data was collected within the area and so this is merely speculation (Langdeau *et al.*, 2009).

2.5 Prevention and Medication

There are multiple options to prevent EIB, all relating to modifying either the stimulus, or the mechanism of EIB (Anderson, 2012). To reduce water loss during exercise, conditioning the inspired air to body temperature (37°C) and 100% relative humidity (RH) prevents EIB in the majority of cases (Anderson, 2012). Stensrud *et al.* (2006) researched the effect of respiratory function at different humidity levels on EIB sufferers.

When exercise was completed at 95% humidity, significant increases were found in both respiratory functions, such as oxygen uptake ($\dot{V}O_2$), with significant reductions in FEV_1 post exercise when compared to exercise completed at 40% humidity.

As EIB is stimulated via the release of mediators, suppression of their secretion via pharmacological agents, such as β_2-agonist, which when used prior to exercise, effectively prevents asthmatics suffering from EIB (Anderson, 2012). A case study by Stoloff *et al.* (2011) on a 45 year old male who had shown symptoms related to EIB, reduced and removed those symptoms after a prescription of a β_2-agonist post lung function assessment. The supplementation of fish oils has also been evaluated as a method of reducing EIB symptoms during exercise. Mickleborough *et al.* (2006) research found that after 3 weeks of supplementation, lung function reduced below diagnosable levels against both a control and a placebo group, creating a 31% reduction in bronchodilator usage. With elite athletes, Mickleborough *et al.* (2003) found similar results to the general population, with all 10 participants experiencing significant reductions in EIB intensity. Supplementation of fish oil has anti-inflammatory potential through eicosapentaenoic acid and arachidonic acid suppressing inflammatory mediators (Mickleborough *et al.*, 2003; Mickleborough *et al.*, 2006).

Preventative measures via controlled exercise programs are regularly prescribed to asthmatic patients as a way to improve aerobic capacity (Matsumoto *et al.*, 1999). Research by Matsumoto *et al.* (1999) found that completing 30 minutes of high intensity swimming at 125% lactate threshold, six times per week for six weeks, subjects with pre-diagnosed asthma and EIB significantly decreased their fall in FEV_1 to levels below EIB diagnosis range. The reduction was due to the increase in aerobic capacity, rather than an increase in histamine responsiveness, with EIB results being reduced on a multifunction format, both on a swimming and cycle ergometer (Matsumoto *et al.*, 1999).

2.5.1 World Anti-Doping Agency Guidelines

For athletes who compete and suffer from asthma or EIB, the WADA have guidelines to follow to allow for the use of β_2-agonists (WADA, 2015). Previously, all β_2-agnoists required proof of airway hyper-responsiveness when submitting therapeutic use exemptions forms, but recent changes have allowed for the exemption of inhaled salbutamol, formoterol and salmeterol, depending on dosage. For salbutamol, a maximum of 1600 micrograms (µg) are allowed over 24 hours. For formoterol, a maximum delivery dose of 54 µg is allowed over 24 hours. Salmeterol can be used in accordance with therapeutic regimen (WADA, 2015).

For those who require an assessment of their asthmatic status, a test for the reversibility of bronchospasm can be performed, whereby an increase of ≥12% in FEV_1 after an inhaled β_2-agonists demonstrates the need for asthma medication. For evaluation of EIB, the ECT and EVH tests are recommended with a ≥10% fall in FEV_1 meeting the criteria for the use of β_2-agonists (WADA, 2015). For those athletes who just require exempt β_2-agonists, the need for EIB evaluation is not necessary, but encouraged to evaluate current medication status (ATS., 1999).

2.6 American Thoracic Society Guidelines for the Diagnosis of EIB

The ATS model is broken down into two predominant areas; an ECT and spirometry assessment. The ECT aims to subject the participant to exercise which induces significant ventilation ($\dot{V}_E$), measured through a percentage of predicted heart rate max (%HR_{max}). An 80-90% HR_{max} (220 - age in years) provides vigorous exercise conditions whilst eliciting a $\dot{V}_E$ rate of 40-60% predicted max (Anderson *et al.*, 2010). ATS. (1999) concur that physical variances such as anaerobic/aerobic fitness and body weight may hinder a participant's ability to obtain a %HR_{max} or percentage of predicted max

ventilation (%$\dot{V}_{Emax}$), but also state the HR protocol is generally effective and practical. However, Trumper *et al.* (2009) suggested that these physical variations, specifically based in HR, means applying a prediction based formula creates an increased risk of misjudging the optimum exercise intensity. Rundell *et al.* (2002) also suggests that due to the increased level of conditioning attained by athletes, there is the potential to under stimulate the ventilatory system through an underestimation of the exercise intensity. Similarly, these theories should be both addressed to the range of physical conditions, with people below their predicted HR_{max} being over stimulated during an ECT, becoming unable to maintain that 80-90% HR_{max}. Although assessment via ECT based on %HR_{max} is an efficient and well documented method, the physical variance can be a factor when it comes to an accurate diagnosis. As EIB occurs within the ventilatory tract, focusing the intensity of exercise upon a subject's %$\dot{V}_{Emax}$ would remove ambiguity caused by fitness levels and physical conditioning.

2.6.1 Evaluation of the Heart Rate Protocol

As previously stated, ATS guidelines suggest that a ventilatory protocol can be completed rather than a HR protocol (ATS., 1999), but it remains unclear whether it is a potentially superseding method. Research by Trumper *et al.* (2009) aimed to equivocally address the proposed statement that a HR based protocol does not ensure sufficient intensity for inducing EIB. The pretence of the study focused on the aerobic to anaerobic pathway shift, with evaluation of lactate concentrations [La^+] and variance between subjects was the key measure within the study. Depending on [La^+], subjects FEV_1 scores were divided into groups who experienced anaerobic conditions, with [La^+] >6mmol/L, against those who stayed within the aerobic pathways with [La^+] <6mmol/L. The results found that out of the 100 subjects, 56 did not reach the anaerobic pathway, remaining below the required conditions. When both groups got compared with FEV_1 data, those who had achieved a >6mmol/L [La^+] had significantly reduced lung function compared with those who remained aerobic.

Although the research by Trumper *et al.* (2009) would claim that a HR based method is insufficient to induce EIB, some stipulations must be made about the research model undertaken, before a true judgement can be formed. Firstly, subject selection, whereby 100 clinically healthy individuals were selected, but literature into EIB is clear that the prevalence of the condition is much greater in those who have a pre-diagnosed asthmatic condition (Lacroix, 1999; Stoloff *et al.*, 2011; Mainardi *et al.*, 2012). The symptoms of EIB have been recorded in those who do not have asthma (Anderson, 2011), but a review of method should seemingly be performed upon those at highest risk. Secondly, data comparison; although the data collected could be enough to conclude that a HR based method lacks intensity in certain populations, choices of physiological markers remained vague, such as the choice of a >6mmol/L [La^+]. Finally, spirometry assessments were completed only once, 10 minutes post exercise. The peak fall in FEV_1 has been recorded between 6 – 12 min post exercise (Rundell *et al.*, 2002), suggesting that the individual time choice would be significant, but the question of physical variances between subjects should seemingly require multiple assessments like the original method (ATS., 1999).

The study by Trumper *et al.* (2009) concluded that a HR protocol was seemingly insufficient when testing a wide range of individuals, but the method deviated from the current standard. To suggest that a method is flawed would require a comparative method design which aims to remove those physiological variances and create and individualist testing protocol, such as analysis of HR_{max} prior, rather than a predicted percentage, or as EIB is a ventilatory condition, focus exercise intensity upon a percentage of $\dot{V}_{Emax}$. As stated, an EIB ECT diagnosis should look more towards a ventilatory method rather than a $\%HR_{max}$, as EIB has the causal link with the respiratory system (Storms, 2003; Trumper *et al.*, 2009; van Leeuwen *et al.*, 2012), although current methods use $\%HR_{max}$ to elicit a sufficient $\dot{V}_E$ (ATS., 1999). Research onto the validity of the respiratory method by Anderson *et al.* (2010) found that the current ATS method is

of sufficient intensity to induce $\dot{V}_E$ rates required to induce hyperventilation, in both adults and children (Adults: 56.8% & 58.0% $\dot{V}_{Emax}$, Children: 54.7% & 56.3% $\dot{V}_{Emax}$).; However, EIB positive diagnoses were only seen in 161 out of 373 of asthmatic subjects, suggesting subjects remained under stimulated when compared to other methods of testing (Cabral *et al.*, 1999; Holzer *et al.*, 2002; Stensrud *et al.*, 2006)

Trumper *et al.* (2009) focused predominantly upon the level of physical exertion during the test, stating that subjects must achieve a state of anaerobic exercise. A current ventilator marker that can be used to assess anaerobic threshold is the gas exchange threshold (GxT). The GxT method for assessing anaerobic threshold is a non-invasive procedure whereby a breakpoint is seen within the breath by breath values of carbon dioxide elimination ($\dot{V}CO_2$) against oxygen consumption ($\dot{V}O_2$), caused by the increase in $\dot{V}CO_2$ from bicarbonate buffering of lactic acid (Magalang & Grant, 1995; McArdle *et al.*, 2009). Research by Haverkamp *et al.* (2005) onto the GxT within asthmatics and diagnosis of EIB found that, when subjects performed an ECT at 90% maximum oxygen consumption ($\dot{V}O_{2max}$), rather than 80-90% HR_{max}, post ECT FEV_1 was significantly reduced in both subjects who are EIB positive and negative, although the gas exchange disturbance took sizably longer in EIB negative subjects (3 min positive; 35 min negative). The GxT method for assessing anaerobic threshold, and subsequently EIB would seem a more refined marker of ventilation than 80-90% HR_{max}, although determination of exercise intensity which induces GxT is required before undertaking and EIB assessment.

The benefits for a ventilation based protocol would be the removal of any individual physiological variations which may occur due to physical background (Trumper *et al.*, 2009), although it would require an initial assessment to define a ventilation level required to elicit a sufficient intensity. Research into the ATS relationship with $\dot{V}_E$ has found that the use of $\%HR_{max}$ is sufficient enough to induce an $\dot{V}_E$ that caused EIB

(Anderson *et al.*, 2010), it remains questionable whether a more specific ventilatory point would be more exacting, such as the GxT, to induce the respiratory and vascular conditions sufficient enough to cause EIB. The stipulation would be whether an initial assessment would be beneficial, when current methods requiring one session with minimal equipment still find positive results.

2.7 Exercise Challenge Test

The ECT uses exercise to stimulate a high minute ventilation through the mouth to increase the airway dehydration stimulus of conditioning inspired air, which can performed both in a laboratory or field environment (Holzer & Brukner, 2004; Anderson, 2011). Field tests benefit from the availability to perform a range of vigorous, sports specific exercises, with the increase in portable spirometry devices making complete assessments much easier, although the large range of equipment needed, and the limit control of air quality may effect results (Anderson, 2011). Laboratory tests, although restrictive of sports specific testing, normally completed on treadmill or cycle ergometer, have the benefit of control of inspired air conditions, as well as online gas meters (Holzer & Brukner, 2004; Anderson, 2011). Limitations and false negative results occur due to the exercise intensity and method undertaken. ATS state that exercise intensity should ramp up rapidly over the first 2 min and be adjusted afterwards to maintain HR at the target level (ATS., 1999). An intensity level too low can lead to the release of bronchodilation constrictors, such as PGE2 which serve to protect the airways (Anderson, 2011).

ATS guidelines for the ECT (ATS., 1999) are currently regarded as the standardised method for testing using exercise as a stimulus. The method design aims to stimulate the airways by exercising at 80-90% HR_{max}, or at a 40-60% $\dot{V}_E$ of predicted MVV for ≥4 min in a dry air environment (ATS., 1999). Control of inspired air is vital as low water

content is needed to stimulate the conditioning process (Rundell *et al.*, 2002; Anderson, 2011). ATS. (1999) state that participants should inspire air of 20-25˚C and <50% relative humidity. Optimally, water content of the inspired air should be <10mg/L. At air temperatures of ≤10˚C, inspired air is sufficiently dry, specifically when testing those who complete exercise in cold environments (ATS., 1999; Rundell *et al.*, 2002; Anderson, 2011).

Exercise duration is also a key factor to the diagnosis of EIB, as time needed to achieve target HR or $\dot{V}_E$ may vary for age, physical fitness and body weight (ATS., 1999; Anderson, 2011). For children, target HR or $\dot{V}_E$ may be achieved quicker than adults, affecting exercise time. For children, an exercise duration of 6-7 min may be needed to elicit EIB, where adolescents or adults may need up to 8 min (ATS., 1999; Rundell *et al.*, 2002; Holzer & Brukner, 2004; Anderson, 2011). Once target HR is achieved, it should be maintained for ≥4 min, but it is important to reach this point rapidly over the first 2-3 min (ATS., 1999; Anderson, 2011). Although two ergometer methods have been developed, the treadmill method is preferred over the cycle ergometer, due to the more rapid increase in $\dot{V}_E$ in response to running, and the higher peak oxygen uptake achieve compared to the cycle method (ATS., 1999).

FEV_1 is the current most reliable marker for the assessment of EIB, with a fall of ≥10% from baseline being regarded as a positive diagnosis, although ≥15% has been used, specifically for field based testing (ATS., 1999; Anderson & Holzer, 2000; Holzer & Brukner, 2004). Rundell *et al.* (2000) has also stated that a fall in FEV_1 of ≥7% indicated an abnormal airway response in the elite athlete population, as to try and reduce the number of false positives. Assessment of FEV_1 should be performed following the ATS and European Respiratory Society (ERS) method for spirometry testing (Miller *et al.*, 2005). An appropriate spirometry testing procedure consists of pre ECT, 5, 10, 15, 20

and 30 min post, although earlier testing at 1 and 3 min may be completed as EIB may appear at the cessation of exercise. On average, the lowest FEV_1 values are achieved between 5-12 min after the completion of the ECT (ATS., 1999; Anderson, 2011). When testing, a minimum of 2, preferably 3 assessments should be obtained, with highest and second highest FEV_1 values not differing by 0.15L (Miller *et al.*, 2005). After 30-60 min, FEV_1 spontaneously recovers to 95% of the baseline value, although if participants do not recover to less than 10% of baseline value, a bronchodilator may be administered if the participant has a previous asthmatic condition.

Although the laboratory ECT has some benefits, such as control of environmental conditions and testing protocols, the field test or sports specific has been used by previous research (Rundell *et al.*, 2000; Wilber *et al.*, 2000; Rundell *et al.*, 2004). The variability in environmental conditions and exercise intensity and duration with field testing makes test-retest difficult to control (Rundell *et al.*, 2004). Similarly, although laboratory tests have a more controlled environment, the environments in air conditioned laboratories may not be sufficiently provocative, as air water content may not be sufficiently low to induce EIB (Dickinson *et al.*, 2006). The use of environmental chambers may impact in the control of air temperature, RH and water content. Limited research has been completed in regards to completing an ECT within a controlled environment. Stensrud *et al.* (2007) used an environmental chamber to evaluate the effect of performing an ECT in room temperature and subfreezing temperatures, 20.2°C±1.1 and -18°C±1.4 respectively, on EIB diagnosis. Using an adjusted ECT protocol, where a 4 min phase was used to reach target HR of above 95% HR_{max}, with 4 min at target HR, significant increases in FEV_1 were found after exposure to cold weather compared to room temperature. During cold weather assessment, peak oxygen consumption ($\dot{V}O_{2peak}$) was reduced in the last 4 min, correlating with participants response that respiration was more difficult compared with regular conditions, possibly

due to starting high intensity exercise in a cold environment without a warm-up, or the reduced running speed (Stensrud *et al.*, 2007).

2.8 Eucapnic Voluntary Hyperpnoea

Indirect challenges to assess bronchial hyper-responsiveness are becoming increasingly used for both research and assessment (Anderson, 2011).The bronchial provocation tests most used to identify EIB include; ECT (Laboratory based or Field), EVH and pharmacological agents such as hypertonic saline and mannitol inhalation (Anderson & Holzer, 2000; Rundell *et al.*, 2004; Anderson, 2011).

The EVH assessment was developed, standardised and validated by the US Army at the Walter Reed Medical Centre, as a surrogate to the ECT, in order to reduce the likelihood of false negatives and increase safety issue related with the ECT (Anderson *et al.*, 2001; Anderson, 2011). The test design is a bronchial provocation test, aiming to stimulate the airways via the respiration of dry air, causing osmotic and thermal consequences due to humidifying the inspired air, without the requirement to complete exercise (Anderson *et al.*, 2001; Rundell *et al.*, 2002). The IOC-MC validated the EVH as the recommended method for analysis of the need for asthmatic medication, specifically in athletes, with the benefits over an ECT such as; lower cost, induced $\dot{V}_E$ rates greater than exercise and increased safety (Argyros *et al.*, 1995; Anderson *et al.*, 2001; Holzer & Brukner, 2004; Rundell *et al.*, 2004; Anderson, 2011). The usefulness of the EVH method is in reducing the over or under diagnosis of EIB (Anderson, 2011).

The current method for EVH involves respiring dry air at 30 times FEV_1 (85% MVV) for 6 min (Anderson *et al.*, 2001; Sue-Chu *et al.*, 2010; Anderson, 2011). Specific aspects should be followed when completing an EVH and ECT assessment. Prior, no

bronchodilators should be used 48hr before, although should not be withheld if needed. Caffeine should not be consumed via food or drink 24hrs prior, although may vary depending on laboratory. Vigorous exercise should not be undertaken 4hrs prior, preferably not on the day of testing (Anderson *et al.*, 2001). The condition of the inspired air is vitally important for a valid assessment, with early studies performing in cold air environments (Anderson, 2011). Medical grade mixture gas cylinder containing 21% oxygen, 4.9-5.1% carbon dioxide with balanced nitrogen can be used (Anderson *et al.*, 2001; Holzer & Brukner, 2004; Rundell *et al.*, 2004; Sue-Chu *et al.*, 2010). The higher CO_2 content is used to maintain eucapnia over the range of ventilation (Holzer & Brukner, 2004; Anderson, 2011).

Certain limitations are present. The need to maintain eucapnia throughout a wide range of ventilation rate means ventilation needs to be between 40-105 $L \cdot min^{-1}$. If ventilation is outside these limits, end tidal CO_2 needs to be monitored, as hypocapnia is a bronchoconstricting stimulus which can cause acidosis (Anderson, 2011). Secondly, the standard protocol of 30 times FEV_1 (85% MVV) for 6 min is only appropriate for athletes or participants who perform regular exercise, as high ventilation rates can cause a severe reduction in FEV_1 (Anderson, 2011). For asthmatics, adaptation to the method by the reduction of ventilation rate to 21 times FEV_1 or duration of 4 min would induce an MVV of ~60% in asthmatics or untrained participants (Brummel *et al.*, 2009).

A positive diagnosis during an EVH assessment is a fall in FEV_1 at ≥10% that of baseline (Holzer & Brukner, 2004). An appropriate spirometry procedure consists of pre EVH, post, 5, 10, and 20 min after testing, with the lowest value at each time point used to calculate percentage fall (Argyros *et al.*, 1996). Other studies have used varying time points during post EVH testing, Sue-Chu *et al.* (2010), tested at 3, 5, 7, 10 and 20 min post EVH, with Rundell *et al.* (2004) testing at 5, 10, and 15 min.

2.9 Exercise Challenge Test vs. Eucapnic Voluntary Hyperpnoea

With the large number of athletes previously diagnosed with EIB, with up to 50%, the most accurate and valid method for assessment should be evaluated (Rundell *et al.*, 2004). The current methods utilised in clinical research are the ECT, developed by the American thoracic society (ATS), and EVH, the current standard by the IOC-MC (Rundell *et al.*, 2004). Research has been completed assessing the sensitivity of both methods, as well as variations of the ECT.

Rundell *et al.* (2004) assessed the sensitivity between the EVH method and a sports specific field ECT (SSECT), aiming to evaluate whether EVH is sufficient enough when compared to an ECT. The ECT design consisted of 6-8 min of either ice skating, cross country skiing or running, in environmental conditions of 5.6˚C±2. The EVH protocol required participants to respire at 30 x FEV_1 for 6 min, with a fall in ≥10% in FEV_1 being regarded as a positive diagnosis for both assessments. From the 38 participants who completed the research, 11 and 17 positive diagnoses were made from the ECT and EVH respectively. Although numerically, more participants were diagnosed with EIB post EVH, the mean fall in FEV_1 was greater post ECT (20.5±7.3% to 14.5±4.5%). $\dot{V}_E$ during EVH was recorded at 104±26 $L \cdot min^{-1}$, with the measured $\dot{V}_E$ being equivalent to 27.7 x FEV_1. They concluded that the EVH has a greater chance of identifying EIB when compared to the ECT, specifically those who perform in cold weather sports.

Similar research to Rundell *et al.* (2004), research by Dickinson *et al.* (2006) evaluated winter sports athletes (10 speed skaters, 4 biathletes) using EVH, laboratory ECT (LECT) and sports specific ECT (SSECT) methods for diagnosis EIB. LECT involved treadmill running for 8 min, at a >90% HR_{max} in environmental conditions of 18°C and 56% RH.

SSECT required speed skaters to skate for 6 min at 11 and 12 s per 250m, with biathletes required to complete a 20 min simulated race in 1-2°C at 31-34% RH. EVH followed standard protocol of 30 x FEV_1 for 6 min, recommended by Anderson *et al.* (2001). Based on the ≥10% fall in FEV_1 being regarded as positive, 10 of the 14 had a positive response to EVH, with 3 and 0 being diagnosed from the SSECT and LECT respectively, concluding that the EVH is the more sensitive challenge for the detection of EIB in athletes. Although EVH has been regarded as the more sensitive by Dickinson *et al.* (2006), stipulations should be made about the control of each study. EVH allows for greater control over the two main contributors; inspired air water content and $\dot{V}_E$ (Dickinson *et al.*, 2006).

Research by Castricum *et al.* (2010) followed the structure that Rundell *et al.* (2004) and Dickinson *et al.* (2006) had previous used. Castricum *et al.* (2010) evaluated a group of 33 elite swimmers using the EVH, field swim test and LECT. Similarly to both Rundell *et al.* (2004) and Dickinson *et al.* (2006), the EVH was the most sensitive test with 18 of the 33 subjects experiencing a positive test compared to the one for the field swim and four for the LECT.

Although colder environmental conditions have proven significantly effect at stimulating EIB mediators (Stensrud *et al.*, 2007), it is not the sole factor in stimulating a positive response. Dickinson *et al.* (2006) reported that even when exercise was performed in temperatures sufficiently low enough for elicit EIB, 1°C and 8°C respectively, only 3 diagnoses were found compared to EVH, performed in 19.1°C, which diagnosed 10. The water content of the inspired air may be a more important factor, rather than cold air alone (Evans *et al.*, 2005).

The ability to control environmental conditions during an ECT, where the inspired air is considered sufficiently dry (<10°C) (Anderson, 2011), could correct for misdiagnosis when comparing LECT and EVH tests. No specific studies have been found where LECT has been performed in a controlled environment following the current ATS guidelines (ATS., 1999), and compared to an EVH assessment following IOC-MC and Anderson *et al.* (2001) guidelines measuring sensitivity, although Evans *et al.* (2005) evaluated cold temperature and room temperature air ECT and EVH respectively, using chilled air from a dry gas cylinder. At approximate temperatures of 22°C and -1°C, a significant difference was found when comparing room temperature EVH and cold temperature ECT (-15.22% vs -10.70%), where other comparisons were non-significant.

2.10 Spirometry

Spirometry is the physiological test which measures how individuals inhale or exhale volumes of air over a function of time, aimed at assessing respiratory health (Miller *et al.*, 2005). Miller *et al.* (2005) describes the current standardised method of spirometry, which consists of three distinct phases; 1) maximal inspiration, 2) a rapid exhalation and 3) continuation of exhalation until test end. Prior to the initial phase, demonstration of the appropriate technique should be performed by the technician, with descriptive information about equipment and usage. During phase one, completion of maximum inspiration should be performed by the subject. During phase two, subjects should be encouraged to push or blast, rather than just blow when exhaling. A standard spirometry assessment should be performed without hesitation, specifically delays when transitioning from inspiration to rapid exhalation which can cause reductions in both peak expiratory flow (PEF) and FEV_1 (D'Angelo *et al.*, 1993). Finally, phase three, where continued encouragement is used so that the subject fully exhales. A sufficient

assessment is achieved when three tests are completed that are free from artefact, such as coughing, with FEV_1 results no less than 0.15 L apart (ATS., 1999; Miller *et al.*, 2005).

A positive diagnosis of EIB is primarily achieved when FEV_1 has a ≥10% reduction post provocation test when compared with a baseline measure (ATS., 1999), with severity of the EIB symptoms being measured through the FEV_1 time curve (Anderson *et al.*, 2010). Guidelines by the ATS. (1999) state that spirometry should be completed at baseline, then periodically post provocation test. Guidelines state appropriate post provocation testing should be completed at 5, 10, 15, 20, and 30 min, although earlier time points of 1 and 3 min have been found to record lowest FEV_1 due to severe EIB becoming present (Anderson *et al.*, 1991; ATS., 1999). Research by Trumper *et al.* (2009) only sampled once at 10 min post-test, due to technical reasons. Although within reported range for greatest reduction in FEV_1, it cannot be disputed that subjects may have been diagnosed negatively, due to the cessation of EIB occurring earlier or later than the 10 min test time. Similarly, Seear *et al.* (2005), completed spirometry at only 5 and 15 min post exercise, and out of the 52 children tested, only 8 were diagnosed with EIB, which, although not stated, could be down to the limited assessment. Wilber *et al.* (2000) research into the prevalence of EIB in Olympic athletes use time points of 5, 10 and 15 min, and even though it was not exact to the ATS guidelines, results remained similar to both Trumper *et al.* (2009) and Seear *et al.* (2005). Although positive results may be found when one or two time points are used post provocation test, individual variances may cause a shift in the time where fall in FEV_1 is greatest (Anderson *et al.*, 1991), so completion of more assessment will remove ambiguity and increase the validity within the data. Spirometry assessments up to 30 min post exercise has been recorded as the time of lowest FEV_1 (Brudno *et al.*, 1994), and although ATS guidelines suggest recording up to 30 min, it remains controversial as a delay of such length is infrequently seen (ATS., 1999).

2.11 Standardisation

Although research has been completed using the ECT, EVH and comparing both provocation tests, it has been brought into question whether the methods they had used were valid and reliable, and following the current guidelines. A review by Stickland *et al.* (2010) found that some previous research had failed to follow the current ECT guidelines, which could have affected the overall results. Out of nine studies, only one followed the current ATS guidelines, with deviations being; uncontrolled room temperature and relative humidity, no nose clip, duration was too long or graded exercise was used. For the four studies which looked to evaluate the ECT against the EVH, none of the studies followed the ATS guidelines, so the sensitivity of the EVH relative to the ECT is unclear.

Alongside provocation method deviations, previous research has lacked adherence to the spirometry guidelines. Current ATS/ERS methods state that spirometry should be completed at 5,10,15,20 and 30 min post provocation test, with optional 1 and 3 min. Of the studies found, none of them completed spirometry at all-time points, ranging from one to three samples (Wilber *et al.*, 2000; Rundell *et al.*, 2004; Seear *et al.*, 2005; Dickinson *et al.*, 2006; Trumper *et al.*, 2009). Although it has been stated that peak fall in FEV_1 occurs between 5-12 min (Anderson, 2011), the physical variances between individuals and response to provocation tests could affect the onset of EIB, causing an under or over diagnosis.

CHAPTER THREE

METHOD

3.1 Participants

Twenty-two participants initially volunteered to participate in this study. Four participants were unable to complete all of the trials due to injury and a fifth was removed from the trial by the investigator due to a severe airway response to the ECT, with a FEV_1 fall of 49%. The remaining seventeen participants (mean (SD) age 25.2 (5.1) yr, stature 1.81 (.06) m, mass 85.4 (13.7) kg), were either University level athletes, who competed in a variety of British Universities & Colleges Sport (BUCS) leagues (n=10; 9 male, 1 female), or were professional rugby league players (n=7 male). Three of the participants had a prior history of asthma of which two were professional rugby league players. Research was completed in accordance with the University of Gloucestershire regulations and the Universities Ethical Committee. Prior to testing, all participants were required to complete a health history questionnaire and a questionnaire to identify any potential contraindications to inhaled salbutamol, and to provide informed consent. By the recommendations of Anderson et al (2010), prior to any testing participants with a pre-diagnosed history of asthma were asked to withhold medication (Short-acting β_2-agonist 8 hr, long-acting β_2-agonist 24 hr). Participants were, however, instructed to use their medication if required and to inform the study investigator so that testing could be rescheduled. A short-acting β_2-agonist (100 mcg salbutamol) was available to participants at all times to offer immediate relief if required. All participants completed testing of their own free will and could stop at any point with no repercussions.

3.2 Procedures

In a repeated measures design participants visited the laboratory on two occasions. At each visit participants first completed baseline spirometry followed by either a randomly assigned ECT or EVH test. Immediately following the provocation test participants performed follow-up spirometry to assess the effect of the provocation test on lung

function. Participants attended the laboratory in their usual exercise clothing and footwear and were asked to maintain similar dietary habits for each visit. In addition to withholding medication, participants were requested to avoid caffeine and strenuous exercise for 12 hr prior to each visit (Anderson et al. 2010).

3.3 Spirometry

Spirometry was performed in accordance with ATS/ERS guidelines (Miller, Hankinson et al. 2005) using an electronic flow measurement spirometer (Microloop, Micromedical, Rochester, Kent, UK) connected to a PC running dedicated software (SPCS, Micromedical, Rochester, Kent, UK). Prior to each test a verbal and visual demonstration was provided. For each manoeuvre, a maximal forced exhalation procedure was performed in a seated position, with the participant wearing a nose clip. Participants began with two to three tidal breaths before fully inhaling, followed immediately with a maximal forced exhalation with limited delay. Verbal encouragement was given during exhalation to encourage maximal effort and to ensure exhalation was complete.

At each visit to the laboratory, and prior to each provocation test participants' baseline lung function was measured. Lung function was also assessed immediately post-test and at 0, 3, 5, 10, 15, 20 and 30 min. At baseline a minimum of three acceptable FVC manoeuvres (maximum of 8) were performed. On completion of three acceptable tests the results were assessed for reproducibility. Specifically, if the difference between the highest and next highest FEV_1 and FVC was ≤ 0.15 L. The largest FVC and FEV_1 were selected from all the useable tests with other indices such as PEF and FEV_1/FVC, derived from the test with the largest sum of FVC and FEV_1. For the post provocation tests, spirometry was performed in duplicate with each test approximately 30 s apart, with the best test being used to assess fall in FEV_1

3.4 Exercise Challenge Test

The ECT protocol followed current ATS guidelines. Each test was performed on a motorised treadmill (ELG 55, Woodway, Gmbh, Weil am Rhein, Germany) within an environmental chamber (9388 Athlete Training Room, Sanyo Gallenkamp PLC, Loughborough, UK) at an ambient temperature of 8 ˚C. This temperature assumed that the water content of the inspired air was < 10 $mg \cdot L^{-1}$. Temperature and humidity was continuously monitored by a temperature and humidity probe (RHT-G-Z5-0, Grant Instruments, Cambridge, UK), which sampled from the centre of the chamber and was interfaced to a Squirrel SQ2020 data logger (Grant Instruments, Cambridge, UK).

The protocol design was chosen to produce 4-6 min of high-intensity exercise with a total duration of 6-8 min (ATS., 1999). Over the first 2 min the treadmill speed and gradient were rapidly increased until the target HR was achieved. Once target HR had been reached the participants completed 4-6 min of continuous exercise, with treadmill speed and gradient adjusted to maintain target HR. HR was recorded via chest heart rate monitor (RCX5, Polar Electro Oy, Kempele, Finland) recording data every 5 s and monitored throughout to ensure HR was within the target zone.

3.5 Eucapnic Voluntary Hyperpnoea

The EVH test was conducted following the recommendations of the IOC-MC (Anderson, Argyros, Magnussen & Holzer, 2001). All tests were performed using the EucapSys™ EVH system (SMTEC SA, Nyon, Switzerland). Participants inhaled a medical grade dry gas mixture of 5% CO_2 and 21% O_2 via a face mask connected to the EVH system via flexible tubing. Participants were instructed to hyperventilate at a rate equivalent to 30 times baseline FEV_1 for 6 min (Anderson, Argyros et al. 2001). Throughout the test,

visual and verbal feedback was provided to encourage the participant to maintain $\dot{V}_E$ at the target rate.

3.6 Treatment of Data

Raw spirometry and HR data was exported into Excel. At each time point FEV_1, FVC, PEF and FEV_1/FVC were recorded into individual spreadsheets. Predicated values for target HR and target ventilation were calculated in Excel. For the ECT, target HR was calculated via (220 - Age in yr) x 0.85. The target ventilation needed for the EVH was calculated prior via (Baseline FEV_1 x 30). For diagnosis of EIB, a fall in FEV_1 of ≥10% from baseline must be observed, calculated via:

$$\frac{(FEV_{1post} - FEV_{1baseline})}{FEV_{1baseline}} \text{ X } 100$$

3.7 Statistical Analysis

Descriptive data are reported as means and standard deviation (Mean (SD)). Statistical analysis was completed on SPSS (version 22, IBM, Armock: NY, United States) with an α value of $p<0.05$. Data was analysed for normality and found to be normally distributed. A series of individual paired samples *t*-tests were performed to compare baseline FEV_1, FVC, PEF and FEV_1/FVC for both positive and negative results. A series of individual paired samples *t*-tests were performed to compare predicted FEV_1, FVC, PEF and FEV_1/FVC for both positive and negative results. A paired samples *t*-test was performed comparing target HR achieved during the ECT for positive and negative results. A paired samples *t*-test was performed comparing target $\dot{V}_e$ achieved during the EVH for positive and negative results. A series Individual paired samples *t*-test were performed to compare the peak delta (Δ) in FEV_{1peak}, FVC_{peak} PEF_{peak}, and FEV_1/FVC_{peak} during the ECT and EVH. A series of repeated measures 2-way analysis of variance (ANOVA) were

performed to determine whether differences were present at each post provocation time-point between percentage Δ in FEV_1, FVC, PEF, and FEV_1/FVC for both the ECT and EVH tests. If a significant difference was present, follow up Bonferroni paired samples *t*-tests were performed to determine whether differences were present at each post provocation time-point (0, 3, 5, 10, 15, 20, and 30) for the ECT and EVH. A Bland and Altman plot was produced to evaluate limits of agreement between the lowest %ΔFEV_1.

CHAPTER FOUR

RESULTS

4.1 Summary

All seventeen participants completed both of the provocation tests. As seen in Table 1, nine had a positive response (those highlighted) to at least one of the challenges based with a fall in FEV_1 ≥10%. Out of those, five experienced a ≥10% fall in both the ECT and EVH, with two during the ECT and two during the EVH alone. For the following analysis all seventeen participants were included unless otherwise stated.

***Table 1*:** Participant response to provocation testing

Participant	Baseline FEV1 (L)	Predicted FEV1 (%)	ECT ΔFEV1 (%)	EVH ΔFEV1 (%)
1	4.36	103	-5	1
2	5.02	111	**-10**	-8
3†	3.83	83	-9	-7
4*	3.67	100	**-13**	**-26**
5†*	5.00	98	-4	-4
6	4.53	101	-1	-4
7†	4.81	103	1	-7
8	4.45	111	**-11**	-7
9	4.34	110	-7	**-10**
10†*	4.13	92	**-16**	**-28**
11	4.57	94	**-29**	**-26**
12†	4.76	102	-4	-8
13†	5.32	104	**-23**	**-23**
14	4.52	103	-5	**-14**
15†	5.07	103	-2	-2
16	3.94	90	2	-6
17	5.20	121	**-16**	**-11**
Mean (SD)	4.56 (0.48)	102 (8.96)	-8.99 (8.35)	-11.19 (9.11)

* Past history of Asthma. † Professional Rugby League Players
Participants with positive responses for EIB are highlighted in bold and greyed.
ΔFEV_1 values rounded to nearest whole number
Baseline FEV_1 taken from second visit.

4.2 Baseline Lung Function

All participants had a baseline FEV_1 that was >75% of the predicted value and an FEV_1/FVC >70%. Consequently, all participants were considered to be free of an airway obstruction at rest and suitable for testing. Actual and predicted values for FEV_1, FVC,

PEF and FEV_1/FVC and $FEF_{25-75\%}$, were not significantly different between EIB positive and EIB negative participants. There was a significant difference in percent predicted FVC between EIB positive and negative participants (p=0.048) but no differences in any of the other percent predicted parameters.

4.3 Achievement of Target Heart Rate during the Exercise Challenge Test

There was no significant difference in percentage of target HR achieved during the exercise challenge test between the ECT positive and negative participants (102 ± 2% vs 100 ± 2%; p=0.20). The HR that was achieved by all participants during the ECT ranged from 96 to 104% of the target HR (Figure 1).

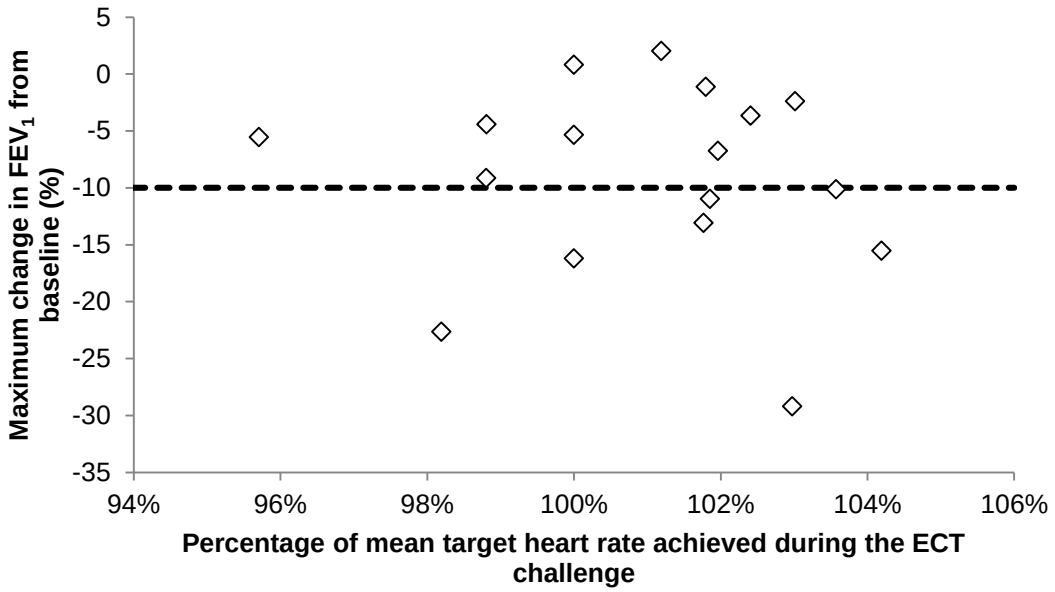

Figure 1: Percentage of target HR achieved against peak fall in FEV_1

4.4 Achievement of Target Ventilation during the Eucapnic Voluntary Hyperpnoea

There was no significant difference in the percentage of target $\dot{V}_E$ achieved during EVH between EVH positive and negative participants (91 ± 4.8% vs 87.6 ± 72%; p=0.29). The percentage of target MVV achieved during the EVH ranged from 79 to 99%. All

participants achieved a level of $\dot{V}_E$ greater than 21 times FEV_1 (range 23.6 – 29.4 x FEV_1), which is considered to be the minimum rate for a test to be considered valid (Brummel *et al.*, 2009). Due to missing data on mean ventilation, three participants were not represented in Figure 2.

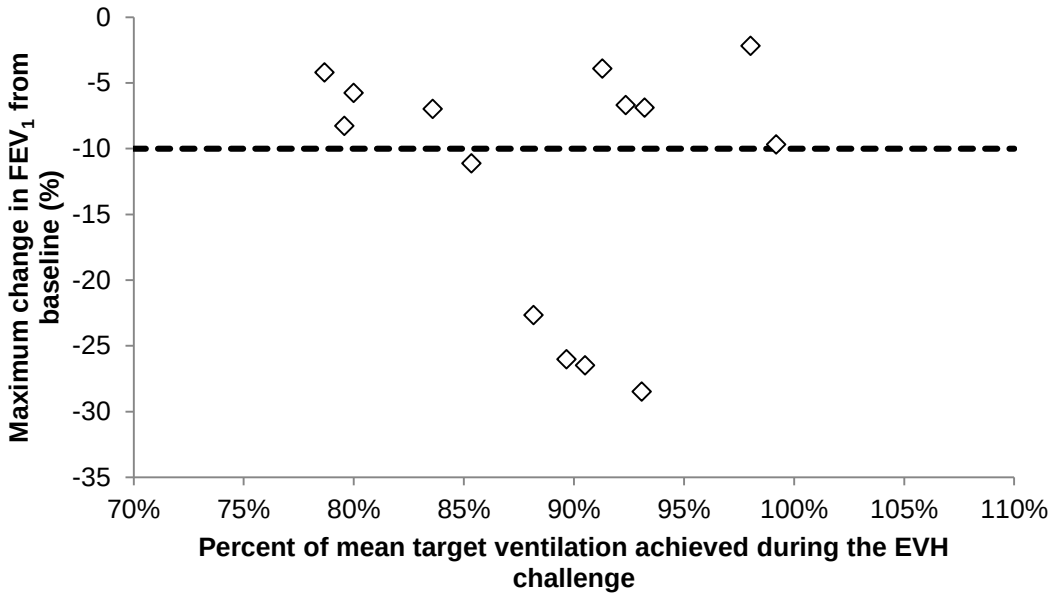

Figure 2: Percentage of target $\dot{V}_E$ achieved against peak fall in FEV_1

4.5 Effect of Provocation Test on the Airway Response: ECT and EVH

There were no significant differences between the provocation tests for ΔFEV_{1peak} (p=0.143, MD = 2.20, 95% CI 0.83 - 5.22, ΔFVC_{peak} (p=0.291, MD = -1.14, 95% CI 3.34 - 1.07), or ΔPEF_{peak} (p=0.300, MD = 2.14, 95% CI -2.09 - 6.36). However, $\Delta FEV_1/FVC_{peak}$ was significantly lower in the EVH compared to the ECT (p=0.018, MD = 5.04, 95% CI 0.97 - 9.11) (Table 2).

***Table 2*:** Mean (SD) for paired samples *t*-tests for peak fall in: ΔFEV_1, ΔFVC, ΔPEF, and $\Delta FEV_1/FVC$.

ΔFEV_{1peak} (%)		ΔFVC_{peak} (%)		ΔPEF_{peak} (%)		$\Delta FEV1/FVC_{peak}$ (%)*	
ECT	**EVH**	**ECT**	**EVH**	**ECT**	**EVH**	**ECT**	**EVH**
-8.99	-11.19	-3.98	-2.85	-8.72	-10.86	-3.62	-8.66
(8.35)	(9.11)	±(5.09)	±(3.43)	(9.05)	(9.62)	(5.58)	(8.18)

* Significant difference ($p \leq 0.005$) between ECT and EVH
Mean and standard deviation as; M (SD)

4.6 Effect of Provocation Test on the Airway Response: Positive and Negative

A series of paired samples *t*-tests were conducted to determine whether was were significant differences between the positive and negative results for both the ECT and EVH for the dependent variables: ΔFEV_{1peak}, ΔFVC_{peak}, ΔPEF_{peak} and $\Delta FEV_1/FVC_{peak}$ (table 3). Significant differences occurred between the positive and negative results for both the ECT and EVH for ΔFEV_{1peak} (ECT $p \leq 0.001$ and EVH $p \leq 0.001$), ΔFVC_{peak} (ECT $p \leq 0.001$ and EVH $p = 0.05$) and $\Delta FEV_1/FVC_{peak}$ (ECT $p = 0.01$ and EVH $p = 0.02$). For ΔPEF_{peak}, significant difference occurred within EVH ($p \leq 0.001$) but not for ECT ($p = 0.27$).

Table 3: Mean (SD) for the paired samples *t*-tests in positive and negative results peak fall in ΔFEV_1, ΔFVC, ΔPEF, and $\Delta FEV_1/FVC$.

		n	ΔFEV_{1peak} (%)*ˣ	ΔFVC_{peak} (%)*ˣ	$\Delta FEV1/FVC_{peak}$ (%)*ˣ	ΔPEF_{peak} (%)ˣ
ECT	+	7	-16.81 (6.85)	-8.03 (3.88)	-15.14 (9.59)	-5.47 (8.23)
	-	10	-3.52 (3.44)	-1.16 (3.80)	-4.23 (5.45)	-2.32 (2.40)
EVH	+	7	-19.78 (7.93)	-4.76 (3.90)	-16.96 (9.06)	-15.65 (8.46)
	-	10	-5.17 (2.79)	-1.51 (2.45)	-6.59 (7.78)	-3.77 (2.60)

*Significant difference between positive and negative means for ECT.
ˣ Significant difference between positive and negative means for EVH.
Mean and standard deviation as; M (SD)

4.7 Post Provocation Lung Function

As seen in Table 4, a series of two-way repeated measures ANOVAs were used to determine possible differences for the main effects (provocation test and time) as well as the interaction (provocation test by time) for the dependent variables ΔFEV_1, ΔFVC, ΔPEF and $\Delta FEV_1/FVC$ across the seven post provocation test time-points (Post 0, Post 3, Post 5, Post 10, Post 15, Post 20, Post 30).

Table 4: Mean (SD) for ΔFEV1, ΔFVC, ΔPEF and ΔFEV1/FVC over the seven post provocation test time points for positive and negative groups.

ΔFEV1*ˣ		**Post 0**	**Post 3**	**Post 5**	**Post 10**	**Post 15**	**Post 20**	**Post 30**
ECT	+	-7.32	-11.79	-11.28	-11.10	-8.10	-7.73	-7.25
		(6.70)	(7.62)	(6.99)	(7.47)	(5.86)	(6.83)	(5.91)
	-	-0.57	-1.67	-2.16	-1.98	-1.11	-0.75	-0.91
		(2.05)	(3.37)	(3.78)	(3.59)	(3.52)	(3.30)	(2.42)
EVH	+	-10.43	-15.64	-13.39	-10.95	-7.14	-7.72	-6.23
		(8.95)	(9.27)	(8.98)	(7.57)	(11.50)	(5.05)	(2.95)
	-	-1.73	-3.97	-3.35	-0.12	-1.70	-1.60	-1.37
		(2.83)	(3.51)	(2.70)	(6.83)	(2.75)	(2.96)	(2.49)

ΔFVC		**Post 0**	**Post 3**	**Post 5**	**Post 10**	**Post 15**	**Post 20**	**Post 30**
ECT	+	-4.86	-3.57	-3.99	-3.07	-1.94	-2.46	-1.75
		(3.93)	(3.52)	(5.17)	(3.83)	(3.80)	(3.32)	(5.02)
	-	-1.10	0.66	0.33	-0.55	0.27	0.28	-0.02
		(3.91)	(3.81)	(3.59)	(4.36)	(4.02)	(4.04)	(3.71)
EVH	+	-2.20	-3.48	-2.65	-1.86	-1.28	-1.18	-0.59
		(1.73)	(2.90)	(2.38)	(2.75)	(3.73)	(2.68)	(4.17)
	-	0.42	-0.37	-0.75	-0.64	0.08	0.30	-0.45
		(1.99)	(2.58)	(2.44)	(2.41)	(3.37)	(3.62)	(2.36)

ΔPEF		**Post 0**	**Post 3**	**Post 5**	**Post 10**	**Post 15**	**Post 20**	**Post 30**
ECT	+	-7.00	-9.97	-9.34	-11.54	-10.36	-10.44	-8.85
		(9.88)	(10.78)	(9.80)	(7.28)	(7.22)	(7.06)	(7.75)
	-	-3.45	-4.74	-2.35	-2.90	-4.34	-2.94	-3.17
		(4.38)	(4.82)	(6.26)	(7.14)	(5.92)	(4.53)	(5.26)
EVH	+	-9.59	-11.27	-11.21	-11.19	-14.08	-9.01	-6.46
		(10.71)	(8.92)	(10.65)	(7.81)	(8.59)	(8.50)	(8.14)
	-	-5.99	-4.49	-3.90	-6.31	-4.47	-4.54	-3.15
		(6.76)	(5.03)	(5.72)	(7.06)	(4.77)	(4.92)	(7.30)

ΔFEV1/FVC*ˣ		**Post 0**	**Post 3**	**Post 5**	**Post 10**	**Post 15**	**Post 20**	**Post 30**
ECT	+	-3.99	-5.36	-7.61	-8.25	-6.17	-5.21	-5.52
		(7.00)	(6.72)	(5.47)	(6.30)	(5.37)	(6.13)	(3.72)
	-	1.18	-2.14	-2.15	-1.05	-0.95	-0.75	-0.41
		(3.37)	(1.83)	(2.51)	(2.34)	(2.07)	(2.48)	(2.84)
EVH	+	-8.60	-12.68	-10.98	-9.22	-8.21	-6.70	-5.52
		(8.46)	(8.77)	(8.24)	(7.67)	(6.49)	(6.16)	(3.48)
	-	-2.12	-3.78	-2.60	-2.42	-1.65	-1.96	-0.91
		(3.56)	(2.84)	(2.02)	(2.71)	(2.57)	(2.66)	(2.88)

* Significant interaction between the two provocation tests.

ˣ Significant main effect for time.

4.7.1 ΔFEV$_1$

The two-way repeated measures ANOVA for the dependent variable ΔFEV$_1$ revealed a significant interaction between the two provocation tests ($F_{(6,90)}$=2.261, p=0.044). Follow up post-hoc Bonferroni paired samples *t*-tests revealed no significant differences between the ECT and EVH at any of the seven time-points. A non-significant main effect was found for the provocation tests ($F_{(1,15)}$=0.139, p=0.715), however there was a significant main effect for time ($F_{(6,90)}$=5.167, p≤0.001). For the ECT test, follow up post-hoc one-way repeated measures ANOVA revealed a significant difference over time ($F_{(6,90)}$=3.629, p=0.003), with follow up Bonferroni tests showing no significant differences between all time-points. For the EVH test, a significant difference over time was found ($F_{(6,90)}$=4.537, p≤0.001), with follow up Bonferroni revealing that post 0 min was significantly higher than post 3 min (MD=3.768, 95% CI=0.13-7.40) and post 3 min was significantly lower than post 20 min (MD=5.182, 95% CI=10.18-0.19).

4.7.2 ΔFVC

The two-way repeated measures ANOVA for the dependent variable ΔFVC revealed a non-significant interaction between the two provocation tests over time ($F_{(6,90)}$=1.301, p=0.265). A non-significant main effect was found for the provocation tests ($F_{(1,15)}$=0.323, p=0.578) as well as the main effect for time ($F_{(6,90)}$=1.874, p=0.094).

4.7.3 ΔPEF

A two-way repeated measures ANOVA for the dependent variable ΔPEF revealed a non-significant interaction between the two provocation tests over time ($F_{(6,90)}$=0.706, p=0.646). A non-significant main effect was found for the provocation tests ($F_{(1,15)}$=0.232, p=0.637) as well as the main effect for time ($F_{(6,90)}$=1.732, p=0.123).

4.7.4 ΔFEV$_1$/FVC

A two-way repeated measures ANOVA for the dependent variable ΔFEV$_1$/FVC revealed a significant interaction between the two provocation tests over time ($F_{(6,90)}$=2.911, p=0.012). Follow up post-hoc Bonferroni paired samples t-tests revealed that the EVH test was significantly lower than the ECT at time-points 0 min (p=0.022, MD = 4.00, 95% CI 0.68 - 7.32) and 3 min (p=0.017, MD = 4.64, 95% CI 0.94 - 8.34). A non-significant main effect was found for the provocation tests ($F_{(1,15)}$=2.598, p=0.128), however there was a significant main effect for time ($F_{(6,90)}$=4.666, p≤0.001). For the ECT test, a follow up post-hoc one-way repeated measures ANOVA revealed a non-significant difference over time ($F_{(6,90)}$=1.969, p=0.078). For the EVH, a significant difference over time was found ($F_{(6,90)}$=7.900, p≤0.001), with follow up Bonferroni revealing that post 0 min was significantly higher than post 3 min (MD=2.874, 95% CI=0.034-5.74) and that post 3 min was significantly lower than both post 15 min (MD = -3.167, 95% CI 6.05 - 0.29) and post 20 min (MD = -3.701, 95% CI 7.19 - 0.22).

4.8 Agreement between ECT and EVH

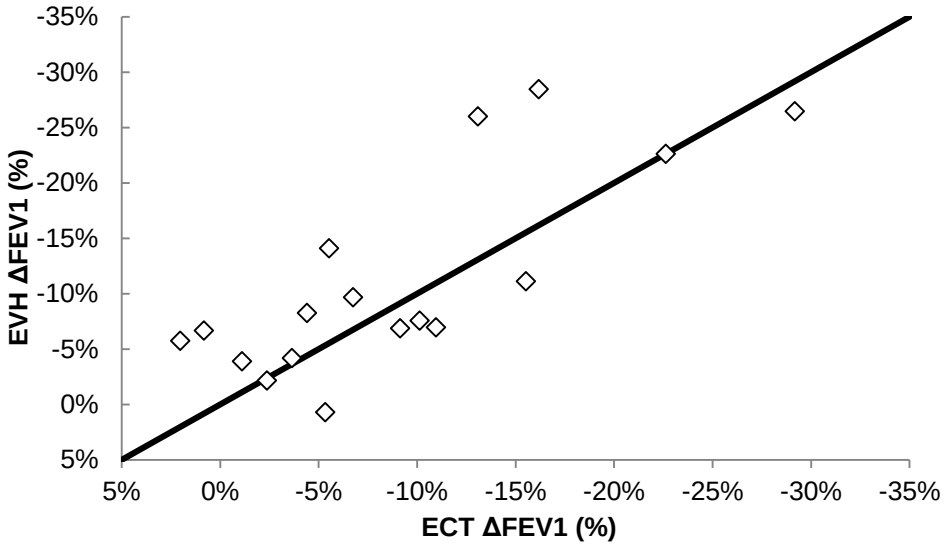

Figure 3: Correlation between peak fall in FEV_1 during the ECT and EVH.

As expected a strong positive correlation (R=0.78) was found between the ΔFEV_1 responses to ECT and EVH. The line of identity provides an indication to the degree of spread amongst the data points, which would all lie along the line if agreement was perfect (figure 3).

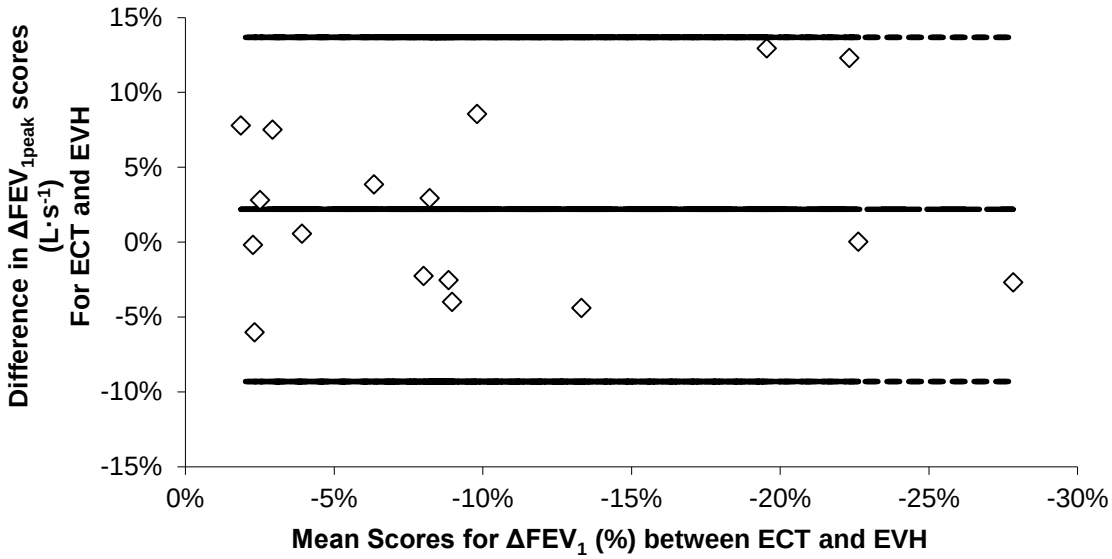

Figure 4: Bland and Altman plot for the mean scores and difference in peak for FEV_1 between the ECT and EVH.

The mean difference is 2.2% indicating that ECT tended to give a ΔFEV_1 reading between 0.8% lower and 5% higher than EVH. The upper and lower limits of agreement (13.7 and -9.3% respectively) were large indicating that the ECT may result in a ΔFEV_1 between 13.7% higher and 9.3% lower than EVH. Furthermore, the confidence intervals for the upper and lower limit of agreement (8.5 to 18.6% and -14.5 to -4.4% respectively) are wide also indicating poor agreement (figure 4).

CHAPTER FIVE

DISCUSSION

5.1 Key Findings

The main findings of our study found that out of seventeen participants who completed both provocation tests, nine experienced a ≥10% fall in FEV_1. Five experienced a fall of ≥10% in both the ECT and EVH, with two in the ECT or EVH alone. Out of the nine, only two had a previous history of asthma, with our screening identifying seven who had no previous respiratory issues. Our study suggests that athletes need to be tested for EIB, regardless if a history of asthma is present or not. Our results, although varied from previous research, may be inconclusive due to limitations (Dickinson *et al.*, 2006; Trumper *et al.*, 2009; Castricum *et al.*, 2010).

5.2 Literature Comparison

The object of the present study was to follow the recommendations of Stickland *et al.* (2010), which called for the need to standardise the methods employed during the screening process within research, specifically with the control of inspired air water content, spirometry and intensity. The methods used during the study followed that of the current ATS guidelines for ECT and current methods for the EVH (ATS., 1999; Anderson *et al.*, 2001). Above the guidelines, ECT testing was performed within an environmental chamber to control air temperature to reduce air water content, and spirometry was completed with increased post provocation test sampling times.

Our research found that there was no significant difference between the ECT and EVH when performing them following the standardised guidelines. Out of the seventeen participants who completed both provocation tests, nine experienced a fall of ≥10% in FEV_1. Five of those had ≥10% falls in both the ECT and EVH, with two in the ECT and EVH alone. Our results showed that, if performed using current guidelines, both the ECT

and EVH are sufficient tests for inducing the EIB response in athletes. The results of this study go against previous research (Rundell *et al.*, 2004; Dickinson *et al.*, 2006; Trumper *et al.*, 2009), which has stated that the EVH was conclusively the more sensitive test when compared to the ECT (Rundell *et al.*, 2004; Dickinson *et al.*, 2006; Castricum *et al.*, 2010).

Trumper *et al.* (2009) research suggested that the ECT was not sufficient for the assessment of EIB. This is due to the predicted HR formula used for the target exercise intensity does not put the body in a state of anaerobic exercise for exercise bronchial provocation. This variance, specifically in the athletic population, could be due to physical fitness or participants sporting event. When comparing the two methods, Dickinson *et al.* (2006) found a greater number of positive EIB assessments during the EVH, stating that this is due to the greater amount of control compared to the ECT. Although we used the predicted HR formula to follow the current ATS guidelines, we focused control of the ECT by controlling the inspired air temperature to reduce the inspired water content. The aim of our research was to assess EIB via ECT and EVH protocols following the standardised methods.

5.3 Pathological Interpretation

The variations from previous research would coincide with the review of Stickland *et al.* (2010) with the lack of adherence to the current guidelines causing a possible under or over estimation of the results. As stated, the key areas which previous research had failed to control was the inspired air water content, exercise intensity and spirometry.

Environmental conditions during the ECT may be the contributing factor when comparing the difference in results. Dickinson *et al.* (2006) stated that the key factors for the EVH

tests superiority was the level of control over the inspired air water content and $\dot{V}_E$. Ensuring that the inspired air is sufficiently dry would stimulate the osmotic hypothesis for the mechanisms of EIB, causing water to move from nearby cells to condition the inspired air (Anderson & Daviskas, 1997). From our results, increasing control over inspired air water content can be completed by performing the ECT in an environmental chamber set at 8˚C. When comparing our results to Dickinson *et al.* (2006) and Castricum *et al.* (2010), who performed the ECT in 18˚C, 46% RH and 21˚C, 60.5 RH respectively, our results suggest that completing the ECT in 8˚C creates an environment with sufficiently low air water content to stimulate the osmotic mechanisms of EIB. The movement from 8˚C during the ECT to 20˚C during post spirometry may have assisted in the rewarming of the airway, aiding to the fall in FEV_1. The range in temperature if not as large as those where rapid rewarming occurs (McFadden *et al.*, 1986), suggesting that it was unlikely that any thermal mechanisms affected the fall in FEV_1. Consideration must be made into the accessibility of environmental chambers or systems which can control environmental conditions such as temperature and air water content. Although controlling temperature and air water content has a direct effect on the stimulation of EIB, more research would have to be done to fully ensure that controlling temperature and water content should be standard in testing.

Control of $\dot{V}_E$ was also a key factor for the superiority of the EVH (Dickinson *et al.*, 2006). During the ECT, target HR ranged between 96 to 104% 85% HR_{max}, with EVH target $\dot{V}_E$ ranged between 79 to 99% 80% MVV. From our data, the ECT had much greater control of target HR compared to target ventilation for EVH. The lack of control comes down to the EVH being a voluntary action compared to a treadmill protocol. Although all the participants achieved a level of ventilation over the minimum rate of 21 times FEV_1 (Brummel *et al.*, 2009), the failure of participants achieving the target 30 times FEV_1 may have caused an under diagnosis in the EVH results. Although target HR during the ECT was controlled, it does not take into account the $\dot{V}_E$ during testing. Research by Trumper

et al. (2009) cast doubt on the HR based protocol for the ECT. Trumper *et al.* (2009) concluded that using a percent predicted HR ECT does not allow for the physical variances seen within the athletic population. Although the ATS state that 80 to 90% HR_{max} protocol is sufficient to induce 40 to 60% MVV, physical variances may affect these ratios.

Spirometry remains a key part of the screening process, and an area in which previous research has failed to follow current guidelines (Stickland *et al.*, 2010). Our results found that peak falls in FEV_1 ranged throughout the sample times emphasising the need to sample regularly. Slightly askew from the current guidelines, the most peak falls post EVH occurred after 3 min, which is currently an optional time point suggested by the ATS (ATS., 1999).

5.4 Clinical Interpretation

With the high prevalence of EIB in the athletic population, it still remains important to screen even those who have no previous history of asthma. Out of the seventeen participants screened, 53% achieved a fall in FEV_1 ≥10%. Our results suggest that both the ECT and EVH are sensitive enough tests for stimulating the EIB response, as long as both are performed under the right conditions.

Trumper *et al.* (2009) suggests that determining an individual's aerobic capacity should be achieved prior to the ECT. Completing this would add an extra step to the current simple screening process, but would create a more accurate ECT as pre-determined exercise intensity would already be known. From our data, this step would not be required for general screening, but for elite athletes, it may provide more accuracy during the screening process.

5.5 Strengths, Limitations and Future Research

The key strength of this research was the testing standards. As suggested by Stickland *et al.* (2010), a key criticism of previous research was the need to standardise the methods when screening athletes. In this study, all tests were performed to a high standard following the current ATS guidelines for the ECT and the guidelines for EVH (ATS., 1999). Previous research also failed to follow the standards for spirometry (Wilber *et al.*, 2000; Rundell *et al.*, 2004; Dickinson *et al.*, 2006; Trumper *et al.*, 2009). This study address this by completing the standard model with two extra sample times at 0 and 3 min post provocation test. During EVH tests, 10 out of the 17 participants experienced their peak fall in FEV_1 at 3 min post, implying the need to perform spirometry earlier than the recommended 5 min post.

Our research had some limiting factors. The main factor was the participant number. During the study, participant recruitment was restricted by the number of University level athletes interested in the study. A larger sample size would be more create a greater image of the current condition of EIB in University level athletes, but also strengthen the data analysis. A larger sample may find a different result compared to our research.

Future research following the practice employed in this study should look to increase the sample size to strengthen the data. Although our data was sufficient, a greater number of participants would allow for a more accurate analysis. Measuring the air water content during the ECT would give a definitive answer to determine whether the environmental conditions matched that seen in the EVH. Although RH can be controlled using an environmental chamber, literature states a temperature of <10˚C is sufficiently dry (Anderson, 2011). Finally, due to software issue we were unable to get the forced

expiratory flow (FEF_{25-75}) values during spirometry. Although not a direct measure of EIB, more respiratory values would have added to the data sets. Overall, the areas for future research should not have caused false readings within our results.

Although the results of our study are different to those found in previous research, some limitations must be considered. The conditions that the ECT was performed in were specifically designed to stimulate the EIB response, rather than related to a sports specific format. The ability to control air temperature

CHAPTER SIX

CONCLUSION

In summation, the current study aimed to determine whether there was a significant difference between the ECT and EVH when testing athletes following current guidelines. Out of the 17 participants who completed both tests, nine experienced a fall ≥10%, suggesting the presence of EIB. When comparing peak falls in FEV_1 achieved during the ECT and EVH, there was no significant difference, which suggests that both methods can be used when testing athletes for EIB. However, our Bland and Altman plot revealed poor agreement, meaning limitation of our study may mean our results are more inconclusive.

Future research needs to develop on from the model used within this study by increase the size of the participants tested to strengthen the data set. Although we found a significant difference between the ECT and EVH for ΔFEV_1 across the post-test spirometry, a larger sample size may provide a clearer answer to the research aims.

Data collection of $\dot{V}_e$ during the ECT may also provide an added perspective on the ventilation achieved compared to the target HR, as it does not allow for physical variances which may occur in high level athletes. Pre testing for HR would add an addition step but also give an accurate target HR for the ECT.

The aim of the study was to compare the ECT and EVH when following current guidelines. From our results, we cannot conclude whether the ECT or EVH is a more superior test. Although we found a significant difference between the ECT and EVH for FEV_1 across the post-test spirometry, peak FEV_1 had no significant difference, and with poor agreement, our results remain inconclusive due to limitations of the study.

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APPENDICES

Appendix 8.1 – Health History Questionnaire

UNIVERSITY OF GLOUCESTERSHIRE

SPORT AND EXERCISE LABORATORIES

Health Questionnaire

About this questionnaire:
The purpose of this questionnaire is to gather information about your health and lifestyle. We will use this information to decide whether you are eligible to take part in the testing for which you have volunteered. It is important that you answer the questions truthfully. The information you give will be treated in confidence. Your completed form will be stored securely for 5 years and then destroyed.

Section 1, which has been completed by the tester, provides basic information about the testing for which you have volunteered. Sections 2 to 7 are for you to complete: please circle the appropriate response or write your answer in the space provided. Please also complete section 8. Sections 9 and 10 will be completed by the tester, after you have completed sections 2 to 8.

Section 1: The testing (completed by tester)

To complete the testing for which you have volunteered you will be required to undertake:

Moderate exercise (i.e., exercise that makes you breathe more heavily than you do at rest but not so heavily that you are unable to maintain a conversation) ☐

Vigorous exercise (i.e., exercise that makes you breath so heavily that you are unable to maintain a conversation) ☐

The testing involves:

Walking	☐	Generating or absorbing high forces through your arms	☐
Running	☐	Generating or absorbing high forces through your shoulders	☐
Cycling	☐	Generating or absorbing high forces through your trunk	☐
Rowing	☐	Generating or absorbing high forces through your hips	☐
Swimming	☐	Generating or absorbing high forces through your legs	☐
Jumping	☐		

Section 2: General information

Name: ..... Sex: M F Age:

Height (approx.): ..... Weight (approx.):

Section 3: Initial considerations

1. Do any of the following apply to you?		No	Yes
a)	I have HIV, Hepatitis A, Hepatitis B or Hepatitis C		
b)	I am pregnant		
c)	I have a muscle or joint problem that could be aggravated by the testing described in section 1		
d)	I am feeling unwell today		
e)	I have had a fever in the last 7 days		

(If you have answered "Yes" to question 1, go straight to section 8)

Section 4: Habitual physical activity

2a.	Do you typically perform moderate exercise (as defined in section 1) for 20 minutes or longer at least twice a week?	No	Yes
2b.	Have you performed this type of exercise within the last 10 days?	No	Yes
3a.	Do you typically perform vigorous exercise (as defined in section 1) at least once a week?	No	Yes
3b.	Have you performed this type of exercise within the last 10 days?	No	Yes

Section 5: Known medical conditions

4.	Do any of the following apply to you? a) I have had Type 1 diabetes for more than 15 years b) I have Type 1 diabetes and am over 30 years old c) I have Type 2 diabetes and am over 35 years old	No	Yes
5.	Have you ever had a stroke?	No	Yes
6.	Has your doctor ever said you have heart trouble?	No	Yes
7.	Do **both** of the following apply to you? a) I take asthma medication b) I have experienced shortness of breath or difficulty with breathing in the last 4 weeks?	No	Yes
8.	Do you have any of the following: cancer, COPD, cystic fibrosis, other lung disease, liver disease, kidney disease, mental illness, osteoporosis, severe arthritis, a thyroid problem?	No	Yes

(If you have answered "Yes" to any questions in section 5, go straight to section 8.)

Section 6: Signs and symptoms

9.	Do you often have pains in your heart, chest, or the surrounding areas?	No	Yes
10.	Do you experience shortness of breath, either at rest or with mild exertion?	No	Yes
11.	Do you often feel faint or have spells of severe dizziness?	No	Yes
12.	Have you, in the last 12 months, experienced difficulty with breathing when lying down or been awakened at night by shortness of breath?	No	Yes
13.	Do you experience swelling or a build up of fluid in or around your ankles?	No	Yes
14.	Do you often get the feeling that your heart is racing or skipping beats, either at rest or during exercise?	No	Yes
15.	Do you regularly get pains in your calves and lower legs during exercise that are not due to soreness or stiffness?	No	Yes
16.	Has your doctor ever told you that you have a heart murmur?	No	Yes
17.	Do you experience unusual fatigue or shortness of breath during everyday activities?	No	Yes

(If you have answered "Yes" to any questions in section 6, go straight to section 8)

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Section 7: Risk factors

18. Does **either** of the following apply to you? a) I smoke cigarettes on a daily basis b) I stopped smoking cigarettes on a daily basis less than 6 months ago	No	Yes
19. Has your doctor ever told you that you have high blood pressure?	No	Yes
20. Has your doctor ever told you that you have high cholesterol?	No	Yes
21. Has your father or any of your brothers had a heart attack, heart surgery, or a stroke before the age of 55?	No	Yes
22. Has your mother or any of your sisters had a heart attack, heart surgery, or a stroke before the age of 65?	No	Yes
23. Do **any** of the following apply to you? a) I have had Type 1 diabetes for less than 15 years b) I have Type 1 diabetes and am 30 or younger c) I have Type 2 diabetes and am 35 or younger	No	Yes

Section 8: Signatures

Participant: ..... Date: .....

Guardian*: ..... Date: .....
(*Required only if the participant is under 18 years of age)

Section 9: Additional risk factors (to be completed by the tester if relevant)

24. Is the participant's body mass index >30 kg/m^2?	No	Yes
25. Has the participant answered no to questions 2a **and** 3a?	No	Yes

Section 10: Eligibility (to be completed by the tester)

26. Is the participant eligible for the testing?	No	Yes

Name (of tester): .....

Signature: ..... Date: .....

N.B. A flow chart showing how to make a decision about accepting or excluding a participant is on the lab wall near the door and in Appendix 7 of the Sport and Exercise Laboratories General Procedures Document.

3

Preparing and processing pre-test health questionnaires

Introduction

These notes should be read in conjunction with the standard Health Questionnaire of the Sport and Exercise Laboratories. They are intended to assist staff and students with a) preparing a health questionnaire for distribution to a potential participant and b) processing the completed questionnaire. The questionnaire is designed to gather the information needed to decide whether an individual is or is not eligible for a particular set of testing. This information is highly confidential and should be handled accordingly. During the course of a project or sequence of testing, it is the tester's responsibility to ensure that all completed health questionnaires are kept under lock and key and that the information they contain remains confidential. On completion of the project or sequence of testing, these questionnaires should be submitted to a technician, who will store them for 5 years for insurance purposes.

Preparing the questionnaire

First you need to summarise the cardiorespiratory demands of the testing by indicating whether it involves moderate or vigorous exercise. You should tick the moderate box for sub-lactate threshold exercise and the vigorous box for supra-threshold exercise or when testing is likely to invoke a marked cardiorespiratory response. For example, it would be appropriate to tick the vigorous box if the testing involves cold-water immersion, sustained isometric muscle actions or sustained exercise in an unusually hot or humid environment. If cardiorespiratory demands of the testing are minimal, you should not tick either box. However, if you are unsure you should err on the side of caution. Similarly, if you are unsure whether the exercise involved in a particular set of testing will be sub- or supra- threshold, tick the vigorous box. Next you need to summarise the musculo-skeletal demands of the testing by naming the activity and giving an indication of the forces involved in the testing so that the participant can make a judgement about whether any physical problem they have is likely to be aggravated. If you are unsure, err on the side of caution.

Processing the completed questionnaire

The process all laboratory users are expected to follow to reach a decision about whether a particular participant is eligible for testing is outlined below. This process is closely aligned with Olds and Norton's (1999) interpretation of the American College of Sport Medicine's Guidelines for Exercise Testing and Prescription (ACSM, 1995, 2000). It is underpinned by two key principles: first that the risk of a cardiac or other potentially fatal event occurring in response to exercise is low in individuals who are accustomed to meeting the cardiorespiratory demands of the exercise; second that the risk of such an event occurring in an unaccustomed individual depends on their age, whether they have particular medical conditions or show signs or symptoms of cardiovascular or pulmonary disease, and how many risk factors for cardiovascular disease they have. The process itself comprises a series of sequential steps.

1) Automatic exclusions

Section 3 covers blood-borne diseases, pregnancy, muscle or joint problems, recent fever or feeling unwell on the day. If the participant answers 'Yes' to any of the criteria they are automatically excluded.

2) Cardiorespiratory demands

Section 4 of the standard questionnaire summarises how often they typically exercise and when they last performed moderate or vigorous exercise. An individual should be deemed to be accustomed to a particular intensity of exercise if they typically experience it at least twice a week for moderate or once a week for vigorous exercise and have done so within the last 10 days. Individuals who show themselves to be accustomed to moderate exercise need to be screened further if the testing involves vigorous exercise but accept them if it involves moderate exercise. All participants are eligible for testing where the cardiorespiratory demands are minimal (for which neither the moderate nor the vigorous box would be ticked in section 1).

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3) Known medical conditions
If there are any "yes" responses in section 5 exclude the participant, otherwise go to step 4.

4) Signs and symptoms of cardiovascular or pulmonary disease
If there are any "yes" responses in section 6 exclude the participant, otherwise go to step 5.

5) Age and sex
If the participant is older than 44 and male, or older than 54 and female, and the testing involves moderate exercise only, accept the participant or if the exercise is vigorous exclude the participant. If they are younger than 45 and male, or younger than 55 and female, proceed to step 6.

6) Risk factors for cardiovascular disease
The tester should have completed section 9. To calculate the individual's body mass index (BMI), divide their body mass in kg by their height in cm squared. A BMI of >30 kg/m^2 constitutes one risk factor. To classify the participant as sedentary or otherwise, use the information from section 4: a "no" response to question 2a **and** 3a constitutes one risk factor.

In sections 7 and 9 if there is one or less "yes" response, accept the participant. If there are two or more "yes" responses and the testing involves moderate exercise only, accept the participant or if it involves vigorous exercise, exclude the participant.

7) Signatures
Accepting or excluding a participant involves answering "yes" or "no" in section 10. Then print your name and sign and date the form. You then need to explain your decision to the participant.

It is sufficient for all participants (except those who report one or more signs or symptoms of cardiovascular or pulmonary disease), to provide a brief oral explanation of why they have been excluded. For those with two or more signs or symptoms, Appendix 8 contains a standard letter warning that the signs and symptoms listed on the questionnaire are not definitive indicators of disease and inviting the excluded participant to discuss with their GP the sensations or events they have reported.

Appendix 7 is a flow diagram showing how to process the completed questionnaire. The principle is that the processing stops when a decision to accept or exclude the participant can be made. Often this point will be reached after two or three steps. Performing all seven steps would only be necessary for testing involving moderate or severe exercise for which the potential participant is young and sedentary, with no known medical conditions or signs and symptoms.

<u>Routine testing vs. specific projects involving special populations</u>
Participants who would normally be excluded from a particular type of testing may be eligible provided the testing is conducted under medical supervision (e.g. in a cardiac rehabilitation programme). Projects involving high-risk populations, or vigorous exercise in moderate risk populations, involve medically qualified personnel and are most likely to be conducted in a hospital environment.

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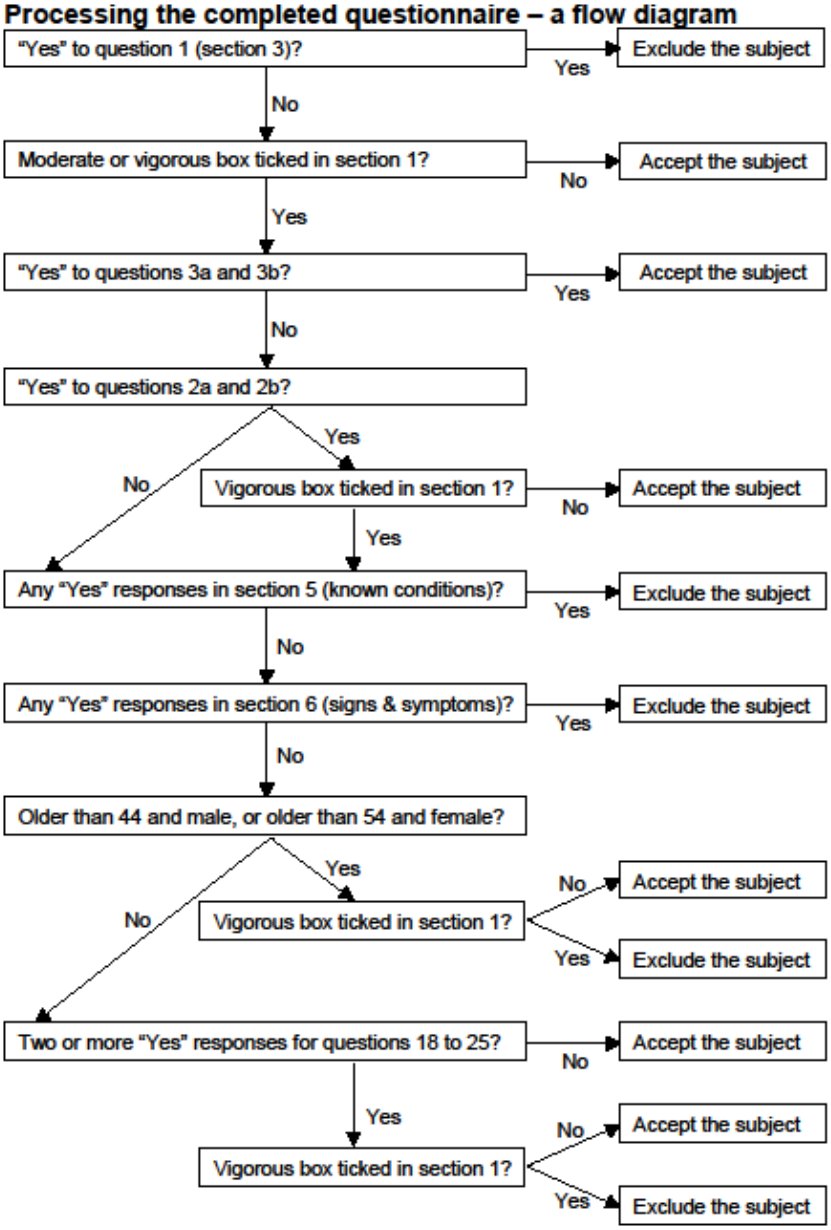
Processing the completed questionnaire – a flow diagram



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Appendix 8.2 – Respiratory Clinic Health Questionnaire

Respiratory clinic health questionnaire
To be completed in addition to the Sport & Exercise Laboratories Health Questionnaire

Contraindications for bronchodilator testing (from Cooper, 2011)

The number of contraindications to administering short-acting β_2-sympathomimetics by inhalation is limited and the risk of a single administration, for diagnostic purposes, is minimal (Insulander *et al.*, 2004; Kallergis *et al.*, 2005). Please consider the following medical conditions and the associated questions below.

1. ***Thyrotoxicosis;***
    a. *Are you being treated for an overactive thyroid gland?*
2. ***Heart failure;***
    a. *Are you being treated for heart failure, have you ever had heart treatment of any sort?*
3. ***Hypertension;***
    a. *Do you have high blood pressure?*
4. ***Tachydysrhythmias (can be provoked by β_2-agonists);***
    a. *Have you ever had any abnormal heart rhythm problems, do you get palpitations?*
5. ***Decreased glucose tolerance;***
    a. *Do you suffer from high blood sugars?*
6. ***Unstable diabetes mellitus; and***
    a. *Are you diabetic; was your doctor happy with your last check up?*
7. ***Concomitant use of cardiac glycosides.***
    a. *Are you taking a cardiac glycoside i.e. digoxin?*

Please indicate below if you have been diagnosed with any of the above medical conditions.

YES ☐ NO ☐

Signatures:

Participant name:..... Signature..... Date:.....

Guardian name*:..... Signature..... Date:.....

(*Required only if the participant is under 18 years of age.)

Eligibility (to be completed by the tester)

A bronchodilator **MUST NOT** be administered to participants who have ticked **'YES'** in response to questions 1 – 7 above. Any **YES** response excludes the participant from airway challenge testing.

Is the participant eligible for the testing? No Yes

Name of tester: Dr Stephen C. How

Signature: ..... Date:

Dr Stephen C. How 2014

Appendix 8.3 – Research Participant Information Sheet

UNIVERSITY OF GLOUCESTERSHIRE
at CHELTENHAM and GLOUCESTER
Exercise and Sport Research Centre
University of Gloucestershire
Oxstalls Lane
Gloucester
GL2 9HW

RESEARCH PARTICIPANT INFORMATION SHEET

EXERCISE AND SPORT RESEARCH CENTRE

Title of Study: A comparison of two challenge methods for the identification of exercise-induced asthma

Study Investigator(s): Mr Joe Ffoulkes and Dr Stephen C. How

Participant Name: _____

SUMMARY OF THE RESEARCH STUDY

The prevalence of exercise-induced asthma (EIA) in elite football may be as high as 30 %. Consequently more and more clubs are screening their players for EIA to ensure airway health is not compromised. This study will compare two recognised screening methods for the assessment of EIA. The first is a six minute, high-intensity, exercise challenge at an ambient temperature of ≤10 °C. The second is a seated breathing challenge that will require participants to breathe fast and deep for six minutes into a face mask. Before and after both challenges lung function will be measured using spirometry. A total of three visits to the laboratory will be necessary; one for each challenge test and another (visit 3) for the assessment of cardiorespiratory fitness.

WHAT WILL MY PARTICIPATION INVOLVE?

Visit 1 and 2

Spirometry

Your lung function will be assessed using a device called a spirometer whilst you are seated in a chair. You will be asked to fill your lungs before blowing out hard through a tube connected to the spirometer. When your lungs are empty, you will be asked to breathe in again as fast as possible until your lungs are full. The spirometer will measure how quickly you can fill and empty your lungs, and how much air your lungs can hold. You will do this on a number of occasions to ensure accurate results.

Eucapnic voluntary hyperpnoea (EVH) test

The EVH test utilises dry air to mimic the airway dehydration associated with heavy breathing during exercise. The EVH test requires you to wear a face mask that is attached to the EVH device by a plastic tube. You will be required to breathe in and out at 85 % of your maximal breathing rate for 6 minutes. The air mixture that you will breathe contains a higher than normal concentration of carbon dioxide (5%) and this will prevent you from experiencing the dizziness normally associated with hyperventilating. The EVH test is quite demanding and will feel unnatural; however, as you progress you will develop a rhythm and start to find the test easier. It is permissible to take short breaks to swallow as at times your throat will feel dry. Your lung function will be assessed before and at various intervals after the EVH test using the spirometry procedures described above. The EVH test is recommended by the IOC for the diagnosis of exercise-induced asthma in elite athletes.

Exercise challenge test

The exercise challenge test will take place in an environmental chamber with an air temperature of ≤10 °C. You will be asked to run on a treadmill for 8 minutes at a speed that invokes a heart rate response of 80-90% of your predicted maximum based on the equation 220-age. You will wear a nose clip throughout the test to promote mouth breathing. Your lung function will be assessed before and at various intervals after the exercise test using the spirometry procedures described above.

Visit 3

Incremental Exercise Test to Exhaustion

You will be asked to run on a treadmill on two occasions separated by 10 minutes. On the first occasion the treadmill speed will start slowly (8-10 km/h) and will increase by 1 km/h every 3 minutes until you are close to exhaustion. During the run we will take continuous measurements of the air you breathe out and your heart rate. Your subjective perceptions of effort and your blood lactate levels will be taken at the end of each 3 minute stage. During the test you will breathe into a rubber mask attached to gas analysis equipment. A heart rate monitor will be placed around your chest to monitor your heart rate and at the end of each stage a small pin prick of blood will be taken from your thumb for analysis of your blood lactate levels. On completion of the first test you will sit for 10 minutes to recover. Next you will complete a maximal treadmill test that will last between 6 and 10 minutes. You will run at a constant speed that is 2 km/h lower than the final speed achieved in the first test. Every minute we will increase the gradient of the treadmill by 1 % until you can no longer continue. We will measure your expired air and heart rate as described above and will take a blood lactate sample on completion of the test.

ARE THERE ANY RISKS?

Lung function tests

Sterile mouthpieces/filters and tubing are used and there are no foreseeable risks associated with performing the lung function tests.

Incremental exercise

As professional athletes you will all be familiar with the sensations and discomfort associated with high intensity exercise. However, it is necessary to point out that there are foreseeable risks associated with this type of exercise. These risks include temporary fatigue, shortness of breath, and muscle soreness. There is also a remote risk of sudden death (approx. 1 per 100,000 tests). You will complete a health questionnaire and will be asked to report any reason why you should not perform exercise. If any reason you should not exercise is made apparent you will not be allowed to participate in the study. You will be monitored continuously during and after exercise and will be advised to stop exercising if experiencing dizziness or chest pain.

Eucapnic voluntary hyperventilation (EVH) test and exercise challenge test

The aim of both of these tests is to create the physiological conditions necessary to be able identify exercise-induced asthma in susceptible individuals. Consequently, participants may experience shortness of breath, wheezing and chest tightness either during, or on completion of these tests. Your lung function will be monitored for 30 minutes after each test and if necessary you will be advised to take an inhaler of bronchodilator medication to relieve any symptoms you may experience.

Blood Sampling

You may feel some discomfort when the incision is made to your thumb. Bruising may occur and there is an unlikely chance of infection at the sampling site. To minimise this risk the sampling site will be cleaned with an alcohol swab and the investigator will wear surgical gloves.

ARE THERE ANY BENEFITS?

By participating in this study we will be able to identify whether you are at risk from exercise-induced asthma. If we identify that you have exercise-induced asthma a treatment programme can be put in place to ensure the health of your lungs, which may also be important from a performance perspective.

ARE THERE ANY COSTS?

No.

WILL I BE PAID FOR MY PARTICIPATION IN THE STUDY?

Your club has requested that you take part in this study as part of a general health screening program and your normal financial arrangement with the club will continue. No additional remuneration will be due to you.

IF I DECIDE TO START THE STUDY, CAN I CHANGE MY MIND?

Your decision to participate in this research is entirely voluntary. You may choose not to participate. If you do decide to participate, you may change your mind at any time without penalty or loss of benefits that you had prior to the study. You will be told of any new and significant findings that may affect your willingness to continue. If you wish to withdraw please contact the study investigator using the contact details below. You should discuss any decision to withdraw with the club medical team in the first instance.

WILL MY CONFIDENTIALITY BE PROTECTED?

The results from your participation in this study will be shared with the Rugby League All Gold's medical doctor. The researchers may also use information learned from this study in scientific journal articles or in presentations. You will be identified by number only and none of the information will identify you personally. The data will be stored for an indefinite period of time at the School of Sport and Exercise (University of Gloucestershire) and will not be released without written permission or unless required by law.

WHAT IF I HAVE QUESTIONS?

If you have questions about this research, please contact the study investigator, Dr Stephen How, on 01242 715317 or email show@glos.ac.uk.

Appendix 8.4 – Medication Withhold Times

Table 1 Required medication withholding periods for medications before exercise tests

	Factor	Withholding Period
Inhaled agents	Short acting bronchodilators (isoproterenol, isoetharine, metaproterenol, albuterol, levalbuterol, terbutaline) (e.g. Proventil® or Ventolin®)	8 hr
	Inhaled anticholinergics or combination products (e.g. Atrovent® or Combivent®)	1 week
	Long acting inhaled bronchodilators (salmeterol, formoterol) (e.g. Serevent® or Foradil®)	2 weeks
	Inhaled corticosteroid/long acting inhaled bronchodilator combination (e.g. Advair®)	4 weeks
Oral bronchodilators	Theophylline	24 hr
	Intermediate-theophylline	48 hr
	Long acting theophylline	48 hr
	Standard β-agonist tablets	24 hr
	Long acting β-agonist tablets	48 hr
Corticosteroids	There is no washout for topical corticosteroids applied to skin unless they are high potency steroids	4 weeks
Other medications	Hydroxyzine, cetirizine (and other antihistamines)	72 hr
	Tiotropium bromide	72 hr
	Nasal corticosteroids	1 week
	β-blockers	1 week
	Cromoyn sodium	2 weeks
	Nedocromil	2 weeks
	Leukotriene modifiers	6 weeks
Foods	Coffee, tea, cola drinks, chocolate (caffeinated foods)	12 hr
Strenuous exercise or exposure to cold air to a level that would be expected to interfere with challenges		12 hr
Tobacco		6 hr

From Anderson et al. (2010) *Respiratory Research*, 11:120