Diagnosis and management of exercise induced bronchoconstriction in athletes

This thesis is submitted for the Degree of PhD in Sport & Exercise Science and Sports Therapy

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Signed: ……. Date: …….

DECLARATION

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University or Institution of learning.

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CONTENTS

LIST OF ABBREVIATIONS

AHR	Airway hyperresponsiveness
ANOVA	Analysis of variance
ARIA	Allergic rhinitis and its impact on asthma
ASL	Airway surface liquid
ATS	American Thoracic Society
bpm	Beats per minute
BHR	Bronchial hyperresponsiveness
BTS	British Thoracic Society
°C	Celsius degree
cm	Centimetre
CO_2	Carbon dioxide
CONT	Control trial
cmH_2O	Centimetres of Water Column at 4°C
EIA	Exercise induced asthma
EIB	Exercise induced bronchoconstriction
EILO	Exercise induced laryngeal obstruction
eNOS	Endothelial nitric oxide synthase
ERS	European Respiratory Society
EX	Exercise challenge
EVH	Eucapnic voluntary hyperpnoea
EVH+	EVH positive

EVH-	EVH negative
FeNO	Fraction of exhaled nitric oxide
FEV_1	Forced expiratory volume in one second
FEV_1/FVC	FEV_1: FVC ratio
FVC	Forced vital capacity
GB	Great British
GP	General practitioner
HDAC2	Histone deacetylase 2
HME	Heat and moisture exchanger
HR	Heart rate
HR peak	Peak heart rate
HR max	Maximum heart rate
ICS	Inhaled corticosteroids
iNOS	Inducible nitric oxide synthase
INT	Intervention
IOC-MC	International Olympic committee – medical commission
IQR	Interquartile range
Kg	Kilogram
Km	Kilometres
Km/hr	Kilometres per hour
L	Litre
LABA	Long acting β_2-agonist
LCM	Leicester cough monitor

LCQ	Leicester cough questionnaire
L/min	Litre per minute
L/s	Litres per second
LOA	Limits of agreement
LTRA	Leukotriene receptor agonist
m	Metre
MASK	HME mask trial
MDI	Metered dose inhaler
min	Minute
MiniAQLQ	Mini asthma quality of life questionnaire
MiniRQLQ	Mini rhinoconjuntivitis questionnaire
mg/L	Milligram per litre
mL/min	Millilitres per minute
mL/s	Millilitres per second
ml/kg/min	Millilitres per kilogram of body weight per minute
mmHg	Millimetres of mercury
MVV	Maximal voluntary ventilation
n	Number
NHS	National health service
NNT	Number needed to treat
N_2	Nitrogen
NI	No intervention
NO	Nitric oxide

NOS	Nitric oxide synthase
nNOS	Neural nitric oxide synthase
O_2	Oxygen
p	Significance level
PEF	Peak expiratory flow
PNIF	Peak nasal inspiratory flow
ppb	Particles per billion
r	Reliability coefficient
RH	Relative humidity
RPE	Perceived exertion
SABA	Short acting β_2-agonist
SHAM	Sham mask trial
SD	Standard deviation
Sig.	Significance level
SPSS	Statistical package for social sciences
TUE	Therapeutic use exemption certificate
UEFA	Union of European Football Associations
UK	United Kingdom
URTI	Upper respiratory tract infection
VAS	Visual analogue score
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_2$ max	Maximum oxygen consumption

$\dot{V}O_2$ peak	Peak oxygen consumption
W	Watts
WADA	World antidoping agency
X^2	Chi-squared
yr(s)	Year(s)
β_2	Beta-2
µ	Nanogram

FIGURES LEGENDS

Chapter 4. The impact of detecting and treating EIB in elite footballers

Chapter 5. Evaluating and managing EIB, respiratory health and overall wellbeing in elite swimmers

Chapter 6. A Heat and Moisture Exchange Mask to Reduce Exercise Induced Bronchoconstriction

Figure 6.2. A: Participant wearing the SHAM during an EX; B: MASK. C: SHAM.

Figure 6.3. Leicester Cough Monitor (LCM).

Figure 6.4. Maximum % fall from baseline in FEV_1 post exercise challenge. * indicates significant difference to CONT. FEV_1, forced expiratory volume in 1 s. Dashed line represents the threshold for a positive challenge.

Figure 6.5. Cough per hour over 24-hour monitoring period.

LIST OF TABLES

Chapter 5. Evaluating and managing EIB, respiratory health and overall wellbeing in elite swimmers

Chapter 6. A Heat and Moisture Exchange Mask to Reduce Exercise Induced Bronchoconstriction

PUBLICATIONS ARISING FROM THIS THESIS

Chapter 3.

Jackson, A. R., Hull, J. H., Hopker, J. G., & Dickinson, J. W. (2017). Diagnosing Exercise Induced Bronchoconstriction: A Comparison of Eucapnic Voluntary Hyperpnoea And Exercise in Low Humidity. *Medicine and Science in Sports and Exercise*, **49(5S)**, 17. **ACSM 2017 International Student Award.**

Chapter 4.

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Chapter 5.

Hull, J. H., **Jackson, A. R**., Hopker, J. G., Greenwell, J., & Dickinson, J. W. (2017). Maximizing Respiratory Health in Elite Swimmers - A Systematic Approach to Optimize Total Airway Health. *Medicine and Science in Sports and Exercise*, **49(5S)**, 318.

Chapter 6.

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ABSTRACT

It is now well established that there is a high prevalence of exercise-induced bronchoconstriction (EIB) amongst athletes. There is still debate however regarding the optimal method of diagnosis in this group and current treatment guidelines are mainly based on recommendations for the general population with asthma. The aim of this thesis was to address these gaps in the literature by investigating methods of diagnosing EIB, the impact of standard asthma treatment upon airway inflammation, EIB severity, health and performance in elite athletes, and the effect of reducing environmental exposure upon EIB severity.

Study 1 compared two objective methods of diagnosing EIB; eucapnic voluntary hyperpnoea (EVH) and an exercise challenge (EX) on a cycle ergometer in a dry (26%RH) environment. Twenty-seven recreational athletes completed both an EVH and EX challenge in a randomised order. Challenges were deemed positive if there was a fall in FEV_1 of $\geq$ 10% from pre to post challenge. Six participants were positive to EVH (% fall in FEV_1 16 ± 5%, range -11 to -25%), of these, only two were positive to Ex (both with an 11% fall in $FEV_{1)}$. These findings demonstrated that EVH provides greater sensitivity than a standardised EX challenge in a dry environment in the diagnosis of EIB.

Study 2 reports results of the largest EIB screening in elite footballers to date and evaluates the prevalence of EIB and the impact of standard asthma therapy on airway health and exercise performance. Ninety-seven male professional football players completed an EVH challenge. Players demonstrating a positive result (EVH+) were prescribed standard asthma therapy and underwent repeat assessment after 9 weeks of treatment. Eight players (3 EVH+, 5 EVH-) completed a $\dot{V}O_2$ peak test at initial and follow-up assessments. Of the 97 players, 27 (28%) demonstrated EVH+. Seven of the 27 (24%) EVH+ players attended follow-up and demonstrated improved post-challenge spirometry (FEV_1 post-test; pre = -22.9 ± 15.4%,

post = -9.0 ± 1.6%, p = 0.018). At follow-up $\dot{V}O_2$ peak improved by 3.4 ± 2.9 ml/kg/min in EVH+ players compared to 0.1 ± 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$ in EVH- players. It was concluded that elite footballers have a high EIB prevalence and treatment with inhaler therapy reduces EIB severity and may also lead to improved exercise performance.

In study 3 a three-part body of work with elite British swimmers was undertaken: (I) To investigate the effects of screening for EIB and treating appropriately on health and availability for training. (II) To monitor lung function, airway inflammation and respiratory symptoms in relation to a change in training environment from an indoor to an outdoor pool. (III) To report the findings of a systematic evaluation of total airway health in elite swimmers with EIB. A 75% prevalence of EIB was found in swimmers entering the GB funded programme. Treating this group with standard asthma therapy led to no differences in the percentage of time swimmers spent carrying out modified training in the 6 months post screening compared to the 6 months pre-screening (p = 0.17). No differences were found in FEV_1 (p = 0.41), FeNO (p = 0.12) or PNIF (p = 0.67) in response to a change in training environment to an outdoor pool. Despite being prescribed treatment for EIB on assessment 3 swimmers still demonstrated airflow obstruction at rest with bronchodilator reversibility of FEV_1 by 12.9 ± 7.7% above baseline. FeNO was reduced compared to initial consult (pre: 27.7 ± 15.1, post: 16.3 ± 6.5 ppb (p < 0.01). It was concluded that respiratory health in elite swimmers can be optimised through systematic assessment, however larger well controlled studies are still required to determine the impact of this approach upon performance and wellness.

Study 4 investigated if a heat and moisture exchange mask (HME) face mask could be effective in protecting against EIB in response to a cycle challenge in a cold, dry environment (9°C, 24% RH) in asthmatic individuals. Seventeen participants completed three EXs on a cycle ergometer wearing either an HME mask (MASK), a sham mask (SHAM) or no mask (CON) in a randomised order. There was a significant difference in the % fall in FEV_1

following EX (MASK: -6.00, SHAM: -10.00, CON: -13.00%, $p < 0.01$), with the % fall following CON greater than that of MASK ($p < 0.01$). Chapter 6 concluded that HME masks can attenuate EIB in individuals with asthma/ EIB when exercising in cold, dry environments.

This thesis concludes that case detection programmes for EIB should be established for athletes training and competing in sports which put them at risk of developing EIB. Standard asthma therapy is effective in the treatment of EIB in athletes, however more work is required to establish the long-term effects of treatment upon overall health and performance.

Chapter 1. Introduction and Literature Review

1.0 OVERVIEW

The benefits of regular exercise for maintaining good health have long been known. Exercise is now even prescribed as medicine in the treatment of various chronic diseases including psychiatric, neurological, metabolic, cardiovascular and pulmonary disease, musculoskeletal disorders and cancer (Pedersen and Saltin, 2015). It might be expected therefore that elite athletes would be the healthiest of all populations, however, this appears not to be the case when looking at respiratory health: One in four athletes have been found to report troublesome respiratory symptoms such as cough, wheeze and dyspnoea (Turcotte, et al. 2003); UK primary care physicians report that they come across an amateur athlete with exercise related respiratory symptoms at a frequency of once a month (Hull et al., 2009); Respiratory illness has been the most common athlete illness within TeamGB at recent Olympic games (Palmer-Green and Elliott, 2015); and there is a wealth of evidence demonstrating a much higher prevalence of exercise induced bronchoconstriction (EIB) amongst elite British athletes (21 – 68% (Dickinson et al., 2005; Levai et al., 2016)) , than their recreational counterparts (13% (Molphy et al., 2014)) and the general population 8 - 10% (AsthmaUK, 2014).

Regular physical activity is recommended for asthmatic individuals, because although exercise cannot improve lung function, an improved level of cardiorespiratory fitness is thought to reduce the risk of exacerbation during exercise due to a reduced ventilation (Ram, 2000) and exercise training may also have an anti-inflammatory effect in the lungs (Silva et al., 2010). At the opposite end of the spectrum however, it seems that there may be too much of a good thing and frequent, repeated periods of high ventilation in certain environmental conditions may in fact be disadvantageous to respiratory health (Weiss and Rundell 2011).

EIB is defined as the transient airway narrowing that occurs in association with physical activity in susceptible individuals. EIB has been reported to have a negative impact on both

health (i.e. deterioration in condition), athletic performance (Price et al. 2014; Stensrud et al., 2007; Brukner et al. 2007; Spiteri et al. 2014) and in severe cases heightened risk of mortality (Becker et al., 2004). With repeated exposure to high intensity exercise, coupled with potentially noxious training environments athletes are more at risk of developing EIB over the course of their careers (Knöpfli et al., 2007). These risk factors make EIB a particularly prevalent condition for many of the high participation sports in the UK such as swimming, athletics, cycling and football, where the ventilatory demand of the sport is high and training and competition often takes place the presence of aero allergens such as trichloramine, pollution, pollens and moulds. Such is the risk to endurance athletes in particular, one group has investigated if in fact this airway dysfunction in elite athletes should be classified as an occupational lung disease (Price et al., 2013).

Regulatory bodies of sport have a mandate of care to athletes and as such when the International Olympic Committee – Medical commission (IOC-MC), noticed an apparent increased prevalence of asthmatic athletes, from an increasing trend in the notification by athletes for the use of inhaled short acting β_2-agonists (SABA) (Fitch et al., 2008), they intervened. There were concerns that athletes without asthma or EIB were using SABAs, and to protect athlete health, in 2002 the IOC made it a requirement that any athlete wishing to use SABAs had to demonstrate evidence of asthma or EIB. The World Antidoping Agency (WADA) incorporate this into their Therapeutic Use Exemption Certificate (TUE) system and SABAs including salbutamol then remained on the prohibited list until 2010.

These changes in regulations, did not affect the prevalence of asthma and EIB within TeamGB between the 2000 and 2004 Olympic games (Dickinson et al., 2005), but did serve to highlight EIB as a relevant health issue amongst athletes and lead to improved guidelines for the management of EIB in athletes (Fitch et al., 2008). By screening TeamGB athletes for EIB using bronchoprovocation challenges, Dickinson et al., (2005) identified a large number of athletes (21%) who had previously been misdiagnosed with EIB and conversely

a high proportion (9%) of previously undiagnosed athletes demonstrating evidence of EIB. The authors concluded that the implementation of the IOC guidelines amongst TeamGB athletes had led to an improved level of care to these athletes.

The discrepancy between a prior diagnosis and objective evidence of EIB highlighted by Dickinson et al., (2005) is in part due to the non-specific nature of common respiratory symptoms such as cough, wheeze and dyspnoea. Research has consistently shown that respiratory symptoms during and after exercise correlate very poorly with objective evidence of airway narrowing (Rundell et al. 2001; Turcotte et al. 2003; Simpson et al., 2015). Despite clear guidelines created following the changes in the prohibited list (Carlsen et al. 2008; Fitch et al. 2008), following WADA relaxing the guidelines regarding SABAs in 2011, athletes continue to be misdiagnosed, with Ansley et al., (2012) reporting that 49% of elite footballers had an inappropriate diagnosis of EIB. Recent studies have also demonstrated a high proportion of athletes who are either asymptomatic or do not report respiratory symptoms as troublesome, when screened are susceptible to EIB (Dickinson et al., 2011; Molphy et al. 2014; Levai et al. 2016).

Due to the high prevalence of EIB, the difficulties with symptom diagnosis, its effect upon health and the potential impact on performance, some authors have called for screening for EIB to be implemented amongst athletic populations (Dickinson et al., 2005; Holzer and Brukner, 2004; Vakali et al., 2017). Before a screening programme can be put in place however, a number of stringent criteria must be met (Wilson and Jungner, 1968), which includes demonstrating the prevalence, having the ability to detect the condition of interest and also having an understanding of the impact in the population of interest (Wilson and Jungner 1968; Ansley et al., 2013; Hull et al. 2007; Hull and Rawlins 2016).

The high prevalence has been demonstrated in many sports (Larsson et al. 1993; Mannix et al. 1996; Helenius et al., 1998; Dickinson et al. 2005; Levai et al. 2016). Many of these

studies however have small sample sizes and have been focussed primarily on Olympic sports. Due to the nature of EIB it would appear to be a relevant condition in many more high participation sports and as such further investigation is required in sports such as football.

The best method and criteria to detect EIB remains somewhat debated. Eucapnic voluntary hyperpnoea (EVH) has been endorsed by the IOC-MC as the optimal bronchoprovocation challenge to diagnose EIB in elite athletes (Anderson et al., 2001) and has previously been reported to possess a high specificity and provide greater sensitivity in comparison to other airway challenges in the diagnosis of EIB in elite athletes (Dickinson et al. 2006; Holzer et al., 2002). However despite EVH often being termed as the gold standard, Hull et al., (Hull et al., 2016) suggested that the wide sensitivity and specificity along with apparent poor repeatability particularly in mild cases still prevent EVH from being termed the gold standard. There is also some discussion regarding the current 10% fall in FEV_1 following EVH being indicative of a positive test for EIB, with some authors suggesting that a cut off of 15% might be a more appropriate threshold in athletes (Price et al. 2016).

The impact of both untreated and treated EIB in athletes and the effects of both upon health and performance is largely unknown. Recommendations for the pharmacological treatment of EIB in athletes is predominantly based on guidelines for standard asthma care alongside expert opinion, due to the absence of adequately powered randomised clinical trials in elite athletes (Boulet and O'Byrne, 2015). Therefore, it is not known whether or not treating a screen detected athlete with EIB with standard asthma treatment will improve their airway health and reduce the risk of further exacerbation, potentially limiting disease progression.

As well as the impact upon health, in an elite athletic population, there is the potential for EIB and its subsequent treatment to have a significant impact on performance and therefore livelihood. It is fairly well established that therapeutic dosing of inhaled asthma therapies

has no ergogenic impact in healthy individuals (Pluim et al., 2011; Dickinson et al., 2014; Kuipers et al., 2008). There is limited evidence however that left untreated, EIB may have deleterious effects of performance (Stensrud et al., 2007) and therefore treatment with appropriate therapy may improve performance in athletes with EIB. The small number of studies examining this have shown promising results (Haverkamp et al. 2007; Brukner et al. 2007; Spiteri et al. 2014), however the paucity of evidence leaves a consensus unable to be drawn (Price et al., 2014).

Addressing undetected and untreated EIB may also have an impact on an athlete's general health. Upper respiratory tract infection (URTI) is the most common medical condition amongst athletes (Bermon, 2007) and there appears to be an association between a high prevalence of EIB (Bonini et al., 2015) and uncontrolled EIB (Helenius and Haahtela, 2000) and recurrent URTIs. There are however no studies which have investigated the effect of treating EIB on overall wellness in athletes.

This thesis will address some of the aforementioned gaps in the current body of knowledge to provide evidence that can be used to develop optimal strategies to manage EIB in athletes. Specifically, this thesis will look to investigate methods of diagnosing EIB, the impact of both short- and long-term standard asthma treatment upon airway inflammation, EIB severity, overall health and performance and the effect of reducing environmental exposure upon EIB severity.

1.1 What is Exercise-induced bronchoconstriction (EIB)?

"If from running, gymnastic exercises, or any other work, the breathing becomes difficult, it is called asthma" Aretaeus (81–138 AD) (Adams, 1856).

This phenomenon is now known as Exercise-induced bronchoconstriction (EIB) and is defined as the transient airway narrowing that occurs in association with physical activity in susceptible individuals (Weiler et al., 2007). In EIB, bronchoconstriction will peak within 3 to 15 minutes following exercise and then return to normal either spontaneously, usually within 30 - 45 minutes (Godfrey and Bar-Yishay, 1993), or with administration of treatment in the form of inhaled short acting β_2-agonist (SABA). EIB can be a sign of uncontrolled asthma, however it can also occur in those without clinical asthma, particularly in athletes (Carlsen et al. 2008) and those with rhinitis or atopy (Bousquet et al., 2012). As such, although EIB has in the past been used interchangeably with exercise induced asthma (EIA), the Thoracic Society Clinical Practice Guidelines (Parsons et al., 2013) recommended abandoning the term 'EIA', because exercise triggers bronchoconstriction and does not induce the clinical condition of asthma. It is now widely accepted that EIB occurs in athletes without accompanying features of asthma, often in the absence of respiratory symptoms (Boulet and O'Byrne, 2015) and it is thought that EIB has particular pathologic and clinical features making it a distinct clinical entity from EIB with asthma (Couto et al., 2017). Consistent with this opinion, Haahtela et al., (2008) identified two distinct phenotypes in elite Finish athletes; 'classical asthma' characterised by childhood asthma, responsiveness to methacholine, atopy and eosinophilic airway inflammation and 'another distinct phenotype' with late onset of symptoms, airway responsiveness to eucapnic voluntary hyperpnoea (EVH) but not always to methacholine, and a variable association with atopy and eosinophilic inflammation. Using latent class analysis, Couto et al., (2015) agreed with this finding and also concluded that there are two distinct phenotypes of asthma in athletes 'atopic asthma' (defined by the occurrence of atopy, increased levels of fraction of exhaled

nitric oxide (FeNO), rhinitis and other allergic co-morbidities) and ‘sports asthma’ (defined by the presence of exercise-induced respiratory symptoms and bronchial hyperresponsiveness (BHR) in the absence of allergic features). They also discovered that the type of sport in which an athlete participates was associated with the different phenotypes, with water and winter sport athletes having a three and nine-fold respectively increased risk of ‘sports asthma’. Indeed, a higher prevalence of EIB is seen within these sports (Dickinson et al., 2005) which supports the hypothesis that high intensity training, particularly in certain environmental conditions may lead an athlete to develop EIB.

Within this thesis, EIB is used to define EIB without accompanying general asthma including both ‘atopic’ and ‘sports’ asthma defined by Couto et al., (2015) above.

1.2 The pathogenesis of EIB

The pathogenesis of EIB is multifaceted and is still not completely understood. Currently, it is widely accepted that it is the exercise induced increase in ventilation which leads to the changes in airway physiology. At rest, inspired air is warmed and humidified through heat exchange in the nasal cavity, however when minute ventilation ($\dot{V}_E$) exceeds approximately 35 L/min there is a switch from a nasal to oral predominant breathing pattern (Niinimaa et al., 1980). As a result, during intense exercise where $\dot{V}_E$ can exceed 150 L/min, the airways are compromised in their ability to condition the air and are exposed to an increase in unconditioned (relatively cold and dry) air and depending upon which sport is being practiced, this may also be coupled with increased penetration from environmental irritants such as airborne allergens, ozone and chloramines. This leads to airway osmotic changes, epithelial injury, airway inflammation and neuronal activation (Couto et al., 2017) all of which are key events leading to EIB in susceptible individuals (Figure 1.1).

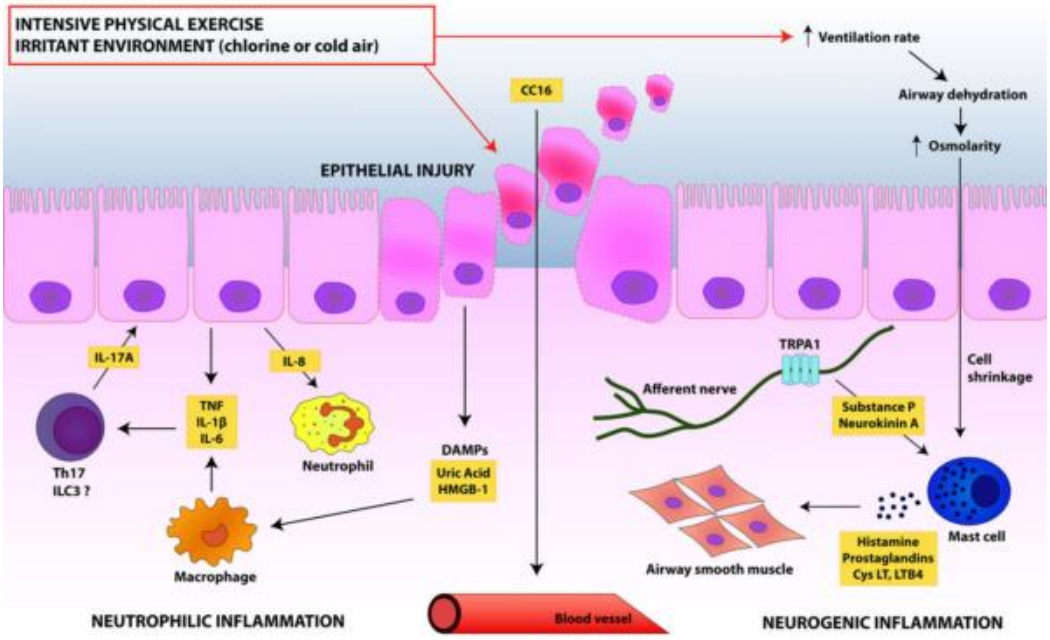

Figure 1.1. Mechanisms of EIB from Couto *et al*., (2017)

Historically there have been two main concepts regarding the acute development of EIB; the 'osmotic' and 'thermal' theories, both of which are built around the key factor being the increased ventilation leading to mucosal cooling and dehydration. Dehydration of the airway surface liquid (ASL) drives a local osmotic stimulus which causes cell shrinkage, triggering the release of inflammatory mediators such as histamine, prostaglandins, and cysteinyl leukotrienes from mast cells and eosinophils (Hallstrand et al., 2005), ultimately leading to airway smooth muscle contraction, airway obstruction (Anderson and Kippelen, 2005) and the release of mucus into the airways (Hallstrand et al., 2007). In addition, the thermal theory suggests that post exercise, following the cooling of the airways a rewarming takes place leading to vasodilation which contributes to the airway obstruction (McFadden Jr, 1990). These mechanisms explain how EIB is triggered in susceptible individuals, however do not explain the occurrence of EIB in athletes with no evidence of general asthma. Recently a hypothesis of airway injury caused acutely by the osmotic and mechanical stress of exercise has been put forward to explain the development of EIB in elite athletes (Anderson and

Kippelen, 2008). Repeated bouts of exercise induced hyperpnoea, particularly in noxious environments have been shown to lead to a continuous cycle of injury and repair of the bronchial epithelium (Karjalainen et al., 2000). This results in an increase of cellular inflammatory mediators, proinflammatory cells, airway remodelling and increase airway hyperresponsiveness (Anderson and Kippelen, 2008; Kippelen and Anderson, 2012). These modifications to airway structure and function may play a key role in the increased prevalence of EIB in endurance athletes who often train and compete in asthmogenic environments.

Airway smooth muscle is innervated by sympathetic and parasympathetic nerves and as such it has been suggested that dysfunction of the nervous system may contribute to the pathogenesis of EIB (Langdeau and Boulet, 2001). During exercise, airway cooling causes parasympathetic stimulation leading to bronchoconstriction and increased cholinergic inflammation (Wessler and Kirkpatrick, 2008). The mechanism behind modulation of bronchial tone and the possible role in the development of EIB in athletes requires further investigation, however it is thought that intensive endurance training increases parasympathetic tonus and modulation as a compensatory response to the sympathetic stimulation associated with frequent, intense exercise (Goldsmith et al., 1997). This not only induces the well-known bradycardia in athletes but may also increase basal bronchomotor tone and it is this autonomic dysregulation which is thought to be an etiologic factor for EIB (Langdeau and Boulet, 2001). Couto *et al*., (Couto et al., 2015) found that amongst swimmers, a positive response to a methacholine challenge (PD_{20}) was correlated with parasympathetic activity. Another group however concluded that this parameter showed only a weak correlation with PD_{20} and suggested that although parasympathetic activity may act as modulator of airway responsiveness, the increased prevalence of EIB observed in elite athletes is mostly due to the nature and content of the inhaled air and not exercise itself or neurogenic mechanisms. (Langdeau et al., 2000).

1.3 The prevalence of EIB

There is substantial data showing that EIB occurs very commonly in athletes at all levels. EIB has been reported to affect around 8-10% of the general population in the UK (AsthmaUK, 2014), whereas recreationally active individuals have been shown to have a 13% prevalence (Molphy et al., 2014) and in elite British athletes this number appears to be higher still; Dickinson *et al*., (2005) reported that the prevalence of EIB at the 2000 and 2004 Olympics was 21.2% and 20.7% respectively, with the highest prevalence (~ 40%) in endurance athletes and more recently Levai *et al*., (2016) found that the prevalence of EIB within elite British swimmers was 68%, which appears to be the highest reported in any athletic group.

With repeated exposure to high intensity exercise, coupled with potentially noxious training environments athletes are more at risk of developing EIB over the course of their careers. Knopfli *et al.*, (2007) concluded that athletes develop EIB quickly, at a rate of increase 195-286 times that of the normal rate for development of asthma. They assessed bronchial reactivity on three occasions over a two-year period in the Swiss national triathlon team; All athletes were free from respiratory disease at entry, but over the course of the study all developed incidence of increased bronchial reactivity and almost half developed EIB. As previously discussed, prolonged hyperpnoea along with environmental influences such as the presence of aero allergens play an important role in the development of EIB. This has implications for the top participation sports in the UK; Swimming, athletics, football and cycling (Sport England, 2016). In the UK athletes training and competing in running, cycling and football spend the majority of this time outdoors and over the course of a year will be exposed to varying environmental conditions: All athletes will be affected by poor air quality from pollution, cold and dry air and allergens such as pollens and moulds are also of concern to atopic athletes (Rundell and Sue-Chu, 2013). These sports also require high ventilation which enables more irritants to reach the distal airways.

There have been a limited number of studies looking into gender differences in the prevalence of EIB. It has been reported that female athletes have a higher prevalence of asthma than their male counterparts (Norqvist et al., 2015). However, despite a higher prevalence of respiratory symptoms in females, studies have shown no differences in evidence of EIB as assessed by a physician's diagnosis (Romberg *et al*., 2017), mannitol or sports specific challenge (Pignataro et al., 2017).

There is a wide range of prevalence of EIB reported in different sports around the world (Table 1.1). As well as this being down to differing demands of sports and the environments in which they are played, this may also be due in part to the methods of accessing EIB.

Table 1.1. Studies highlighting the prevalence of EIB in athletes

Population	n	Methodology	Prevalence (%)	Reference
Olympic winter sport athletes	170	Field test	23	(WILBER et al., 2000)
Cross-country skiers	42	Methacholine	55	(Larsson et al., 1993)
Cross-country skiers	171	Methacholine	14 - 43	(Sue-Chu et al., 1996)
Figure skaters	124	Sports specific	35	(Mannix et al., 1996)
National runners	32	Field test	25	(Helenius, et al., 1996)
National swimmers	29	Histamine	48	(Helenius et al. 1998)
National runners	58	Field test	9	(Helenius *et* al., 1998)
Figure skaters	29	EVH & Sport specific	55	(Mannix *et* al., 1999)
Elite winter sport athletes	158	Sports specific	26	(Rundell et al., 2001)
Olympic summer sport athletes	77	EVH	21 - 44	(Dickinson et al., 2005)
Elite swimmers & winter sport athletes	64	Methacholine	69 28	(V. Bougault et al., 2009)
Swimmers & Boxers	44 38	EVH	68 8	(Levai et al., 2016)
Swimmers	16	EVH	50	(Pedersen et al. 2008)
Professional footballers	54	Field test	7	(Mousinho et al. 2018)

EVH, eucapnic voluntary hyperpnoea.

EIB and swimming

Swimming is one of the most popular participation sports in the UK (Jones et al., 2011) and has been considered particularly beneficial for patients with asthma and other respiratory diseases due to the warm, humid pool environment lowering likelihood of EIB compared to other sports (Bar-Yishay et al., 1982; Bar-Or and Inbar, 1992). Amongst elite swimmers however, there is now a substantial body of evidence demonstrating a high incidence of respiratory disorders, including asthma, EIB, rhinitis and allergic diseases compared to the general population and other elite athletes (Helenius and Haahtela 2000; Levai et al. 2016; Bougault et al., 2010; Bougault et al. 2009). Whereas once thought the result of an early selection bias, due to the recommendation to parents of asthmatic children to take them swimming, a study of adolescent swimmers has also shown that elite swimmers do not begin their careers with EIB (Pedersen et al. 2008). It is thought that this high prevalence is likely to arise due to the combined effects of the high ventilatory requirement of swimming, the large training volume swimmers complete, and the noxious environment in which training is carried out (Bougault et al. 2009).

Swimmers in the UK generally train in indoor pools all year round. This is a unique training environment not least because the majority of pools use chlorine as the main disinfectant agent and often have inadequate ventilation in place. Although effective in controlling microbial growth, chlorine reacts with nitrogen containing products in the water, such as urine and sweat resulting in the release of chloramines and nitrogen trichloride into the local atmosphere. Chloramines are heavy gases and will sit just above the water surface and it is these which have been suggested to be harmful for respiratory health (Drobnic et al. 1996). Swimmers inhale this air which is just above the water surface at high ventilation rates and Drobnik *et al*., (1996) found that the levels of chlorine a swimmer was exposed to in a two hour session was above that recommended for a worker in an eight hour shift.

Athletes who regularly train in chlorinated swimming pools for a prolonged period of time have a higher risk of developing respiratory health problems than the general population and non-aquatic elite athletes (Bougault et al. 2009). It has been proposed that with repeated exposure to trichloramine there may be a sensitisation process which induces airway inflammation (Anderson and Kippelen, 2008) and a recent finding by Bougault *et al*., (2012) showed that there was significant airway inflammation and remodelling on bronchial biopsies in swimmers similar to what is found in those with mild asthma. In contrast to indoor competitive swimmers in whom the prevalence of EIB has been found to be up to 67% (Levai et al., 2016), sea swimmers are reported to demonstrate a much lower prevalence of 14% (Bonsignore et al., 2003), highlighting the potential impact of the training environment. The phenomenon of high rates of airway hyperresponsiveness also appears to be transient in nature; Bougault *et al*, (2011) found that after a period of 15 days without intense swim sessions, airway responsiveness was significantly reduced and Helenius *et al*, (Helenius et al., 2002) reported that following retirement from competitive swimming heightened airway hyperreactivity appeared to resolve.

In addition, to EIB, competitive swimmers have also been found to have a high prevalence of rhinitis, thought to occur due to chlorinated water irritating the nasal mucosa. Current thinking is that rhinitis precedes the development of EIB and managing rhinitis can improve asthma control (Brożek et al., 2017). The Allergic Rhinitis and its Impact on Asthma (ARIA) recommends that all patients with rhinitis are screened for asthma and as such Bonini *et al*., (2006) have suggested that this should be extended to athletes.

Football and EIB

The majority of previous research investigating EIB in athletes has focussed its attention on Olympic sports, however the risk factors for the development of EIB as previously discussed

make it a relevant condition for many more. Football is the world's most popular sport and within the UK it is the sport with the highest number of professional athletes. The nature of elite level football suggests that EIB may pose a risk for this group of athletes; during a game elite footballers will cover between 10 – 13 km (Bangsbo et al., 2006), at an average work rate of approximately 70% $\dot{V}O_2$max (Bangsbo, 1994) resulting in a high ventilatory requirement. Players are also exposed to high training loads from a young age (Read et al., 2016) whilst often training and competing in asthmogenic environments, for example in cold air, high pollen and in areas of high pollution. Despite this, very little is currently known regarding the nature of EIB in professional footballers. One small scale study suggested that the prevalence of EIB in football is likely to be around 30% (Dickinson et al., 2013) and demonstrated that the use of objective airway testing identified Premier League football players with EIB who had no previous history of asthma or EIB (Dickinson et al., 2013). Recent data has also demonstrated considerable level of mis-diagnosis of asthma/EIB in English Premier League and Championship footballers. Ansley *et al*., (2012) reported that only 33 of 65 (51%) Premier League and Championship level players who had received a previous diagnosis of EIB could provide a positive response to a bronchoprovocation challenge. This finding is striking and indicates professional football players reporting exercise respiratory symptoms are not always receiving adequate care.

1.4 Respiratory symptoms and EIB

Athletes frequently report respiratory symptoms during and after exercise, in particular cough, wheeze, chest tightness, dyspnoea and excess mucus production (Dickinson et al. 2005; Dickinson et al., 2011). Research has consistently shown however that respiratory symptoms during and after exercise correlate very poorly with objective evidence of airway narrowing, thereby limiting the accuracy of symptom based diagnosis (Rundell et al., 2001;

Dickinson et al., 2005). In fact, when respiratory symptoms are used to diagnose EIB in elite football players independently of indirect airway challenges, they have been shown to result in 49% mis-diagnosis (Ansley et al., 2012). Despite this well-known discrepancy between the absence of symptoms and the presence of EIB, when questioned, one third of primary care physicians indicated that they would initiate treatment based on clinical information alone (Hull et al., 2009). It is common to encounter athletes who experience a significant reduction in lung function following exercise but perceive few respiratory symptoms; Turcotte *et al*., (2003) found that in a group of 698 athletes only a minority of asthmatic athletes reported troublesome respiratory symptoms and Simpson *et al*., (2015) found that self-reports of respiratory symptoms in conditions of induced and inhibited bronchoconstriction do not correlate with changes in airway calibre in athletes with EIB. This group found that 48% of the athletes they assessed reported at least one respiratory symptom despite their fall in FEV_1 post bronchoprovocation challenge being blunted by the use of inhaled terbutaline, and in fact 28% had a higher symptom score when this fall was blunted. Despite this, some authors have shown that questionnaires could still provide a role in the diagnosis of EIB; Turcotte *et al*., (2003) concluded that questions on symptoms and associated nociceptive sensations may help to detect airway hyperresponsiveness (AHR), however for some athletes, in particular swimmers and triathletes, there is a risk of false negatives being observed. Price *et al*., (2016) built on this in their in depth qualitative approach to the assessment of breathlessness and discovered that there are several features which can differentiate between EIB and non-EIB causes of exertional dyspnoea in athletes. These are: The location of symptoms, recovery time following exercise and response to β_2-agonist therapy.

To confound the problem with the diagnosis of EIB in athletes, baseline spirometry is also poorly predictive of EIB, in most cases being within the normal ranges with disease present (Bonini et al., 2007), or even above the predicted normal (114 - 121%) (Rundell et al., 2001).

Thus, in order to establish a secure diagnosis of EIB it is important to perform objective testing to confirm dynamic changes in airway function (Parsons et al., 2013).

2.5 Diagnosis of EIB

As highlighted above the diagnosis of EIB cannot reliably be made by symptoms alone and as such requires objective testing by way of a bronchial provocation challenge. There are two main types of airway challenge; direct and indirect challenges both of which require measurement of lung function using spirometry before and after a challenge to monitor any changes in lung function. Direct challenges are predominantly used to exclude current asthma, when the test result is negative, whereas indirect challenges are more specific for the diagnosis of asthma and EIB, however are less sensitive in excluding asthma (Randolph, 2011).

Direct airway challenges

The majority of direct challenges are pharmacological. In these a pharmacological agent such as methacholine or histamine is administered which acts directly on the airway smooth muscle receptors causing contraction in susceptible individuals (Holzer and Brukner, 2004). Methacholine directly interacts with muscarinic receptors on smooth airway muscle by mimicking the neurotransmitter acetylcholine, resulting in contraction and airway narrowing (Coates et al., 2017). During a methacholine challenge increasing doses of methacholine is delivered to the participant via a hand-held nebuliser. The initial dose of methacholine is inhaled and forced expiratory volume in 1 second (FEV_1) measured at 60- and 90-seconds post with the highest value recorded. The dose of methacholine is then increased and FEV_1

measurement repeated. This continues until either the fall in FEV_1 reaches > 20%, or the participant reaches the maximal dose.

A low sensitivity has been shown for methacholine in the diagnosis of EIB in summer athletes (Holzer et al., 2002). It is common however for winter athletes to be positive to methacholine but negative to either exercise, eucapnic voluntary hyperpnoea (EVH) or mannitol challenge (Rundell et al., 2004). Due to this disparity, Anderson and Kippelen (2012) warn against using methacholine in the diagnosis of EIB in athletes training and competing in cold or polluted environments. The reason behind this phenomenon is still unclear however it is thought that the hyperresponsiveness may be due to airway remodelling and an increase in parasympathetic tone to cold air (Anderson and Kippelen, 2005). Support for this hypothesis was shown in a study of cross country skiers who despite a positive methacholine challenge showed no improvement with inhaled corticosteroid (ICS) therapy (Sue-Chu et al., 2000).

Indirect airway challenges

Indirect airway challenges include exercise, eucapnic voluntary hyperpnoea (EVH) and osmotic challenge tests such as mannitol and hypertonic saline. These stimuli act indirectly causing bronchoconstriction by contraction of the airway smooth muscle via the release of mediators such as prostaglandins, leukotrienes and histamines from inflammatory cells in the airways (Fitch et al., 2008). The response to indirect challenges has shown to be inhibited by inhaled corticosteroids (ICS) and as such this process is thought to be a result of active airway inflammation (Anderson, 2016). Indirect challenge tests require measurement of forced expiratory volume in one second (FEV_1) prior to and following the challenge. The result of the challenge is determined by calculating the maximal percentage change in FEV_1 as a consequence of the challenge and this is used to determine the presence and also the

severity of EIB. Measurement of FEV_1 is required as it has good repeatability (Enright et al., 2004). At least two reproducible FEV_1 manoeuvres are measured at set time intervals following the challenge, with the highest acceptable value recorded at each time point (Crapo et al., 2000).

Osmotic challenges

Osmotic challenge tests such as hypertonic saline and mannitol induce hyperosmolarity and hypertonicity of the airways in the absence of exercise, resulting in the release of inflammatory mediators that lead to bronchoconstriction (Holzer and Brukner, 2004). A mannitol challenge requires administration of increasing doses of dry mannitol powder through an inhaler device with spirometry measured after each dose. The threshold for a positive mannitol challenge is a fall in FEV_1 of $\geq$15%, or a fall of $\geq$10% between consecutive doses (Anderson et al., 2009). This challenge has been shown to possess both sensitivity and specificity in the diagnosis of EIB in elite athletes (Holzer et al., 2003). Mannitol has the advantage that it can be performed with minimal equipment. The disadvantage of the Mannitol challenge is that is has been shown to provoke cough during testing, which on occasion can prevent participants from effectively inhaling the next dose (Brannan et al., 2005).

Exercise challenges

Exercise challenge testing is often considered the most logical method to detect EIB, after all it is an 'exercise' induced condition. Despite clear guidelines however (Crapo et al., 2000; Weiler et al., 2016), there remains no single standardised protocol for exercise testing, meaning that there remains limitations when employing this approach in an athletic population. The sensitivity of an exercise challenge is highly dependent on control over the two main contributors to the airway response: the water content of inspired air (Evans et al.,

2005) and minute ventilation ($\dot{V}_E$) (Carlsen et al., 2000) and as such any challenge needs to make efforts to maintain strict control over these two variables.

Laboratory exercise tests may be performed on a cycle ergometer or a treadmill. Early studies suggested that treadmill tests were preferable to cycling (Cropp, 1979), however, more recently cycle tests have proven effective, providing the work rate is able to raise $\dot{V}_E$ to the target within four minutes of exercise initiation (Crapo et al., 2000). Current thinking suggests the best protocol to detect EIB in lab conditions with exercise is to achieve a rapid increase in exercise intensity over 2 – 4 minutes in an attempt to achieve a high level of ventilation and to continue this intensity for a further 4 – 6 minutes. As pulmonary ventilation is more closely related to the stimulus of bronchoconstriction than heart rate, it is suggested that $\dot{V}_E$ is measured to guide exercise intensity. During the final 4 – 6 minutes of exercise, $\dot{V}_E$ should be sustained between 40 – 60% of predicted maximal voluntary ventilation (MVV), calculated as $FEV_1 \times 35$ for a clinical population (Anderson, 2016) or > 25 times the FEV_1 (70% MVV) in an athlete population (Anderson and Kippelen, 2012), or 80-90% of predicted maximal heart rate (220-age) (Bonini and Palange, 2015). Standardisation of exercise intensity is crucial as Carlsen *et al*., (2000) found that the mean fall in FEV_1 after an exercise challenge was more than doubled after achieving 95% predicted maximum compared to 85%. It is vital also to maintain control over the water content of inspired air. Dry air is required for exercise challenges and a water content of 10 mg/L is recommended (Rundell and Slee, 2008) whilst a nose clip is worn to ensure oral breathing. Crapo *et al*., (2000) advises that this can be accomplished by using an air-conditioned lab with an ambient temperature of 20-25 °C and relative humidity of $\leq$ 50%. To highlight this importance, Stensrud *et al*. (2006) demonstrated a 50% reduction in severity of EIB (24% and 12% falls in FEV_1) when comparing exercise challenges in conditions of 40% and 95% RH at 20°C. As a way of controlling the water content of inspired air, some authors recommend using medical grade dry air (Anderson et al., 2010). Anderson *et al*., (2010); found that using a

standardised exercise challenge where participants inspired medical dry air, there was agreement in results of even mild responders to the exercise (< 15% fall in FEV_1) when two exercise challenges were completed within four days.

Standardised lab challenges may provide an insufficient stimulus to induce a positive response in some athletes, particularly if it is certain environmental conditions which appear to trigger bronchoconstriction in these athletes. As such, sports specific challenges can also be employed. For these challenges the same guidelines regarding the exercise intensity apply. Although this type of challenge is highly specific for the diagnosis of EIB, the disadvantages are the lack of control over the environmental conditions. However in one study by Rundell *et al*., (2000) a comparison of lab and field based challenges was carried out and they reported that in cold weather athletes, carrying out tests in the outdoor environment is important so as not to have a large number of false negative tests. The authors found that 18 (78%) of the cold weather athletes who demonstrated EIB positive following a field test, demonstrated no evidence of EIB following a laboratory challenge. The lab conditions in this study however were 21°C and 60% RH and so were not as recommended.

Exercise challenges whilst intuitive and highly specific may lack sensitivity if strict control of $\dot{V}_E$ and water content of inspired air is not adhered to thereby creating the potential for high rates of false negative diagnoses. The lack of a standardised protocol for an exercise challenge may in part explain the wide range in the reported prevalence within specific sports and the reported rate of false-negative tests (Rundell et al., 2000).

Eucapnic Voluntary Hyperpnoea EVH

The variability in exercise tests prompted the development of surrogate methods which could more easily be standardised for use in a laboratory or clinic setting. Exercise itself was found not necessary to elicit an airway response and as such a challenge utilising eucapnic voluntary hyperpnoea (EVH) was developed by members of the US army (Hurwitz et al.,

1995). A full detailed description of the EVH challenge can be found in chapter 2, however, in brief the EVH challenge requires ventilation of medical grade dry air containing 5% carbon dioxide (CO_2), 21% oxygen (O_2), and the balance nitrogen (N_2). This concentration of gas has been shown to maintain normal end-tidal CO_2 levels throughout the challenge. Six minutes of breathing at a high level of ventilation equivalent to 85% MVV (calculated by 30 ×FEV_1) is used as a target for athletes to reduce the possibility of false negative results. Lung function is then measured for up to 15 minutes following the challenge. Because high ventilation can lead to large decreases in FEV_1 EVH is recommended for use only in those who exercise regularly and intensely at high ventilation (Anderson et al., 2001), and guidelines state that an EVH challenge should not performed if resting FEV_1 is less than 70% of predicted (Weiler et al., 2016). EVH has previously been reported to possess a high specificity and provide greater sensitivity in comparison to other airway challenges in the diagnosis of EIB in elite athletes (Dickinson et al., 2006; Holzer et al., 2002). On this basis, EVH has been endorsed by the International Olympic Committee Medical Commission (IOC-MC) as the optimal bronchoprovocation challenge to diagnose EIB in elite athletes (Anderson et al., 2001). Because of its high potency, EVH can be used to exclude EIB in elite athletes particularly in those with suspected dysfunctional breathing (Anderson and Kippelen, 2013). There are numerous advantages in using EVH to identify EIB in athletes, which Anderson *et al*., (2001) summarises succinctly (Table 1.2).

Table 1.2. Advantages to EVH as a bronchoprovocation method (Anderson et al., 2001)

EVH is a potent challenge test for provoking bronchoconstriction in clinically recognised asthmatics responsive to exercise
Symptoms provoked by EVH (cough, dyspnoea, and wheeze) are the same as those reported with exercise.
EVH requires less expensive equipment and fewer personnel than exercise testing.
EVH can induce $\dot{V}_E$ equivalent to, or higher than most modes of exercise, and can be sustained over six minutes.
The maximum airway response to EVH is similar to that achieved by exercise and occurs within the first 10 minutes of cessation of the hyperpnoea.
As with exercise β_2-agonists are effective in inhibiting the airway narrowing to EVH.
As with exercise, inflammatory mediators, including histamine, prostaglandins and leukotrienes are involved in the response to EVH.
EVH has a very high specificity for identifying persons with clinically recognised asthma.
Guidelines are readily available on the standardisation, application, and interpretation of EVH testing.
EVH has been used safely in many thousands of subjects.
EVH has been used with some success to identify EIB in cold weather athletes.

EVH, eucapnic voluntary hyperpnoea; $\dot{V}_{E,}$ minute ventilation.

Comparison of indirect challenges

Several authors have carried out comparison studies between EVH and different exercise challenge for screening of EIB in athletes. Dickinson *et al*., (2006) reported that out of 14 winter sports athletes tested, 10 were EVH positive, however only three were positive to a sport specific challenge (8°C and 35% RH or 1.5°C and 33%RH), and no athletes were positive to a lab based challenge (18°C and 56% RH). Rundell *et al*., (2004) also screened 38 winter sport athletes for EIB; they identified 17 athletes by EVH and only 11 by exercise

challenge. Similarly, Mannix *et al*., (1999) compared an on ice skating challenge to EVH in 29 ice skaters and found 12 skaters were positive by EVH, 9 by on ice testing and 5 individuals were positive for EIB on both tests. The authors concluded that 'for the evaluation of EIB in athletes who train and compete in cold environments, exercise testing in the cold air along with a challenge test such as EVH should be conducted to increase the yield of positive responders.'

Holzer *et al*., (2003) assessed the sensitivity of a mannitol challenge to identify responsiveness to EVH in 50 asymptomatic elite summer sport athletes, reporting that mannitol had a sensitivity of 96% and specificity of 92% to identify a positive response to EVH. This group used a 10% threshold however as opposed to the recommended 15%. In a comparison between mannitol, exercise and methacholine in 509 participants, Anderson *et al*., (2009) found that mannitol was equivalent to methacholine in identifying EIB, with the sensitivity and specificity being 59% and 65% respectively. The authors reported that there was a similar positive response for both exercise (43.5%), and mannitol (44.8%), with a test agreement of 62%. They concluded that in this group with mild EIB, the sensitivity and specificity of mannitol to identify EIB was lower than previously documented (Brannan et al., 1998).

Eucapnic voluntary hyperpnoea is often quoted as the 'gold standard' challenge, however as exercise is the provocative stimulus to induce bronchoconstriction in athletes, the true gold standard may be a sport and environment specific exercise challenge. Such discrepancies in the methods above however show that a standardised, sensitive exercise protocol still needs to be found before exercise testing can be used as a robust screening tool.

Threshold for a positive test

There remains some debate regarding which percentage fall in FEV_1 defines a positive challenge indicative of EIB. Currently, a positive test for EIB when employing either EVH or EX is defined as a pre-to post challenge fall in FEV_1 of ≥10% (Weiler et al., 2016). For elite athletes to demonstrate evidence of EIB, a decrease of 10% or more in the FEV_1 is preferable at two consecutive time points post challenge, reducing the possibility of a false positive due to a reduced FEV_1 immediately post challenge as a result of respiratory muscle fatigue (Johnson et al., 1993). EIB severity can be graded based on the percentage fall in FEV_1 post EVH or exercise challenge, these grades are mild, moderate, or severe based on a percent fall in FEV_1 of ≥ 10% to < 25%, ≥ 25% to <50% ≥ 50% respectively (Parsons et al., 2013).

The 10% diagnostic threshold for a positive EVH test was proposed following a study of army recruits with asthma (Hurwitz et al., 1995). This study demonstrated that a drop of 14% was 100% specific for asthma but had a sensitivity of only 53% and as such a threshold of 10% was recommended on the basis of an improved relationship between specificity (90%) and sensitivity (63%). This 10% cut off is also the most widely used in exercise studies (Crapo et al., 2000). Hull *et al*., (2016) conducted an analysis of EVH test results from 860 athletes across 12 studies and discovered a mean drop of approximately 9%. They noted that the large standard deviation of 8.4% may suggest that a greater fall in FEV_1 from baseline may be more appropriate. A recent retrospective analysis of data from asymptomatic athletes also challenged the widely accepted 10% threshold suggesting that 15% might be a more appropriate threshold in this population (Price et al. 2016), particularly because they have found poor diagnostic reproducibility of EVH suggestive of mild EIB (10-15% fall in FEV_1 following EVH) has been demonstrated in recreational athletes over a short time period (Price et al., 2015). Others however, have found a good reproducibility of EVH at all severities in physically active participants in both the short term (21 days) and long term (70

days) (Williams et al., 2015). These discrepancies may in part be due to the transient nature of EIB (Cockcroft and Davis, 2006).

It is not only the threshold for a positive EVH test which has been challenged, but also the response to exercise. The 'normal' response to exercise is mild bronchodilation (Todaro, 1996) and as such, there have been suggestions that the criteria for a positive exercise challenge should be less than the 10% threshold originally suggested. A 6.5% fall in FEV_1 following an exercise challenge has been shown to be the threshold of an abnormal response in elite runners (Helenius et al., 1998), and a 7.1% fall in a population of winter athletes (Rundell et al., 2000).

Anderson and Kippelen (Anderson and Kippelen, 2013) suggest that the appropriate cut-off for both EVH and Exercise will ultimately be dependent on the investigator's need to be either more sensitive or more specific and whereas a 10% fall in FEV_1 may be an appropriate threshold for demonstrating the need for inhaled β_2-agonists for antidoping purposes, a value of 20% may be more appropriate for subjects to be included in clinical trials to assess new drugs for EIB. It would also be pertinent to consider whether or not small falls in FEV_1 (~ 10%) are of functional significance (Dickinson et al., 2006).

1.6 Treatment for EIB

Treatment for EIB can be broken down into pharmacologic and non-pharmacologic therapy. Recommendations for the pharmacological treatment of EIB in athletes is largely based on guidelines for standard asthma care and expert opinion, due to the absence of adequately powered randomized clinical trials in elite athletes (Boulet and O'Byrne, 2015), in fact Weiler *et al*., (2016) recommends using the recommended general treatment for asthma to treat athletes with EIB alone in a similar way to those with EIB and asthma.

Reliever therapy

The initial step in treatment for those with EIB and still the most common therapeutic recommendation for athletes is the administration of an inhaled short-acting β_2-agonist (SABA) 15 - minutes prior to exercise. SABAs act on the adrenergic β_2-receptors that are distributed in the lungs (primarily and heart and skeletal muscles) and relax the smooth muscle cells surrounding the airways, causing bronchodilation and relieving symptoms such as coughing, wheezing and chest tightness (Davis et al., 2008). The use of inhaled SABA in sports is restricted by anti-doping regulations. Over the years inhaled β_2-agonists have alternatively been allowed and prohibited by the World Anti-Doping Agency (WADA). Currently the 2018 prohibited list it states "All selective and non-selective β_2-agonists, including all optical isomers, are prohibited. Including, but not limited to: fenoterol, formoterol, higenamine, indacaterol, olodaterol, procaterol, reproterol, salbutamol, salmeterol, terbutaline, tulobuterol, vilanterol. Except: Inhaled salbutamol: maximum 1600 micrograms over 24 hours in divided doses not to exceed 800 micrograms over 12 hours starting from any dose. Inhaled formoterol: maximum delivered dose of 54 micrograms over 24 hours and inhaled salmeterol: maximum 200 micrograms over 24 hours."

Although still common practice, the advice for a SABA to provide the mainstay of treatment in an athlete should be obsolete (Price and Hull, 2014). Parsons *et al*., (2013) recommends that treatment for EIB should be stepped up if athletes 'continue to have symptoms despite using an inhaled SABA before exercise, or who require a SABA daily or more frequently'. Discussion previously in this chapter showed that athletes rarely correctly recognise or identify the symptoms of EIB and elite athletes are often training twice daily. Furthermore, the sole use of SABAs can have adverse effects such as tremor and tachycardia and there is also the potential for the development of tachyphylaxis.

Preventative therapy

Due to the adverse effects of the sole use of SABAS, guidelines recommend that controller pharmacotherapy for athletes who have EIB should include daily inhaled corticosteroids (ICS) (Weiler et al., 2016). ICS are the most effective treatment of asthma and the only drugs which successfully supress airway inflammation in asthmatic airways. This suppression of inflammation is mainly achieved due to ICS switching off multiple activated inflammatory genes by reversing histone acetylation through the recruitment of histone deacetylase 2 (HDAC2) (Barnes, 2010).

Regular use of ICS has been shown to provide significant prevention against airway inflammation and bronchoconstriction in those diagnosed with mild airway hyperresponsiveness (Boushey et al., 2005) and to reduce the number and severity of exacerbations and thereby asthma mortality in asthmatics (Suissa et al., 2000). It is thought however that ICS may be less protective in elite athletes without asthma who experience EIB (Sue-Chu et al., 2000), but relatively few studies on the effect of ICS on EIB have been performed in athletes, and no studies have been carried out in elite athletes (Carlsen et al. 2008).

In moderate to severe EIB, combination therapy (an inhaler containing both an ICS and a long acting β_2-agonist (LABA)) can have added benefit (Weiler et al., 2016). Combination therapy of fluticasone and salmeterol has been shown to be more effective than fluticasone alone in preventing EIB (Weiler et al. 2005; Reynolds et al., 2005) and Noonan *et al*., (2006) showed better asthma control with treatment of budesonide and formoterol in combination compared with budesonide alone. It is thought that this additive effect may be due to the ICS increasing the gene transcription of β_2-receptors, resulting in increased expression of cell surface receptors (Barnes, 2010). The drawback of these studies is that they were all conducted in an asthmatic population.

Leukotriene receptor antagonists (LTRAs) are an additional add on therapy. LTRAs are a non-steroidal oral medication and have both bronchodilator and anti-inflammatory effects which are as a result of by interfering with the activity of leukotrienes. LTRAs act by blocking the specific leukotriene receptors on bronchial tissue, preventing bronchoconstriction, mucus secretion, and oedema. They also reduce the influx of eosinophils, which acts to limit inflammatory damage in the airway. Daily therapy with montelukasts have also been found to reduce EIB (Duong et al., 2012), particularly when caused by exposure to pollutants (Rundell et al., 2005). Steinshamn *et al*., (2002) demonstrated that montelukast reduced the maximum post-exercise fall in FEV_1 and in addition improved the running time to exhaustion in 11 of 16 adults with EIB. However, Helenius *et al*. (2004) could not find any effect upon asthma-like symptoms in response to a histamine challenge or fraction of exhaled nitric oxide (FeNO) in 16 ice-hockey players with EIB using a randomized placebo-controlled cross-over study.

Treatment for coexisting conditions

It is also important that athletes are treated for any co-existing conditions. Conditions and symptoms such as nasal obstruction, rhinorrhoea, sneezing, congestion and itching are reported by 74% of competitive elite swimmers (Bougault et al., 2010). The 'united airways disease' theory (Rimmer and Ruhno, 2006; Daabis, 2016) suggests that it is a single inflammatory process within the respiratory tract which leads to manifestations in both the upper and lower airways. As previously discussed, it is recognised that chronic rhinitis is a contributing factor to the development of asthma and may affect its control (Pedersen and Weeke, 1983), as such in the case of athletes with rhinitis, nasal inhaled glucocorticoids and nasal ipratropium antihistamines may be considered. It should be understood however these are not effective against EIB (Boulet and O'Byrne, 2015).

Does therapy have an ergogenic action?

With the increase in the number of athletes using inhaled β_2-agonists and the changes with the exclusion/ inclusion on the WADA prohibited list, there have been questions as to whether asthma therapy, in particular β_2-agonists have performance enhancing effects. Many studies have been conducted using non-asthmatic, well-trained individuals and a systemic review by Pluim *et al.* (2011) concluded that 'no significant effects were detected for the inhaled β_2-agonists salbutamol, formoterol, terbutaline or salmeterol on aerobic or anaerobic capacity or strength in healthy athletes. Additionally, more recently Dickinson *et al*., (2014) reported that there was no improvement in 5 km time-trial performance following the inhalation of up to 1600 µg of salbutamol in non-asthmatic athletes, and a study by Kuipers *et al*., (2008) investigating the effect of four weeks of ICS or placebo inhalation in healthy, well-trained athletes found no effect on maximal power output or mood state.

Non-pharmacological therapy

Alongside pharmacological interventions, there are a number of alternative ways in which an athlete may be able to reduce EIB severity and exacerbations.

<u>Warm-up</u>

A pre-exercise warm up is well documented to lead to a significant reduction in post exercise bronchoconstriction in some athletes (Elkins and Brannan, 2013; Stickland et al., 2012). This has been attributed to a refractory period during which time the airways are less likely to constrict again. It is thought that this is induced by the release of prostaglandins and airway smooth-muscle tachyphylaxis to mediators of bronchoconstriction (Rosenthal et al., 1990).

Dietary modification

Dietary modification may have the potential to reduce the severity of EIB (Mickleborough, 2008) and a number of dietary factors have been investigated because of their role in inflammatory reactions, activities of airway smooth muscle and modulation of pulmonary and microvascular volume and pressure. In a recent review Dickinson *et al*., (2018) showed that there are numerous dietary factors which may have the potential to effectively reduce the severity of EIB, including; Omega-3 fatty acid supplementation, vitamin and antioxidant supplementation and caffeine. The majority of these studies however have been conducted on subjects with mild to moderate persistent asthma, who also exhibited EIB. Despite this Weiler *et al*., (2016) suggest that although evidence is weak, consideration of the reduction of sodium intake and supplementation with fish oil and vitamin C should be made.

Avoidance of triggers

Avoidance of potential triggers is standard advice given to asthma patients to control EIB. It is impossible for athletes to avoid training particularly in environmental conditions such as cold, dry, high pollen levels or pollutants, however where possible it is recommended that athletes avoid training in areas with levels of high pollution or particular allergens to which they know they are sensitised (Kippelen et al., 2012). The pool environment in which swimmers train in should also be optimised for example by ensuring adequate ventilation (Bougault and Boulet, 2012).

Facemasks

An increase in temperature and water content of inspired air has long been shown to prevent EIB in asthmatic subjects (Chen et al., 1979). One method of increasing inspired air temperature thereby potentially diminishing airway dehydration is by the use of a face mask. There are a number of studies which have investigated the use of facemasks and have shown a protective effect against EIB, following exercise (Beuther and Martin 2006; Brenner et al.

1980; Millqvist et al., 2000; Nisar et al. 1992). However, Parsons *et al*., (2013) suggest recommendations to use facemasks are weak based on the current availability of low-quality evidence. Finally, education of an athlete with EIB is a crucial element and should include EIB self-management and inhaler use and technique (Page et al., 2017).

1.7 Impact of treating athletes for EIB

There are numerous studies regarding the high prevalence of EIB in athletes and the potential mechanisms for its development. Despite this however, there is a distinct lack of well-designed studies investigating the impact of treating EIB or what the consequences may be of leaving EIB untreated on both health and performance.

Impact on health

Undetected and uncontrolled EIB can lead to fatal consequences in both elite and sub-elite athletes (Becker et al., 2004). Left untreated, an element of irreversible airflow obstruction may also develop, which is thought to be the result of chronic inflammation (Barnes, 2010). An association between uncontrolled asthma/ EIB has also been suggested to predispose athletes to upper respiratory tract infection (URTI) (Helenius and Haahtela, 2000). In the general population asthma is associated with an increased incidence of pneumococcal disease and pneumonia, with a decreased risk when asthma is well controlled (O'Byrne et al., 2013). However, there are no studies investigating the effects of treating EIB in athletes on overall wellness.

Impact on performance

Left untreated, EIB may also have deleterious effects of performance; Stensrud and colleagues (Stensrud et al., 2007) reported impaired oxygen uptake in individuals with EIB, however in a cohort of army recruits EIB was found not to hinder physical performance including peak oxygen uptake (Sonna et al., 2001).

As previously discussed in this chapter, there appears to be no or limited ergogenic effect of asthma/ EIB therapy in healthy athletes. There are very few studies however in athletic populations with EIB. This lack of literature was recently highlighted by Price *et al*., (2014) who concluded that although it would seem logical that EIB would affect performance, there is not sufficient evidence to give a definitive answer. In fact, there was such little evidence that the authors reported an n = 0 of studies which could be included in their meta-analysis. EIB may compromise not only performance during competition but the capacity to train effectively (Mannix et al., 2003). It has been hypothesised that airway narrowing during exercise compromises ventilatory capacity and efficiency; Haverkamp *et al*., (2007) showed that bronchoconstriction during and following exercise can result in reduced exercise performance, alveolar ventilation and efficiency of alveolar-to-arterial blood O_2 exchange and Price *et al*., (2014) provided a detailed discussion as to the potential mechanisms for the performance impact and summarised this concisely (figure 1.2).

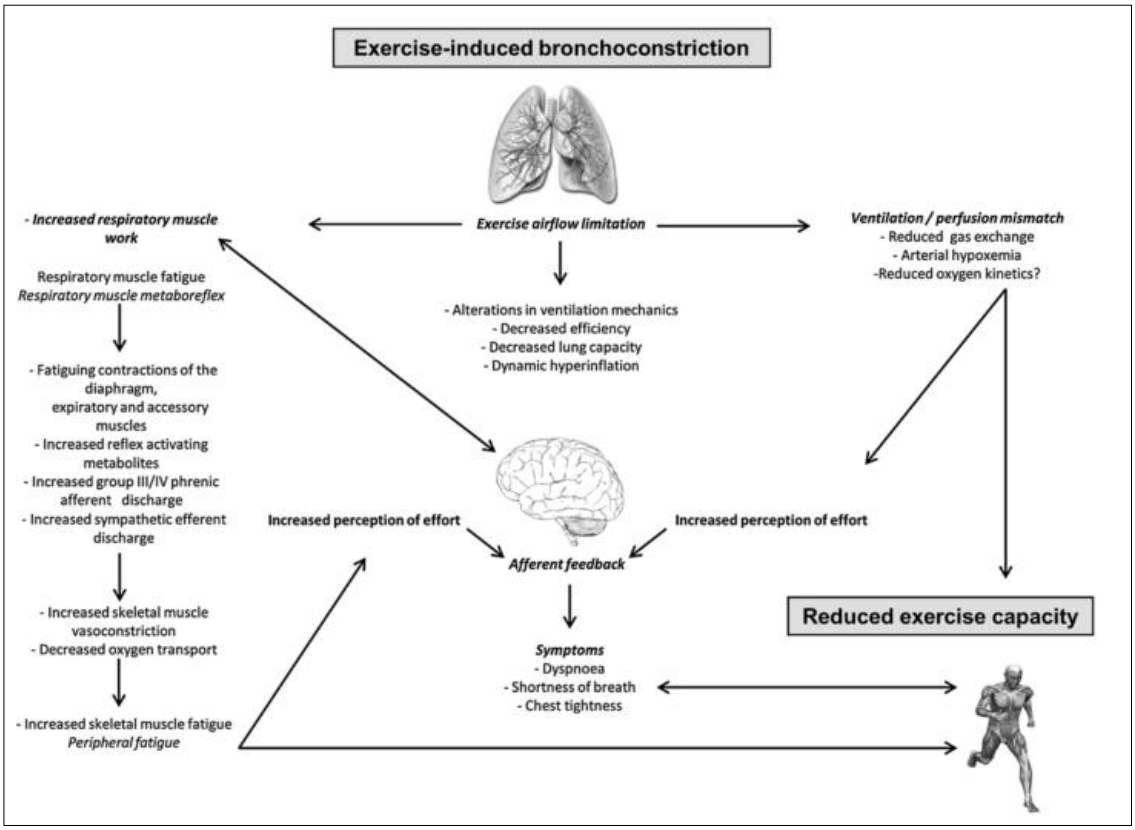

Figure 1.2. Potential mechanisms for the performance impact of EIB (Price et al., 2014).

The acute effect of treatment on performance in athletes with EIB has been examined in a limited number of studies; Koch *et al*. (2013) concluded that the inhalation of salbutamol induced a significant increase in resting lung function in EVH positive and negative athletes, but this improvement did not translate to improved exercise performance. There were however several limitations to this study: although athletes were asked to withhold β_2-agonists for at least 12 hours prior to arriving at the laboratory, they were allowed to continue their ICS treatment and it is well known that ICSs taken on a daily basis may reduce the severity of EIB (Subbarao et al., 2006). Athletes were wearing a facemask during their performance, which may have reduced any EIB (Millqvist et al., 2000). Athletes were also allowed a warm up prior to their performance trial, and prior warm is known to induce a refractory period for around 4 hours. Conversely, although inhalation of SABA in permitted

doses, did not improve swim performance in elite swimmers, swim ergometer sprint performance has been found to increase (Kalsen et al., 2014).

There is a paucity of studies looking at the long-term effects of standard asthma preventative treatment. Haverkamp *et al*., (2007) showed that in habitually active asthmatic participants treatment with ICS significantly improved arterial blood oxygenation in exercise. Furthermore, all but one subject in the ICS treatment group was able to exercise longer in a time to exhaustion trial after treatment. From a very small number of studies there appears be a potential positive impact upon aerobic performance when athletes with EIB are treated with standard therapy. Brukner *et al*. (2007) found that Australian rules football players with newly diagnosed EIB had a significant improvement (9%) in the $\dot{V}O_2$ max following six weeks of treatment compared to controls. In addition, Spiteri *et al*., (2014) demonstrated that appropriately medicating elite rugby players with previously undiagnosed EIB improved their performance in a rugby specific aerobic exercise challenge by 8% over the course of 12 weeks compared to 6% in the control group, however this was not a statistically significant finding and both of these studies had a small number of participants. If this performance benefit is demonstrated additional encouragement for sports to look after the respiratory health of their athletes may be provided.

1.8 Should we screen for EIB?

Due to the high prevalence of EIB, its effect upon health and the potential impact on performance, some authors have called for screening for EIB to be implemented (Dickinson et al., 2005; Holzer and Brukner, 2004; Vakali et al., 2017). Screening for EIB in an athletic population may be important, mainly due to the difficultly in relying on symptoms as a method of diagnosis as discussed previously in this chapter.

Numerous studies have shown that screening for EIB using objective testing identifies EIB in asymptomatic athletes with no asthma history who may benefit from treatment: Dickson *et al*., (2011) screened 228 elite athletes using EVH and found 78 (34%) presented as EVH positive (EVH+), 57 (73%) of whom had no previous history. The same group (Dickinson et al., 2013) also found a similar prevalence in a small sample of English premier league football players; 29% demonstrated as EVH+ and 66% of these players had no prior history. Recently 67% of swimmers with objective evidence of EIB had no previous history, whereas 12% of the swimmer who had no objective evidence of EIB had a prior diagnosis of asthma/ EIB (Levai et al., 2016). More alarmingly Ansley *et al*., (2012) found that almost half of the professional football players they tested with a physician's diagnosis of asthma/EIB presented no objective evidence of either condition.

There are implications for both the under and over diagnosis of EIB. Being susceptible to EIB means an athlete may be vulnerable to exacerbations and evidence suggests that a high proportion of asthma related deaths occur in high level athletes in conjunction to training and competition (Becker et al., 2004; Lang, 2005). Asymptomatic EIB as well as being associated with airway inflammation and remodelling has also been linked to an increased risk for the later development of asthma (Boulet, 2003) and as such the timely detection and treatment of EIB may prevent this.

Failing to implement appropriate treatment presents a potential for deterioration and exacerbation of EIB, however there are also implications when prescribed medication is prescribed unnecessarily. The long term use of SABAs have been associated with degenerative changes in lung function and the development of tachyphylaxis (Bonini et al., 2013) and appropriate diagnosis would also enable athletes to seek treatment for differential diagnoses where necessary which often present with asthma like symptoms (Hull, 2015).

Before a screening programme can be put in place, a number of stringent criteria must be met (Wilson and Jungner, 1968), this includes demonstrating the prevalence, having the ability to detect the condition of interest and an understanding of the impact in the population of interest (Wilson and Jungner 1968; Ansley et al., 2013; Hull et al. 2007; Hull and Rawlins 2016). In the ATS workshop report regarding screening for asthma (Gerald et al., 2007) it was concluded that although the adoption of case detection programmes were unwarranted due to a lack of evidence, it was felt that 'limited case detection programmes may be appropriate in areas where there is a high prevalence of undiagnosed asthma and where newly identified patients have access to quality care'. This report looked at a population of children but could also be relevant to elite athletes. Parsons *et al*., (2013) reported that they were unable to find any well conducted studies evaluating the overall efficacy of screening programmes for EIB on either health or performance in athletes.

1.9 Summary

It is clear that there is a high prevalence of EIB in the elite athlete population, with this prevalence seemingly particularly high in the UK amongst swimmers and athletes who train outdoors all year round, in sports with a high ventilatory demand. Much work has been undertaken into the mechanisms of how EIB occurs in this population, however what isn't clear is how best to make a firm diagnosis and manage these athletes appropriately to optimise both their health (short and long term) and their sporting performance. There are already best practice guidelines in these areas, however much of these are founded on guidance for care in a general asthmatic population and expert opinion. Based on previous evidence for screening for EIB in the athletic population, more work needs to be undertaken before this can be recommended more widely.

1.10 Aims and Hypotheses

The overall aim of this thesis was to investigate the optimal way to diagnose and manage EIB within an athletic population. More specifically the main aims were to:

1. Investigate if a positive EVH test is predictive of a positive standardised exercise challenge.
2. Investigate the impact of appropriate standard asthma therapy on airway inflammation, EIB severity, performance and wellness in athletes with screen detected EIB.
3. Investigate the effect of methods to reduce exposure to an asthmogenic training environment upon airway inflammation, EIB severity, and athlete wellness.

It was hypothesised that:

1. A positive EVH challenge would be predictive of a positive exercise challenge.
2. Treatment with appropriate standard asthma therapy would reduce airway inflammation, decrease EIB severity, improve exercise performance and increase athlete wellness.
3. Reducing exposure to an asthmogenic training environment would lead to a reduction of airway inflammation and EIB severity and an increase in athlete wellness.

Chapter 2. General Methods

This chapter describes the general methods used in the respiratory assessments in the experimental chapters of this thesis. These details are for methods common to two or more chapters. Tests and procedures which are used only in one chapter are defined within the chapters themselves.

2.0 Preparation for respiratory assessment

Participants were asked to adhere to the following criteria in preparation for testing: They were required to be free of any chronic medical condition apart from asthma or EIB, and free of any respiratory infections for four weeks prior to assessment (Bolger et al., 2011; Spiteri et al., 2014; Levai et al., 2016). Participants were required not to undertake any exercise for at least four hours before their assessment. This was because following exercise, there may be a refractory period which occurs (McNeil et al., 1966) and Edmunds *et al.* (1978) demonstrated that it is at four hours following exercise that the initial airway response to an exercise challenge is re-established. Participants were instructed not to consume any food or beverages containing caffeine in the morning of their assessment as caffeine has been found to decrease bronchoconstriction following eucapnic voluntary hyperpnoea (EVH) (Duffy and Phillips, 1991) and moderate to high doses of caffeine have been found to be as effective as albuterol in providing a protective effect against exercise induced bronchoconstriction (EIB) (Vanhaitsma et al., 2010). In addition, participants with a prior diagnosis of asthma and/ or EIB were required to withhold medication for the relevant time (Table 2.1) to ensure wash out of their medication. The exception to this was in experimental chapters where repeat testing occurred to look at asthma/ EIB control and this is highlighted within the relevant chapters. Finally, participants were advised not to consume a large meal within 2 hours prior to their assessment to ensure comfort during spirometry and any use of nitrate

supplementation was recorded due to their potential to elevate fraction of exhaled nitric oxide (FeNO) (Kroll et al., 2017).

Table 2.1. Required times to withhold medication use prior to respiratory assessment (Anderson et al. 2001; Dickinson et al., 2011)

Medication	Minimum time to withhold medication
Inhaled SABA, sodium cromoglicate, nedocromil sodium or ipratropium bromide.	8 hours
Inhaled LABA or antihistamines	48 hours
ICS	72 hours
LTRA	4 days

SABA, short acting β_2-agonists; LABA, long acting β_2-agonists; ICS, inhaled corticosteroids; LTRA, leukotriene receptor antagonists.

2.1 Respiratory assessment

Assessment of respiratory symptoms

Prior to respiratory assessments participants were asked to complete a questionnaire (appendix 1) which asked if they experienced coughing, chest tightness, dyspnoea or excess mucus during or after exercise and if exposure to the cold, dry air, high pollen levels, high pollution, altitude or any other environmental conditions exacerbated these symptoms (Dickinson et al., 2011).

Assessment of airway inflammation

Airway inflammation was assessed by determining the fraction of exhaled nitric oxide (FeNO) using a NIOX VERO (NIOX, Aerocrine, Sweden). Nitric oxide is a gaseous molecule is produced in the airways and therefore present in exhaled breath. Within the respiratory system, NO regulates vascular and bronchial tone, acts as a neurotransmitter for non-adrenergic and non-cholinergic neurons in the bronchial wall and facilitates the synchronised beating of ciliated epithelial cells. It is formed by the action of one of three isoforms of the enzyme nitric oxide synthase (NOS); namely endothelial (eNOS), inducible (iNOS), and neuronal (nNOS) (Hart, 1999). In atopic asthmatics high concentrations of NO have been found in expired air due to the upregulation of iNOS in the respiratory epithelium by pro-inflammatory Th2- cytokines interleukin (IL)-4 and IL-13 (Alving and Malinovschi, 2010). FeNO has previously been regarded as a surrogate marker of eosinophilic airway inflammation, however it is now thought FeNO is more representative of the Th2-driven local inflammation, specifically of the bronchial mucosa, rather than general eosinophilic inflammation (Bjermer et al., 2014). Measuring FeNO has been found useful to distinguish asthma from other respiratory conditions, assess the etiology of respiratory symptoms (Dweik et al., 2011), to predict responsiveness of treatment with inhaled corticosteroids (Smith et al., 2005) and it also correlates with bronchial hyperresponsiveness and atopy (Tinkelman et al., 2009) rendering it a useful marker of airway health.

Assessment of FeNO was performed in accordance with ATS/ERS recommendations (Dweik et al., 2011). Participants remained seated and were instructed to put the NIOX mouthpiece between their lips forming a tight seal and to inhale fully. They were then instructed to exhale against the slight resistance of the machine for 10 seconds at a steady rate (a flow rate of 50 mL˙s-1 + 10% at a pressure of 16 cmH_2O) guided by an on-screen animation. This test was performed in duplicate and a mean of the two measurements recorded. Interpretation of results was as follows: <25 ppb Normal; eosinophilic airway

inflammation unlikely. 25-50 ppb Elevated; eosinophilic airway inflammation likely. >50 ppb High; eosinophilic airway inflammation significant (Dweik et al., 2011).

Assessment of maximal lung function

Lung function was assessed by maximal flow volume spirometry using a Spiro-USB and MicroLab software (CareFusion, Germany). Prior to each day of testing, and after 4 hours of use the spirometer was calibrated using a 3 L syringe at three different flow rates; low, medium and high (0 - 0.9 L/s, 1.6 - 4.5 L/s and 7 - 12 L/s respectively), with the procedure repeated three times at each flow rate. Verification was accepted if the accuracy of the volume was within 3% at all flows.

Prior to testing, participants were assessed for any contraindications to spirometry (Table 2.2) and excluded from participation where necessary. Spirometry was performed in accordance with ATS/ERS recommendations (Miller et al. 2005). The full procedure was first explained to the participant and a demonstration provided. Participants were asked to sit in an upright position throughout the manoeuvre. They were instructed to take a deep breath, ensuring they reach total lung capacity. Without pausing, they sealed their lips around the mouthpiece and exhaled with the maximum possible effort, continuing to exhale to residual volume. On reaching residual volume, they finally inhaled maximally to total lung capacity. A soft nose clip was worn to prevent air escaping through their nose and encouragement was provided throughout to ensure maximal effort. Measurement was repeated with a minimum of 30 seconds rest in between efforts until three acceptable (Miller et al. 2005) attempts had been obtained (Table 2.3), with a maximum of eight attempts performed.

For each flow-volume manoeuvre the following parameters were recorded: forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), peak expiratory flow (PEF) and

FEV_1: FVC ratio (FEV_1/FVC). The best of the three acceptable measurements of FEV_1 and FVC were selected in accordance with the acceptability criteria in Table 2.3.

Table 2.2. Contraindications to spirometry extracted from Cooper (2011)

Contraindication	Recommendation	Reason
Thoracic/ abdominal surgery	Relative	Rupture of injury site, pain, discomfort
Brain/ eye/ear/ EN surgery	Relative	Rupture of injury site, pain, discomfort
Pneumothorax	Relative	Worsen pneumothorax, pain, discomfort
Myocardial infarction	Absolute/ relative	Induce further infarction leading to cardiac arrest
Ascending aortic aneurysm	Absolute/ relative	Rupture of aneurysm, catastrophic/ fatal event
Haemoptysis	Absolute/ relative	Pulmonary emboli or myocardial infarction
Acute diarrhoea	Relative	Discomfort, embarrassment, infection risk
Angina	Absolute/ relative	May lead to cardiac arrest in severe cases, discomfort
Severe hypertension (systolic > 200mm Hg, diastolic > 120 mm Hg)	Measure BP before if suspected	Risk of blackout/ collapse, rupture of cerebral blood vessels
Confusion / dementia	Balance need for test against obtaining results	Lung function tests are volitional and require patient cooperation

Table 2.3. Criteria for acceptable flow volume loops, from Miller *et al*., (2005)

Within-manoeuvre criteria	Between-manoeuvre criteria
Free from artefacts: Cough during the first second of exhalation Glottis closure that influences the measurement Early termination or cut-off Effort that is not maximal throughout Leak Obstructed mouthpiece They have good starts: Extrapolated volume <5% of FVC or 0.15 L, whichever is greater They show satisfactory exhalation: Duration of 6 s or a plateau in the volume–time curve	The two largest values of FVC must be within 0.150 L of each other The two largest values of FEV_1 must be within 0.150 L of each other

Bronchoprovocation Challenges

Eucapnic voluntary hyperpnoea (EVH)

As discussed in Chapter 1, eucapnic voluntary hyperpnoea (EVH) is a surrogate for exercise and has previously been reported to provide greater sensitivity in comparison to other airway challenges in providing a diagnosis of EIB (Dickinson et al. 2006; Holzer et al., 2002). On this basis, EVH has been endorsed by the International Olympic Committee Medical Commission (IOC-MC) as the preferred bronchoprovocation challenge to diagnose EIB in elite athletes (Anderson et al., 2001).

EVH challenges were performed using the methods described by Anderson *et al*., (2001) following baseline spirometry to establish maximal lung flow volume loops. An EVH challenge was not performed if a participant's baseline FEV_1 was less than 70% of predicted normal for age, gender and height (Weiler et al., 2016). In this case they instead performed a reversibility challenge as described below. The target $\dot{V}_E$ the participant was required to reach during the EVH challenge was 85% of their maximal voluntary ventilation (MVV). This was calculated by multiplying their best baseline FEV_1 by 30 (Argyros et al., 1996) to establish a target $\dot{V}_E$. Participants were seated in front of the EVH set up (figure 2.1) and sealed their lips around the mouth piece, they also wore a soft nose clip. Participants inspired a medical grade dry gas comprising 5% CO_2, 21% O_2 and 74% N_2, <2% RH at 19°c (BOC, UK). The 5% CO_2 is to ensure eucapnia is maintained and reduce the chances of experiencing adverse effects from prolonged hyperventilation such as syncope or light-headedness. Participants were instructed to breathe 'hard and fast' for 6 minutes, aiming for the target $\dot{V}_E$. Encouragement was provided throughout, and participants were able to see the elapsed time and the total volume of expired air through the dry gas meter. $\dot{V}_E$ was recorded throughout and a minimum threshold for an acceptable test was considered if the participant achieved at least 60% of their predicted MVV (Anderson et al., 2001).

On completion of the EVH challenge, maximal flow volume loops were measured in duplicate at 3, 5, 7, 10 and 15 minutes using the methodology described above and the highest FEV_1 at each time point recorded. The test was deemed positive if there was a fall in FEV_1 of $\geq$ 10% from baseline at two consecutive time points (Hurwitz et al., 1995). At this point 4 ×100 µg of inhaled salbutamol was self-administered using a metered dose inhaler (MDI) and spacing device (van der Palen et al., 1995) after which maximal flow volume loops were recorded 15 minutes post inhalation. Severity of EIB was classified as mild, moderate or severe dependant on the fall in FEV_1 post EVH: $\geq$10% to <25%, $\geq$25% to

<40% and ≥40% respectively (Anderson and Kippelen, 2013). Participants were required to remain in the laboratory until their FEV_1 had returned to within 10% of baseline.

1: Medical grade dry air. 2: Gas regulator. 3: High pressure tubing. 4: Flow meter. 5: Reservoir. 6: Flexible 35mm tubing. 7: Mouthpiece attached to chamber with one-way valve. 8: Dry Gas Meter

Figure 2.1. EVH set up

Exercise Challenge

Within the experimental chapters of this thesis two exercise challenges have been utilised. Two slightly different methods were used as the first was designed as a one-off diagnostic test and the second was within a study which required an initial $\dot{V}O_2$peak test. The cycle ergometers used, and the environmental conditions of the environmental chamber also differed. Described below is the general methodology employed for exercise testing and the differing details are defined within each chapter.

An exercise challenge would appear to be the logical choice of test to diagnose an exercise induced conditions, however, despite clear guidelines (Crapo et al., 2000; Weiler et al., 2016) there remains a lack of one definitive standardised test. We followed recommended guidelines to create a cycle ergometer challenge in a dry environment without the use of

medical grade dry air. A cycling challenge was decided upon due to the facilities available to us and the practicalities of administering the challenge.

As described for the EVH challenge above, baseline spirometry was performed to establish maximal lung function. Exercise challenges were conducted in an environmental chamber (TIS Services, Hampshire, UK) on a cycle ergometer. Following a standardised four minute incremental warm up, participants were given a target power output which was then maintained for the final six mins of cycling at this work rate. There was no break between the warm up and the main challenge and the warm up was purposely short to ensure that no refractoriness occurred. Heart rate was recorded throughout (Polar RS400; Polar Electro Oy, Kempele, Finland) and $\dot{V}_E$ was recorded for the final six mins of exercise via expired air passing directly through a dry gas meter using a mouth piece with a one-way valve (figure 2.2) and participants wore a nose clip throughout to ensure oral breathing. As with the EVH challenge, the threshold for an acceptable test was considered if the participant achieved at least 60% of their MVV (Weiler et al., 2016). On completion of the exercise challenge participants were seated outside the chamber and maximal lung function was once again measured after 3, 5, 7, and 10 and 15 minutes using the methodology described above. If a participant demonstrated a fall in their FEV_1 of $\geq$ 10% following the exercise challenge at two consecutive time points, this was deemed a positive test. At this point they self-administered 4 ×100 µg of inhaled salbutamol using an MDI and spacing device (van der Palen et al., 1995) and maximal flow volume loops were once again performed 15 minutes post inhalation. Participants were required to remain in the laboratory until FEV_1 returned to within 10% of baseline. In keeping with the EVH challenge participants were not permitted to undergo exercise challenge testing if their baseline FEV_1 was < 70% of their predicted value and where this occurred they carried out a reversibility challenge instead.

1: Environmental Chamber. 2: Mouth piece with one-way valve. 3: Flexible 35mm tubing. 4: Dry gas meter.

Figure 2.2. Exercise challenge set up

Reversibility challenge

Participants were unable to undergo bronchoprovocation challenges (either by EVH or exercise) if their baseline FEV_1 was below 70% of predicted. Instead, they undertook reversibility challenge by self-administering 4 ×100 µg of inhaled salbutamol using a metered dose inhaler (MDI) and spacing device. Maximal flow volume loops were then performed 15 minutes post inhalation. A positive bronchodilator response was defined as a ≥ 12% increase in FEV_1 from the baseline (Fitch et al., 2008).

Chapter 3. A comparison of EVH and a standardised exercise challenge in a dry environment

3.0 ABSTRACT

OBJECTIVES: To compare an exercise challenge (EX) in a controlled dry air environment to EVH for the diagnosis of exercise induced bronchoconstriction (EIB) in athletes. METHODS: Seventeen male and ten female participants (age: 36 ± 11 yrs, height: 174.3 ± 7.8 cm, mass: 72.6 ± 11.3 kg, exercising 7.6 ± 3.0 hrs per week) were included in the final analysis. Seven had a history of asthma/EIB, 4 of whom were taking medication in the form of SABAs. Participants completed an EX on a cycle ergometer and an EVH challenge in a randomised order. EXs were conducted in an environmental chamber at 16.5°C and 26% RH. Following a 4-min warm up, participants completed 6-min of cycling at a work rate associated with 85% HRmax. EVH required participants to breathe a gas mixture (5% CO_2, 21% O_2 and 74% N_2, <2% RH) at 85% predicted MVV. Challenges were deemed positive if there was a fall in FEV_1 of ≥ 10% from baseline. RESULTS: Six participants were positive to EVH (% fall in FEV_1 16 ± 5%, range -11 to -25%), of these, only two had a positive response to EX (11 % fall in FEV_1). There was a moderate correlation between the % fall in FEV_1 post EVH and EX (r_s = 0.50, p = 0.01), however agreement analysis showed no useful agreement between methods. The % fall in FEV_1 post EVH was significantly greater than post EX (EVH: -7.7 ± 5.4 %, EX: - 2.0 ± 4.0 %, p < 0.01). CONCLUSION: EVH challenge provides greater sensitivity than a cycle exercise challenge in a cold, dry environment (16.5°C and 26% RH) in the diagnosis of EIB, which may be due to the lower water content of inspired air and a greater $\dot{V}_E$.

3.1 INTRODUCTION

Securing a diagnosis of exercise-induced bronchoconstriction (EIB) in athletes as discussed in chapter 1 remains a challenge. This is principally due to the poor relationship between the presence of classic airway centric respiratory symptoms (e.g. wheeze, cough, dyspnoea), and objective evidence of EIB (Simpson et al., 2015; Rundell et al. 2001; Turcotte et al., 2003; Price et al. 2016).

As highlighted in chapter 1, EIB is highly prevalent in both elite (21 – 68% (Dickinson et al., 2005; Levai et al., 2016)) and recreational (13% (Molphy et al., 2014)) athletes. Unrecognised or inadequately treated EIB can have significant health consequences and is linked to exercise related mortality (Becker et al., 2004). There is also evidence to demonstrate that EIB has the potential to impair exercise performance (Price et al. 2014; Stensrud et al., 2007; Brukner et al. 2007; Spiteri et al. 2014). For the aforementioned reasons it is important to secure a diagnosis of EIB through objective airway challenge testing (Parsons et al., 2013) to ensure optimal treatment or differential diagnosis.

As exercise is the provocative stimulus to induce bronchoconstriction in athletes, it would seem logical to implement an exercise challenge (EX) as way of diagnosis, however despite clear guidelines (Crapo et al., 2000; Weiler et al., 2016) there remains limitations when employing this methodology as the sensitivity of EX is highly dependent on control over the two main contributors to the airway response: the water content of inspired air (Evans et al., 2005) and minute ventilation ($\dot{V}_E$) (Carlsen et al., 2000).

Eucapnic voluntary hyperpnoea (EVH) can be used as a surrogate for exercise and has been shown to provide greater sensitivity in comparison to EX and direct airway challenges (Dickinson et al. 2006; Holzer et al., 2002). On this basis, EVH is currently endorsed by the International Olympic Committee Medical Commission (IOC-MC) as the optimal bronchoprovocation challenge to diagnose EIB in elite athletes (Anderson et al., 2001). At

present a positive result, suggestive of EIB, in both EVH and EX is currently defined as a 10% pre-to post challenge fall in FEV_1 (Weiler et al., 2016). This cut-off value remains much debated, with a cut off of 15% for EVH being proposed by some authors (Price et al. 2016) and a fall of 6.5% (Helenius et al., 1998) and 7.1% (Rundell et al., 2000) suggested more appropriate for EX in an athletic population.

Due to the lack of a standardised EX, athletes can currently demonstrate EVH positive, without knowing if exercise is likely to trigger the same degree of bronchoconstriction. With this in mind, the aim of the current study was to compare a standardised EX in a dry environment to EVH for the detection of EIB in athletes.

3.2 METHODS

Study population

Following approval from the School of Sport & Exercise Sciences Research Ethics Committee (Prop 29_2014_2015). Thirty-three recreationally active volunteers provided written informed consent to participate (appendix 3). Inclusion criteria were: male or female, aged 18 – 65 years, training ≥ 3 times a week, healthy or exercise induced asthmatics whose only medication was inhaled short-acting β_2-agonist (SABA).

Study design

In a randomised order, participants were required to attend the laboratory on two separate occasions to complete either an EVH or EX on a cycle ergometer. Each visit was separated by at least 48 hrs and was completed within one week at the same time of day. All participants reported that they were free from illness in the two weeks prior to assessment. Participants were instructed to maintain their usual diet throughout the duration of the study, to avoid exercise and caffeine for 24 hrs and 4 hrs respectively before each visit and arrive at the laboratory at least 2 hrs postprandial. Participants with a prior diagnosis of asthma and/ or EIB were asked to refrain from taking any SABA on the day of testing.

At their initial visit participants completed a questionnaire to determine their medical history and evaluate the presence of respiratory symptoms (appendix 1). Airway inflammation was assessed prior to both challenges by determining fraction of exhaled nitric oxide (FeNO) (NIOX VERO, NIOX, Aerocrine, Sweden) (Dweik et al., 2011). Resting lung function was assessed by maximal flow volume spirometry (Spiro-USB and MicroLab, CareFusion, Germany) (Miller et al. 2005). Maximal flow volume loops were then measured in duplicate

at 3, 5, 7, 10- and 15-mins post challenge, with the flow loop with the best FEV_1 accepted at each time-point. A positive diagnosis for EIB was defined by a fall of 10% or more in FEV_1 at two consecutive time-points. Following a positive diagnosis of EIB, inhaled salbutamol (4 ×100 µg) was self-administered and maximal flow volume loops were recorded 15 mins post inhalation to ensure lung function returned to within 10% of baseline.

Eucapnic voluntary hyperpnoea

The EVH challenge was conducted as described in chapter 2. In brief, participants inspired a medical grade dry gas (5% CO_2, 21% O_2 and 74% N_2, <2% RH) for 6 mins at a target ventilation rate equivalent to 85% predicted maximal voluntary ventilation (MVV) (30 ×FEV_1) (Argyros et al., 1996). $\dot{V}_E$ was recorded and a minimum threshold for an acceptable test was considered 60% MVV (Anderson et al., 2001).

Exercise challenge test

The EX was conducted in an environmental chamber (TIS Services, Hampshire, UK) (16.5°C, 25.5% RH) on a Wattbike cycle ergometer (Wattbike Ltd, Nottingham, UK). Further details are provided in chapter 2, however following a standardised 4 min incremental warm up (1 min at 40, 75, 110 and 145 Watts), participants were given a target power output which was a work rate associated with 85% HRmax and they went straight into 6 mins of cycling at this work rate. This work rate was determined using submaximal heart rate data from the first three stages of the warm up and the algorithm described by Ansley *et al*., (2010). Heart rate was recorded throughout (Polar RS400; Polar Electro Oy, Kempele, Finland) and $\dot{V}_E$ was recorded for the final 6 mins of exercise via expired air passing directly through a dry gas meter using a mouth piece with a one-way valve to match

the method in the EVH test (Figure 3.1). Again, the threshold for an acceptable test was considered 60% MVV (Weiler et al., 2016).

Figure 3.1. Equipment set up during exercise challenge (EX)

Statistical Analysis

Data are presented as Mean ± SD unless otherwise stated. Results for FeNO, pre to post challenge spirometry, challenge and exercise data were analysed using paired *t*-tests, or Wilcoxon signed rank test where the data was not normally distributed. Correlation between challenges was determined by Spearman's rank correlation and agreement by Bland Altman analysis (Bland and Altman, 1986). Associations between gender and challenge result were assessed using Chi-squared analysis. All analysis was conducted using SPSS software, V.23 (SPSS, IBM, Armonk, NY, USA) with significance accepted at $P < 0.05$.

3.3 RESULTS

Seventeen male and ten female participants (age: 36 ± 11 yrs, height: 174.3 ± 7.8 cm, mass: 72.6 ± 11.3 kg, exercising 7.6 ± 3.0 hrs per week), were included in the analysis (n = 1 excluded due to inability to perform reliable spirometry and n = 5 due to not achieving an acceptable EX i.e. did not achieve a $\dot{V}_E$ of ≥ 60% predicted MVV). Seven participants reported a history of asthma/EIB, five of these had a current diagnosis and four were currently taking medication for this in the form of a SABA when required.

Baseline lung function

All participants demonstrated normal spirometry at baseline with no evidence of obstruction (FEV_1 ≥70%, FEV_1/FVC ≥70% predicted). Eleven participants had an elevated FeNO (>25ppb) under resting conditions prior to EVH and/or EX (Nine had an elevated FeNO prior to both tests, one prior to EVH only and one prior to EX only). There were no differences in resting lung function or FeNO prior to each challenge (Table 3.1).

Minute ventilation, power output and heart rate

The percentage of predicted MVV achieved in the EVH challenge was greater than that achieved during EX (p = 0.03) (table 3.1), although not all participants had a greater $\dot{V}_E$ during EVH than EX; 18 achieved a greater $\dot{V}_E$ in EVH, 8 during EX and one participant had an equal $\dot{V}_E$. Power output attained during EX was lower than the target power (244.5 ± 57.2 W compared to 288.4 ± 71.3 W, P < 0.001). All participants achieved a HR greater than 80% of their predicted HRmax (89.1 ± 4.8%, range: 80.8 – 96.6%).

Table 3.1. FeNO, pre to post challenge spirometry and challenge data.

	EVH	EX	Sig.
FeNO (ppb)	18 (29)	19 (23)	0.17
FEV_1 (L)	3.89 ± 0.61	3.88 ± 0.62	0.90
FVC (L)	5.00 ± 0.88	4.97 ± 0.89	0.33
PEF (L/min)	556 ± 96	557 ± 87	0.89
FEV_1/FVC (%)	78 ± 7	78 ± 7	0.34
%MVV during challenge	83.0 ± 10.3	77.2 ± 10.7	0.03*
Maximal % change in FEV_1 post challenge	-6.0 (6.0)	-1.0 (5.0)	<0.01*

Data mean ± SD. * indicates a significant difference between EVH and EX. Data for FeNO and % fall in FEV_1 post challenge were not normally distributed and therefore analysed with Wilcoxon Signed Rank tests and are presented as the median score (IQR).

FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow; %MVV, percent of predicted maximal voluntary ventilation.

EVH vs. EX

Of the 27 participants, six had a positive EVH challenge (EVH+) (16 ± 5% fall in FEV_1; figure 3.2a), and of those, two participants were also positive to EX (EX+) (both with 11% fall in FEV_1; figure 3.2b). There were no participants who were positive to EX but not EVH (figure 3.3). There was no association between gender and response to EVH ($p = 0.7$) or EX ($p = 0.69$).

There was a moderate positive correlation between the % fall in FEV_1 post EVH and EX (*r* = 0.50, p = 0.01). Bland Altman analysis revealed poor agreement between methods (Figure 3.4), with wide limits of agreement (LOAs) (+ 3.5% to - 15%). The % fall in FEV_1 post EVH was greater than post EX (- 7.7 ± 5.4% compared to -2.0 ± 4.0% respectively; p < 0.01; table 3.1). The sensitivity, specificity and likelihood ratios for EVH and EX to diagnose EIB are shown in tables 3.2a and 3.2b respectively.

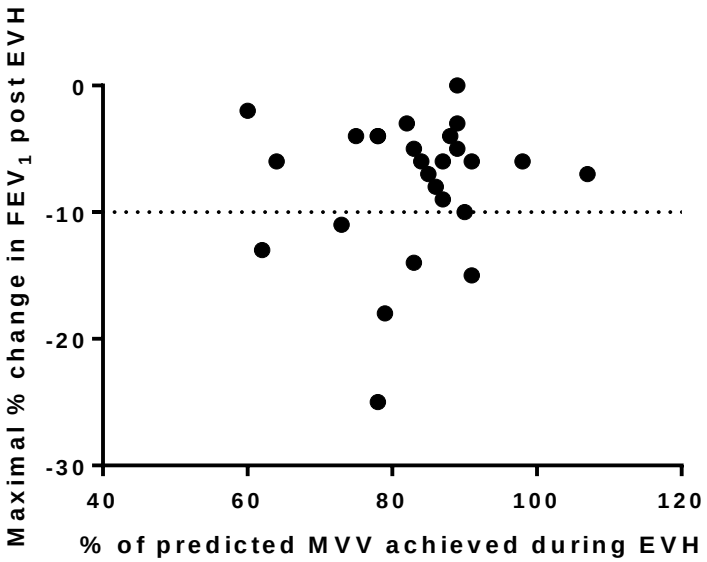

Figure 3.2a. Maximal % change in forced expiratory volume in 1 s (FEV_1) post eucapnic voluntary hyperpnoea (EVH) challenge and % of maximal voluntary ventilation (MVV) achieved during EVH. Dashed line indicates the threshold for a positive EVH test.

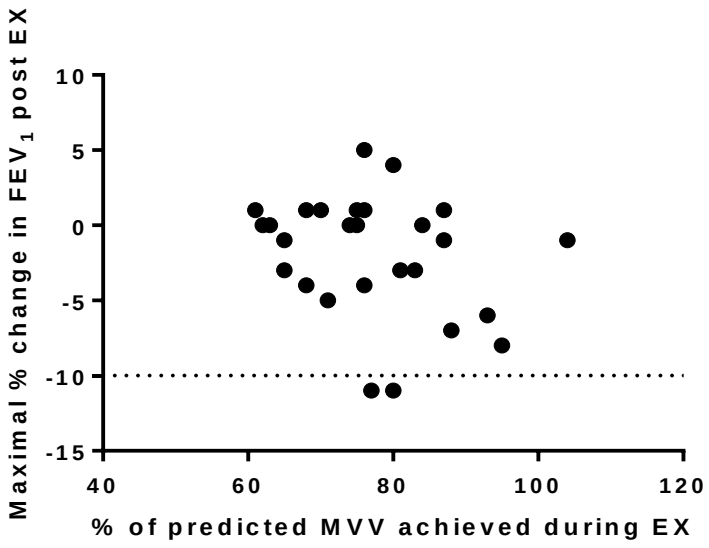

Figure 3.2b. Maximal % change in forced expiratory volume in 1 s (FEV_1) post exercise challenge (EX) and % of maximal voluntary ventilation (MVV) achieved during EX. Dashed line indicates the threshold for a positive EX test.

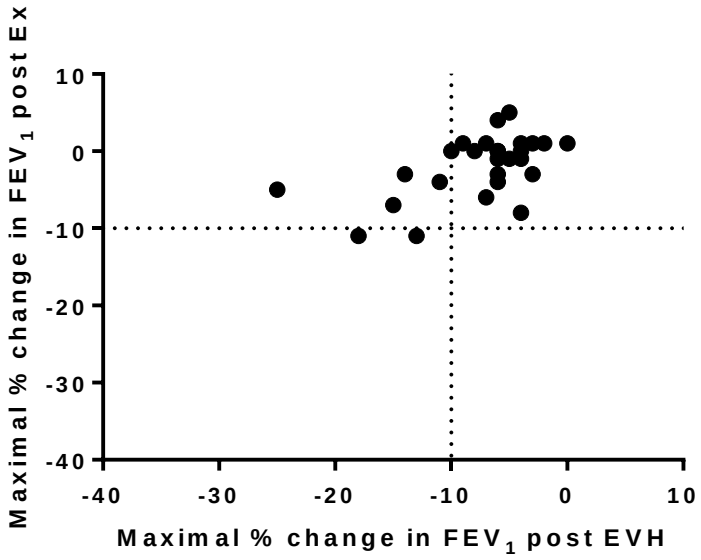

Figure 3.3. Maximal % change in forced expiratory volume in 1 s (FEV_1) post eucapnic voluntary hyperpnoea (EVH) and exercise (EX) challenges. Dashed lines represent the thresholds for a positive test.

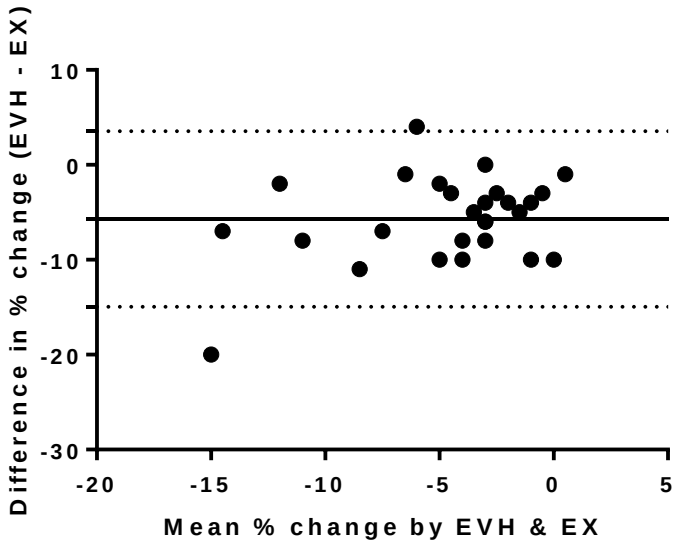

Figure 3.4. Bland-Altman plot of difference in % change in forced expiratory volume in 1 s (FEV_1) between eucapnic voluntary hyperpnoea (EVH) and exercise (EX) challenges. Solid line represents the mean difference. Dashed lines represent limits of agreement (LOAs).

Table 3.2a. Sensitivity, specificity and likelihood ratios for EVH to diagnose EIB.

	EX+	**EX -**
EVH +	2 (true positive)	4 (false positive)
EVH -	0 (false negative)	21 (true negative)
	Sensitivity = 100%	Specificity = 84%
	Positive likelihood ratio = 6.25	Negative likelihood ratio = 0
	Accuracy = 85%	

EVH, Eucapnic voluntary hyperpnoea; EX, Exercise challenge.

Table 3.2b. Sensitivity, specificity and likelihood ratios for EVH to diagnose EIB.

	EVH+	**EVH -**
EX +	2 (true positive)	0 (false positive)
EX -	4 (false negative)	21 (true negative)
	Sensitivity = 33.3% Positive likelihood ratio = 0 Accuracy = 85%	Specificity = 100% Negative likelihood ratio = 0.67

EVH, Eucapnic voluntary hyperpnoea; EX, Exercise challenge.

Reversibility

All EVH+ participants demonstrated a subsequent improvement in FEV_1 following administration of bronchodilator treatment (improvement in FEV_1 = 18 ± 8%, with a range of 8-28%). Following the EX these participants had a 22 ± 6% improvement with a range of 17 and 26% following the administration of salbutamol.

Respiratory symptoms

A total of 19 out of 27 (70%) participants reported troublesome respiratory symptoms including cough, chest tightness, excessive mucus production, dyspnoea and wheeze at a frequency of 37, 22, 30, 19 and 26% respectively. Although respiratory symptoms were frequently reported, there was no relationship between the presence of symptoms and the likelihood of a positive challenge result (Table 3.3).

Table 3.3. Association between symptoms and EVH and EX result.

	Reporting at least 1 symptom	**Reporting no symptoms**	**Sig.**
EVH+	4 (5%)	3 (11%)	
EVH-	15 (56%)	5 (19%)	0.34
EX+	0 (0%)	2 (7%)	
EX-	19 (17%)	6 (22%)	0.42

EVH, Eucapnic voluntary hyperpnoea; EX, Exercise challenge.

3.4 DISCUSSION

Our findings demonstrate that when compared with an EVH challenge, exercising on a cycle ergometer for 6 minutes at 85% predicted HRmax in a cold, dry environment (16.5°C and 26% RH), resulted in fewer participants presenting with bronchial hyper-responsiveness in line with a diagnosis of EIB. Specifically, we found that only two of the five EVH+ participants were also EX+. Indeed, Dickinson *et al*., (2006) report that out of 14 winter sports athletes tested, 10 were EVH+, but only 3 were positive to a sport specific challenge where conditions were either 8°C and 35% RH or 1.5°C and 33% RH, and no athletes were positive to a lab based challenge where the conditions were 18°C and 56% RH. Similarly, Mannix *et al*., (1999) compared an on ice skating challenge to EVH in 29 ice skaters and found 12 were positive by EVH, 9 were positive by on-ice testing, with 5 individuals positive for EIB on both tests. They concluded that EVH had greater sensitivity than on-ice exercise for identifying EIB in competitive figure skaters. Rundell *et al*., (2004) screened 38 winter sports athletes for EIB; they identified 17 athletes by EVH and only 11 by EX. Despite our control of both humidity and $\dot{V}_E$, results of this study also suggest that EVH provides a more sensitive diagnosis of EIB than EX.

We found that there was a greater fall in the % of FEV_1 relative to the baseline following EVH than after EX. Despite standardising the humidity of the environment that the EX challenges were conducted in, the medical grade dry air with which EVH is conducted is <2% RH, which will never be possible to match during an EX, unless the dry air is inspired directly as carried out by some groups (Anderson et al., 2010). We were keen to avoid this method, opting for more realistic environment in which an athlete may train or compete, and the lowest humidity we could achieve at 16°C, which was 25% RH. This is a lower humidity than Dickinson *et al*., (2006) whose lab conditions were 18°C and 56% RH, and would therefore account for why we had a greater number of athletes positive to a lab exercise challenge. The % of MVV achieved during 6 minutes of EVH was significantly higher than

that achieved during the 6 minutes of EX. The target workload during Ex was a workload equating to 85% predicted HRmax; participants in this study struggled to achieve this workload and their achieved workload was significantly lower than this threshold. Despite this, average HR for the EX challenge was 89 ± 5% of predicted, and as such was above that of 80-90% which is recommended (Crapo et al., 2000). Due to the aforementioned differences in the two challenges, the greater sensitivity with EVH is most likely due to the lower water content of inspired air and greater $\dot{V}_E$.

A ≥10% fall in FEV_1 is the cut off value for a positive test recommended in the ERS (Sterk et al., 1993) and ATS (Crapo et al., 2000) guidelines for the diagnosis of EIB by EVH or EX challenges. The 10% diagnostic threshold for a positive EVH test was proposed following a study of army recruits with asthma (Hurwitz et al., 1995). Recent retrospective analysis of data from asymptomatic athletes suggested that a cut off of 15% might be a more appropriate threshold in this population (Price et al. 2016), particularly because poor reproducibility of mild EIB (a 10-15% fall in FEV_1 following EVH) has been demonstrated in recreational athletes (Price et al., 2015). Others however, have found a good reproducibility of EVH at all severities in physically active participants (Williams et al., 2015) and these discrepancies may be due to the transient nature of EIB (Cockcroft and Davis, 2006). There have also been suggestions that the criteria for a positive EX should be lower than the 10% threshold originally suggested. A 6.5% fall in FEV_1 following EX has been shown to be the threshold of an abnormal response in elite runners (Helenius et al., 1998), and a 7.1% fall in a population of winter athletes (Rundell et al., 2000). A change in criteria for a positive EX to two consecutive falls of ≥6.5% in FEV_1 would have led to no additional participants being identified as EX+ in our study. If we used an amended threshold of ≥15% fall in FEV_1 for a positive EVH challenge, 3 participants would no longer be classed as EVH+. One of these athletes was EX+, which highlights the increased risk of detecting false negatives with this threshold change. It is therefore clear that EVH is more sensitive than EX, however further

work is required in athletic individuals to evaluate what comprises an 'abnormal' response in this population. The transient nature of EIB means that individuals with a small (10-15%) fall in FEV_1 following EVH in a one-off test, may be susceptible to EIB. Therefore, prior to considering any changes in threshold, further research is required to look at the effect of mild EIB on airway health and performance.

In agreement with previous studies in athletes (Simpson et al., 2015; Rundell et al. 2001; Turcotte et al. 2003; Price et al. 2016), we found that there was no association between the presence of symptoms and evidence of EIB through either an EVH or EX challenge. This combined with the negative effects of EIB on athlete health (Price et al., 2013) highlights the importance of having a robust objective airway challenge to secure a diagnosis of EIB by confirming any reversible change in airway function (Parsons et al., 2013). The EVH challenge is often quoted as 'gold standard' challenge, however a recent review by Hull *et al*. (2016) challenged this assumption and concluded that 'the wide sensitivity and specificity indices and poor repeatability preclude EVH being termed a 'gold standard' test for EIB'. As exercise is the provocative stimulus to induce bronchoconstriction in athletes, the true gold standard may be a sport and environment specific exercise challenge. However, it may be an athlete has to perform multiple exercise challenges on separate days before they are able to present with a positive challenge, due to variable factors such as environment on the day of test and the athlete's ability to sustain exercise to achieve significant airway ventilation. Our study highlights this difficulty in establishing a standardised exercise which is sensitive enough and more work is needed to establish appropriate diagnostic thresholds. With the absence of a reliable sport specific standardise challenge, EVH remains a valuable indirect bronchoprovocation challenge (Hull et al., 2016). The majority of participants (78%) in the current study were negative to both types of challenge, meaning we had a limited number of comparisons for EVH+ and EX+ results. Most of our subjects who did demonstrate EVH+ were deemed as having a mild response (10 – 15% fall in FEV_1). It

would be beneficial to look at the comparison of EX and EVH in participants with a current diagnosis of EIB to get a much wider spread of the EIB spectrum in this population. The environmental conditions for the EX challenge in this study were able to be standardised through use of an environmental chamber which means to carry out the EX described in this study specialised equipment is required, which involves significant costs and is not transportable. Moreover, achieving a suitable $\dot{V}_E$ remains easier to standardise with EVH. From a practical standpoint, conducting multiple challenge tests remains far easier with EVH.

In conclusion, the EVH challenge provides greater sensitivity than a cycle exercise challenge in a cold, dry environment (16.5°C and 26% RH) in the diagnosis of EIB, which may be due to the lower water content of inspired air and a greater $\dot{V}_E$. The ability of EVH to rule out EIB if a test is negative makes EVH a superior objective test for screening for EIB in athletes with a one-off test. It is not known however whether EVH is too sensitive, or whether athletes diagnosed with EIB through EVH screenings experience improvements in airway health and EIB with appropriate therapy.

Chapter 4. The impact of detecting and treating EIB in elite footballers

4.0 ABSTRACT

OBJECTIVES: To evaluate the prevalence of exercise induced bronchoconstriction (EIB) in elite football players and assess subsequent impact of therapy on airway health and exercise performance. METHODS: Ninety-seven male professional football players completed an airway-health assessment with a eucapnic voluntary hyperpnoea (EVH) challenge to diagnose EIB. Players demonstrating a positive result (EVH+) were prescribed inhaler therapy depending on the severity including inhaled corticosteroids and inhaled short-acting β_2-agonists and underwent repeat assessment after 9 weeks of treatment. Eight players (3 EVH+, 5 EVH-) completed a $\dot{V}O_2$ peak test at initial and follow-up assessment. RESULTS: Of the 97 players, 27 (28%) demonstrated a positive EVH result. Of these, ten had no prior history (37%) of EIB or asthma. EVH outcome was not predictable by respiratory symptoms. Seven of the 27 (24%) EVH+ players attended follow-up and demonstrated improved post-challenge spirometry (FEV_1 post-test; pre = -22.9 ± 15.4 %, post = -9.0 ± 1.6 %, p = 0.02). At follow-up $\dot{V}O_2$ peak improved by 3.4 ± 2.9 $ml \cdot kg^{-1} \cdot min^{-1}$ in EVH+ players compared to 0.1 ± 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$ in EVH- players. Magnitude of inference analysis indicated treatment was possibly beneficial (74%) for exercise capacity. CONCLUSION: Elite football players have a high EIB prevalence. Treatment with inhaler therapy reduces EIB severity.

4.1 INTRODUCTION

Eucapnic voluntary hyperpnoea (EVH) is a well standardised and widely accepted bronchoprovocation methodology (Hull et al., 2016) and was demonstrated in chapter 3 to be a highly sensitive test in the diagnosis of exercise induced bronchoconstriction (EIB). As discussed in chapter 3, the ability of EVH to rule out EIB in the event of a negative challenge makes it a suitable objective tool for screening for EIB in athletes with a one-off test. It is not known however if athletes diagnosed with EIB as a result of a positive EVH challenge will experience any health or performance benefits from treatment with standard asthma medication.

In chapter 1 it was highlighted that the nature of elite-level football would suggest that exercise induced bronchoconstriction (EIB) may be relevant and pose a particular risk to this group of athletes. Specifically, elite football players regularly sustain high ventilation rates (Bangsbo, 1994), from a young age (Read et al., 2016), whilst often training and competing in an asthmogenic environment i.e. in cold air, high pollen and areas of high pollution. Despite this, little is currently known regarding the nature of EIB in professional football players.

Previous studies suggest that football players are often misdiagnosed or incorrectly labelled as having EIB or asthma (Ansley et al., 2012), highlighting that respiratory symptoms are poorly predictive in the diagnosis of EIB and therefore limit the accuracy of a symptom based approach to diagnosis (Rundell et al., 2001). There have however, been no prospective studies evaluating the prevalence of EIB or indeed the benefits of treating EIB in this group of elite athletes.

It is recognised that EIB has deleterious effects on gas exchange efficiency (Haverkamp et al., 2007), and therefore can impair performance. Indeed, Stensrud *et al*., (2007) report impaired oxygen uptake ($\dot{V}O_2$ peak) in individuals with EIB, and an improvement in peak

oxygen uptake was reported in Australian Rules football players following the initiation of treatment for previously undetected EIB (Brukner et al., 2007b).

Football clubs have a duty of care for their players; to protect their health whilst at the same time optimising their performance. There are rigorous medical guidelines in place which include screening players for certain medical conditions (e.g. cardiac abnormalities) (UEFA, 2014), however clubs do not routinely assess players for EIB. Some authors have called for screening for EIB to be implemented (Dickinson et al., 2005; Holzer and Brukner, 2004), however before a screening programme can be put in place, a number of stringent criteria must be met, including demonstrating prevalence, ability to detect the condition of interest and an understanding of the impact of intervention in the population of interest (Wilson and Jungner, 1968; Hull et al., 2007).

The aim of this chapter was therefore to address these deficiencies by using robust objective tests to provide a comprehensive assessment of the impact of EIB in professional football players by: (I) determining the prevalence of EIB in elite footballers; (II) assessing the impact of appropriate therapy on airway inflammation and EIB control and (III) investigating the effect of treating players with EIB on exercise performance.

It was hypothesised that EIB would be highly prevalent and that initiation of standard asthma therapy would be beneficial for airway health, as assessed by physiological measures of airway hyper-reactivity and inflammation and for exercise performance.

4.2 METHODS

Study population

Male professional football players from the English Premier League, Championship and League One were invited to participate in a detailed respiratory health assessment, as a component of their pre-season medical examination in July 2016, prior to their preseason training period.

Study design

All players attended for a detailed respiratory assessment, including screening for EIB using an EVH challenge (part I). Subsequently, players with EIB were treated for 9 weeks, after which they had a follow-up respiratory assessment (part II). A subgroup of players completed maximal exercise testing at both baseline and follow-up (part III). All players were free from illness in the two weeks prior to assessment and were requested to avoid exercise and caffeine for at least 4 hrs before assessment. Ethical approval for the study was obtained from the School of Sport & Exercise Sciences Research Ethics Committee (Prop 144-2014_2015) and participants provided written consent (appendix 4).

Part I: Baseline assessment

Players completed a detailed respiratory assessment as described in Chapter 2 and briefly below. They initially completed a questionnaire to determine their medical history and evaluate presence of respiratory symptoms (appendix 2). Players previously prescribed medication for asthma / EIB (n = 7) were asked to withhold treatment at the time of assessment, in accordance with guideline recommendations as described in chapter 2.

Respiratory Assessment

Airway inflammation was assessed by determining fraction of exhaled nitric oxide (FeNO) (NIOX VERO (NIOX, Aerocrine, Sweden) (Dweik et al., 2011) and lung function was assessed by maximal flow volume spirometry (Spiro-USB and MicroLab, CareFusion, Germany) (Miller et al. 2005).

An EVH challenge was then conducted as described in chapter 2. In brief, players were required to inspire a medical grade air (21% O_2, 5% CO_2 and 74% N_2 with <2% humidity) for 6 minutes at a target ventilation rate of 85% Maximal voluntary ventilation (MVV). Target MVV was calculated as 30 × FEV_1 (Argyros et al., 1996) and $\dot{V}_E$ was recorded. Maximal flow volume loops were measured in duplicate at 3, 5, 7, 10- and 15-minutes post EVH, with the flow loop with the best FEV_1 accepted at each time-point. A test was considered positive for EIB if FEV_1 fell by ≥ 10% from baseline at two consecutive time points. At this point 4 × 100 μg of inhaled salbutamol was administered by a metered dose inhaler (MDI) and maximal flow volume loops were recorded 15 minutes post inhalation.

Severity of EIB was classified as mild, moderate or severe dependant on the fall in FEV_1 post EVH (≥10% to <25%, ≥25% to <40% and ≥40% respectively) (Anderson and Kippelen, 2013). Players with a positive EVH challenge (EVH+) test were then prescribed medication by their team physician, in accordance with recommendations (Parsons et al., 2013) with a spacing device and inhaler technique advice (van der Palen et al., 1995) (*see below*).

Treatment

Medication was prescribed according to EIB severity; players with ‘mild EIB’ were prescribed a daily inhaled corticosteroid (ICS), in conjunction with an inhaled short-acting b_2-agonist (SABA) as needed. Those with ‘moderate’ EIB were prescribed a combination inhaler of ICS and a long-acting b_2-agonist (LABA), with a SABA as needed. Finally,

players with 'Severe' EIB were prescribed the combination inhaler with the addition of a daily Montelukast and a SABA as required.

Part II: Follow-up assessment

Players continued with their usual pre-season training and no change was made to training load. After 9 weeks, EVH+ players underwent a further respiratory assessment (*as above*).

Part III: Performance assessment

A subgroup of players (n = 8) completed a $\dot{V}O_2$ peak test on a motorised treadmill with simultaneous gas analysis (Oxycon Pro, Jaeger, Germany). Initial running speed was set at 11 $km.h^{-1}$ and a gradient of 1%. Speed increased 1 $km.h^{-1}$ every 3 minutes until 16 $km.h^{-1}$, at which point the gradient was increased by 1% every minute until volitional exertion. $\dot{V}O_2$ peak was determined as the single highest 30 s average in $\dot{V}O_2$.

Statistical analysis

Data are presented as Mean ± SD unless otherwise stated. Players were grouped according to the result of their EVH challenge (EVH+, EVH-). Group differences in player characteristics and baseline respiratory assessment data were analysed using independent *t*-tests, or Mann Whitney U tests where the data was not normally distributed. Respiratory symptom data and association between league and EVH result were analysed by Chi-square analysis and Fisher's Exact test. Differences in lung function data and EVH results pre and post treatment were assessed using paired *t*-tests and Sign Exact tests where data was not normally distributed and symmetrically shaped. Given the small number of subjects

completing performance assessment magnitude-base inference analysis was also utilised using the excel spreadsheet from Hopkins (2007) to assess the clinical impact of detection and treatment of EIB. All other analyses were conducted using SPSS software, V.23 (SPSS, IBM, Armonk, NY, USA) with significance accepted at $P < 0.05$.

4.3 RESULTS

Ninety-eight players (age: 24 ± 4 yrs, height: 183 ± 7 cm, mass: 80.3 ± 7.2 kg) completed the initial respiratory assessment however one player was subsequently excluded due to his inability to perform reliable spirometry (Table 4.1). Seventeen players reported a previous diagnosis of asthma and/or EIB, of whom only seven were currently prescribed medication. A total of 16 players reported troublesome respiratory symptoms, including cough, wheeze and dyspnoea at a frequency of 5%, 3% and 8% respectively.

(I) Baseline Assessment

Pulmonary function, inflammation and EIB

Lung function was normal in all players at the baseline assessment with no evidence of significant airflow obstruction (FEV_1>70% predicted). In 39 (40%) players the FeNO was raised above normal (> 25 ppb), with 19 players (20%) having a value above 50 ppb (Table 4.1).

A positive EVH result was found in twenty-seven (28%) players, of which the majority (n = 21, 78%) were classified as having a mild response and fewer having a moderate (n = 4 (15%) or severe (n = 2 (7%) response (Figure 4.1). There was no association between the league the footballers played in and their EVH result (p = 0.31). All EVH + players demonstrated a subsequent improvement in FEV_1 following administration of bronchodilator treatment (ΔFEV_1 = 20.6 ± 11.6%, with a range of 6 - 40% (p = <0.001)). There was a weak inverse relationship between FeNO and % fall in FEV_1 post EVH (r_s = -0.24, p = 0.02), i.e. the greater the FeNO the larger the reduction in lung function post EVH.

Table 4.1. Player characteristics and baseline respiratory assessment data for the 97 players who performed the baseline EVH challenge.

	All player (n = 97)	**EVH+ (n = 27)**	**EVH- (n = 70)**	**Sig.**
Age (yrs)	24 ± 4	24 ± 4	24 ± 4	0.56
Height (cm)	182.6 ± 6.8	183.0 ± 6.7	182.5 ± 6.9	0.77
Weight (kg)	80.3 ± 7.2	80.7 ± 6.3	80.2 ± 7.5	0.73
FeNO (ppb)	21 (25)	36. (63)	19 (17)	<0.01*
FEV_1 (L)	4.71 ± 0.65	4.51 ± 0.55	4.78 ± 0.68	0.07
FVC (L)	5.67 ± 0.76	5.68 ± 0.64	5.67 ± 0.81	0.95
PEF (L/min)	630 (135)	616 (102)	635 (149)	0.29
FEV_1/FVC	83 ± 7	79 ± 8	84 ± 6	0.01*
%MVV during EVH	77.7 ± 11.8	83.1 ± 10.9	75.5 ± 11.5	<0.01*
% fall in FEV_1 from EVH	-8 (5.5)	-13 (10.0)	-6 (4.0)	<0.01*

Data presented as mean ± SD. * indicates a significant difference between EVH+ and EVH-. Data for FeNO, PEF and % fall were not normally distributed and therefore analysed with Mann Whitney U tests and data presented as the median score (IQR).

EVH, eucapnic voluntary hyperpnoea; FeNO, exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow; %MVV, percent of predicted maximal voluntary ventilation.

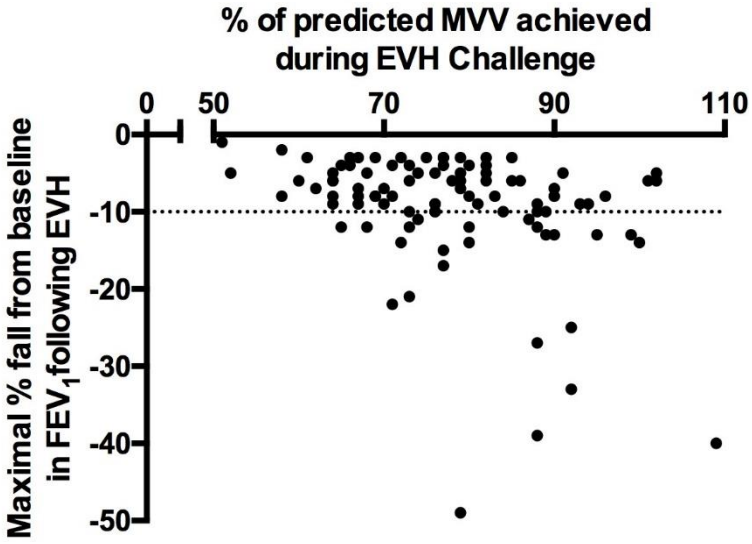

Figure 4.1. Maximum fall in forced expiratory volume in 1 s (FEV_1) post eucapnic voluntary hyperpnoea (EVH) challenge. Dashed line indicates the cut off for a positive EVH test. MVV, maximal voluntary ventilation.

Relationship between EVH result and prior diagnosis and symptoms

Only ten (37%) of the EVH+ players reported a history of asthma and/or EIB and four (15%) of these were currently prescribed asthma medication. Although respiratory symptoms were frequently reported, there was no relationship between the presence of symptoms and likelihood of a positive EVH result (Table 4.2). Moreover, the most severe EVH result (49% reduction in FEV_1) was observed in a player with no prior history of airways disease or current respiratory symptoms.

Table 4.2. Respiratory symptoms reported by players (n = 95).

	Reporting no symptoms	Reporting at least 1 symptom	Cough	Excessive mucus production	Chest tightness	Difficulty breathing	Wheeze
EVH +	19 (70%)	8 (30%)	1 (4%)	1 (4%)	2 (7%)	4 (15%)	1 (4%)
EVH -	60 (89%)	8 (11%)	4 (6%)	1 (1%)	2 (3%)	4 (6%)	2 (3%)
Sig.	0.06	0.06	1.00	0.50	0.32	0.22	1.00

NB. n = 2 did not complete questionnaires.

EVH, Eucapnic voluntary hyperpnoea; EX, Exercise challenge.

(II) Follow-up Assessment

Following nine weeks, only eleven of the twenty-seven (41%) EVH+ players attended for the follow-up visit. Reasons for not attending included: club not permitting time for the medical team to schedule follow-up, not believing it was necessary, away for international competition, injured, and not wanting to repeat the EVH test. A further four players were excluded from follow-up analysis due to them not taking medication as prescribed.

No difference was seen in resting FEV_1 between baseline and follow-up visits (Table 4.3), however FeNO was lower at follow-up ($p = 0.04$) (Table 4.3, Figure 4.2a). There was also a reduction ($p = 0.02$) in the % fall in FEV_1 following EVH and this was apparent despite players achieving a similar total ventilation during the challenge tests (Table 4.3, Figure 4.2b). Moreover, all but two players had a negative EVH test at the second visit (i.e. fall in

FEV_1 <10% post challenge). Despite these findings two of the seven (29%) players reported an increase in symptoms.

Table 4.3. Differences before and after treatment in EVH+ players on medication (n = 7).

	Pre-Treatment	**Post-Treatment**	**Sig.**
FeNO (ppb)	85 ± 61	28 ± 11	0.04*
Baseline FEV_1 (L)	4.41 ± 0.55	4.25 ± 0.32	0.23
%MVV during EVH	85.3 ± 13.6	87.8 ± 13.6	0.38
% fall in FEV_1 post EVH	14 (28)	8 (9)	0.02*

Data presented as mean ± SD * indicates a significant difference between pre-treatment and post treatment. Data for % fall was not normally distributed and therefore analysed with Sign Exact tests and data presented as the median score (IQR).

EVH, eucapnic voluntary hyperpnoea; FeNO, exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; %MVV, percent of predicted maximal voluntary ventilation.

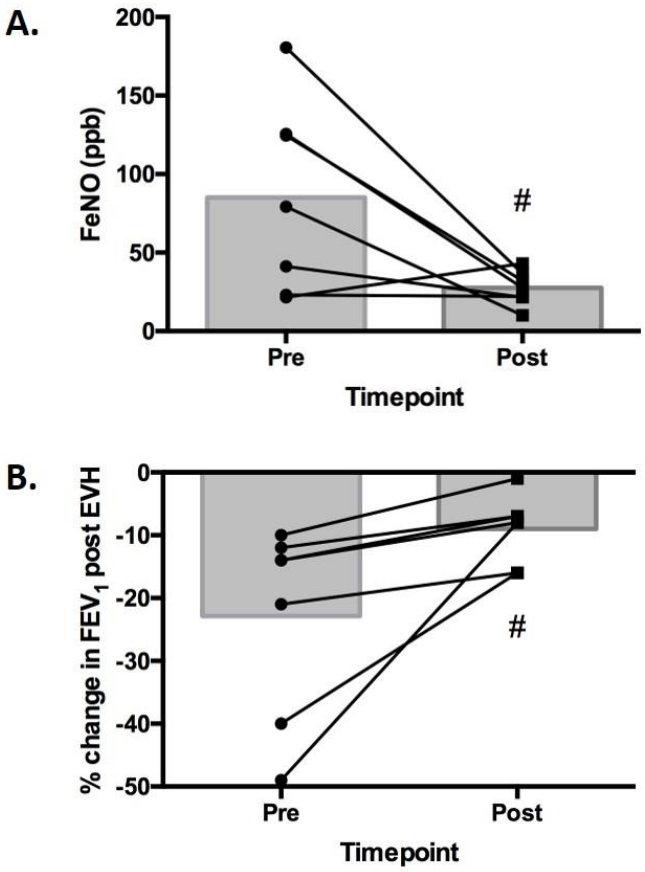

Figure 4.2. A. Exhaled nitric oxide (FeNO) before and after 9 weeks treatment. **B.** Percentage change in forced expiratory volume in 1 s (FEV_1) following eucapnic voluntary hyperpnoea (EVH) challenge before and after 9 weeks treatment. Grey bars indicate mean data. # indicates a significant change from pre-treatment ($P < 0.05$).

(III) Performance Assessment

All EVH+ treated players (n = 3) showed an improvement in $\dot{V}O_2$ peak (Pre: 50.60 ± 5.65 to Post: 53.98 ± 2.80 $ml \cdot kg^{-1} \cdot min^{-1}$ (Figure 4.3), however the magnitude of this change was not significant (p = 0.18). Three EVH- players had a decrease in $\dot{V}O_2$ peak and two demonstrated an improvement (Pre: 53.06 ± 2.14 to Post: 53.15 ± 2.38 $ml \cdot kg^{-1} \cdot min^{-1}$). No significant difference was found in the change in $\dot{V}O_2$ peak between the two groups (p =

0.13); EVH+ players had an improvement of (3.38 ± 2.93 $ml \cdot kg^{-1} \cdot min^{-1}$) and EVH- players (0.09 ± 2.30 $ml \cdot kg^{-1} \cdot min^{-1}$) however, the effect of treatment on maximal exercise capacity, from magnitude-base inference analysis, was found to be possibly beneficial (74%).

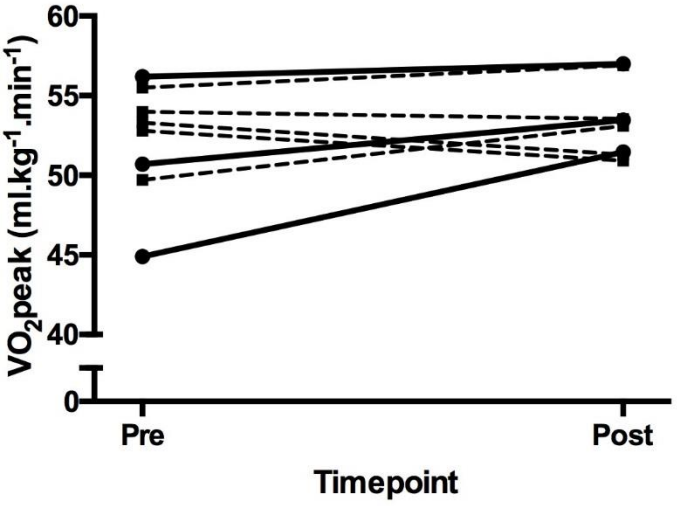

Figure 4.3. $\dot{V}O_2$ peak before and following 9 weeks of treatment. Solid lines represent EVH+ with medication and dashed lines represent EVH-.

EVH, Eucapnic voluntary hyperpnoea.

4.4 DISCUSSION

In a cohort of elite football players completing a pre-season medical screening a high prevalence of both respiratory symptoms and airway dysfunction was found. Indeed, by employing a widely accepted bronchoprovocation methodology (Weiler et al., 2016), namely EVH testing, it was found that approximately one third of this prospectively screened cohort of elite football players had evidence of EIB. Moreover, subsequent treatment with appropriate therapy was associated with a clear attenuation in airway dysfunction and improvement in markers of airway inflammation and hyper-reactivity. In addition, all treated players demonstrated an improvement in peak exercise capacity.

The high prevalence of EIB in elite footballers observed in this study concurs with previous but smaller scale studies that have reported a prevalence of between 29% (Dickinson et al., 2013) and 51% (Ansley et al., 2012) in this group of athletes. Dickinson *et al*., (2013) included only a small sample of one elite football team (n = 21), whilst Ansley *et al*., (2012) studied a highly selected population of football players using inhaler therapy. Thus, this is the first study to truly assess prevalence in a prospective 'medical screening' type scenario across several elite football teams.

The study findings are consistent with other studies demonstrating a heightened prevalence of airway dysfunction in 'running-based' sports (Dickinson et al., 2011). They also, yet again highlight the poor predictive value of symptoms in the diagnosis of EIB (Rundell et al., 2001; Turcotte, Jean Bruno Langdeau, et al., 2003). Indeed 'undetected' EIB was found in 63% of players, and no single symptom or combination of symptoms was predictive of the presence of EIB. Accordingly, it is concerning that there appears to be a high proportion of professional footballers currently training and playing with undiagnosed and uncontrolled EIB. Moreover, in keeping with Ansley *et al*., (2012), evidence of players with a prior diagnosis of EIB / asthma that was incorrect and not supported by the objective test findings

was seen. Indeed 71% of players with a prior diagnosis and treatment plan were either over or under-medicated.

It is also apparent that respiratory symptoms are not useful in predicting the airway response following treatment (Brannan et al., 2007). Specifically, it was found that despite a reduction in the FEV_1 fall post EVH challenge, almost one third of players with EIB reported an increase in symptoms at the follow-up assessment. This is in keeping with the findings of Simpson *et al*., (2015) who reported that half of athletes assessed reported at least one ongoing respiratory symptom despite their fall in FEV_1 post EVH being blunted by the use inhaled terbutaline. Indeed, in this study 28% had a higher symptom score when this fall was blunted. These findings act to emphasis the poor relationship between the presence of respiratory symptoms and airway dysfunction and highlight our current limited understanding of the pathophysiological mechanisms underpinning airway-centric symptoms in athletes (Hull et al., 2017). It also serves to promote the importance of other conditions, such as exercise-induced laryngeal obstruction (EILO), which may co-exist with EIB and cause symptoms (Nielsen et al., 2013; Hall et al. 2016).

In the current study, objective testing was repeated nine weeks after EIB therapy was initiated to ensure adequate control of EIB (Weiler et al., 2016). Data from this follow-up assessment reveals that standard asthma therapy can improve EIB and airway inflammation. Although treatment was recommended for all twenty-seven EVH+ athletes, only seven took the medication as prescribed. This represents an attrition rate of 74%, which suggests a degree of resistance to football players engaging in therapy for this condition. Certainly, anecdotally, EIB often appears to be discounted as medically relevant by medical staff in football squads and viewed as of secondary importance to cardiac screening (Hull and Rawlins, 2016). These findings will hopefully challenge this presumption and provide the supporting basis to educate both players and team clinicians regarding the importance of EIB

in elite football players. This work acts to address some of the concerns regarding screening athletes for airway dysfunction (Hull et al., 2007) and certainly suggests that a focus on airway health is important in football players, given the work suggesting that EIB in endurance athletes could be considered akin to an 'occupational lung disease' (Price et al., 2013).

A key driving factor in elite sport is the impact of any intervention (e.g. screening or new treatment) on athlete performance and in addition to improving airway health, treating EIB may also be beneficial for performance. Studying performance impact at the elite level is clearly complex with multiple potential confounding factors and very few footballers completed this component of this study for the reasons aforementioned, meaning this section of data is difficult to interpret. Although not statistically significant, EVH+ players had mean increase in $\dot{V}O_2$ peak of 7.2%, whereas EVH- players had only a 0.2% increase over the same time, with the observed increases in $\dot{V}O_2$ peak bringing the performance of the EVH+ players in line with EVH- players. As previously stated, due to the small sample size, these results should be interpreted with caution and future research should aim to follow this up with an adequately powered study. Similar findings have been reported in sports with similar demands. Brukner *et al*., (2007) found that Australian Rules football players with newly diagnosed EIB had a significant improvement (9%) in $\dot{V}O_2$ max following six weeks of treatment compared to players without EIB. In addition, Spiteri *et al*., (2014) demonstrated that appropriately medicating elite rugby players with previously undiagnosed EIB improved their performance in a rugby specific aerobic exercise challenge by 8% over the course of 12 weeks compared to 6% EIB negative control group, however this was not a significant finding. The potential impact of treating EIB on performance may be due in part to attenuating bronchoconstriction during and following exercise which has been shown to result in reduced alveolar ventilation and efficiency of alveolar-to-arterial blood O_2 exchange (Haverkamp et al., 2007).

Methodological considerations

A key limitation of this study is that the findings represent the result of a cross-section assessment. Specifically, players were not studied in a prospective randomised placebo-controlled fashion and thus our results have to be interpreted in view of their pragmatic limitations. Due to the elite nature of the football players involved, clubs would not allow medication to be withheld from those who needed it or provide a placebo medication to those who did not. Players are also subject to anti-doping regulations and need to know exactly what they are taking at all times. It is also recognised that diagnosis of EIB from a one-off test is not entirely robust (Price et al., 2015), however due to time restraints from the clubs and the large numbers of players to screen, it would not have been practical to carry out multiple tests. This study design also replicates how a screening programme would work in 'real-life' practise and is similar to the way football players are currently screened for cardiac conditions.

During the EVH test, the %MVV achieved was significantly lower among EVH- players than among EVH+, however both groups achieved a %MVV greater than that of 60% which is required for an adequate test (Anderson et al., 2001) and it is most unlikely that the mean difference of 8% between groups would explain the major findings of this study.

The study was initially powered based on approximately 30% of players being found to have evidence of EIB. Initial power calculations showed that 15 EVH+ players were required to detect a 10% change in FEV_1 in follow-up assessments. To accommodate for an anticipated high drop-out rate in this population, a higher number of players were deliberately recruited. However, even with a sample size of 97 players, the high drop-out rate meant that the study was under powered.

As discussed above, it was difficult for us to follow-up EVH+ players and clearly it would have been desirable to significantly increase the number of players undergoing repeat

assessment, including performance assessment. It may be possible to reduce the attrition rate by working with sub-elite cohorts or academy players, however this limits potential generalisation to the elite population. Players and medical staff at clubs are under huge pressures from clubs and it is hard for them to allocate time for follow-up testing. At the follow-up assessment, it became evident that despite support from coaching and medical staff, many players had decided not to take the inhalers, and there was some confusion amongst these players regarding what they should be taking and when. Miller *et al*., (2005) highlights the importance of educating coaching staff in the management of asthma and this is maybe an area which needs to be addressed when establishing a screening programme. For a screening programme to be effective in elite level football, time needs to be invested in educating players about EIB and its treatment. Although there is a lack of evidence in the athletic population, it is clear educational interventions can have a positive effect on clinical patients' asthma management (Gibson et al., 2002). It is vital therefore to have the cooperation of players at the start of the treatment.

Conclusion

In conclusion, this study revealed that approximately one third of a cohort of elite football players appear to have EIB when screened for respiratory dysfunction at a pre-season medical assessment, which in the majority of cases was not predictable by symptom-based assessment. Treatment of screen-detected EIB positive players resulted in improved airway inflammation and reduced airway-hyper-responsiveness.

Overall the findings of this study support the use of screening for EIB in elite athletes and treating with appropriate asthma inhaler therapy. What this study does not investigate is the long-term impact of treatment upon wellness of the athletes and if the improvement in airway health as shown by a reduction of airway inflammation and reduced severity of EIB translates into a reduction in respiratory illness.

Chapter 5. Evaluating and managing EIB, respiratory health and overall wellbeing in elite swimmers

5.0 ABSTRACT

OBJECTIVES: To report the findings of a 3-part body of work designed to investigate optimal management of respiratory health in elite British swimmers. METHODS: *Part 1*: 14 swimmers entering the GB funded programme (age 19 ± 1.5 yrs, training for 10.8 ± 2.5 yrs) gave informed consent to take part in a one-year study. Swimmers completed an online training and wellness diary daily for 6 months either side of a detailed respiratory assessment which included measurement of FeNO, EVH and PNIF. Following assessment, swimmers were treated for EIB where necessary, or had previous treatment adjusted where appropriate. Swimmers were grouped as EVH+ and compliant with the treatment intervention (INT) and EVH– or EVH+ not compliant with treatment (NI). *Part 2*: 21 elite swimmers training ≥ 25 hrs per week in an indoor pool gave informed consent. 10 swimmers underwent monitoring of their respiratory health including spirometry, FeNO and PNIF before, during and 2 weeks following a 7-week warm weather training camp where training was undertaken in an outdoor pool. The remaining 11 swimmers continued training at their usual indoor pool throughout this period. All swimmers completed a daily online training and wellness diary for the 11-week period. *Part 3*: 15 elite swimmers underwent a systematic assessment of total airway health three months prior to competing at the Rio 2016 Summer Olympic Games. All swimmers had a prior diagnosis of EIB, confirmed by EVH and all were prescribed inhaler therapy and educated on inhaler technique. At the assessment spirometry, FeNO, inhaler flow-rate and PNIF was measured, and they underwent an assessment with a pulmonologist. Data was statistically analysed against their initial diagnostic assessment data. RESULTS: *Part 1*: 75% of swimmers entering the GB funded swim programme demonstrated evidence of asthma/ EIB. No differences were found between the INT and NI groups in the 6 months post respiratory assessment for the % of days the swimmers carried out modified training due to illness ($p = 0.17$), the % of days that swimmers reported symptoms of respiratory illness, or for scores for energy ($p = 1.00$) and sleep quality ($p =$

1.00). *Part 2*: No differences were found between pre, during or post the period of outdoor pool training in FEV_1 ($p = 0.41$), FeNO ($p = 0.12$) or PNIF ($p = 0.67$). Data from the training and wellness diaries showed no significant interaction for the % time swimmers carried out modified training due to illness ($p = 0.45$). Stress levels of the swimmers attending training camp group were found to be significantly higher than the swimmers remaining at home ($p < 0.01$). *Part 3*: FeNO was significantly reduced compared to their initial screening visit ($p = 0.01$). All swimmers were found to have at least one co-existing condition in addition to EIB. All swimmers demonstrated sub-optimal inhaler technique. Despite being prescribed treatment for EIB, three swimmers had on-going airflow obstruction with bronchodilator reversibility of FEV_1 by 12.9 ± 7.7 % above baseline. CONCLUSION: It appears that on an individual level, respiratory health in elite swimmers can be optimised through a systematic assessment of airway health and modification of training environment. Larger, well controlled studies are still required in this area and should investigate the impact of this approach upon performance as well as wellness.

5.1 INTRODUCTION

Results in chapter 4 demonstrated support for screening for exercise induced bronchoconstriction (EIB) and subsequent treatment with appropriate standard asthma medication. It was shown that over a relatively short time period (9 weeks), in athletes with screen detected EIB, treatment led to a decrease in airway inflammation and EIB severity. It is not known however if prolonged treatment to control EIB will have an effect on an athlete's overall health and wellbeing.

A sport in which a high prevalence of EIB and other respiratory disorders has been demonstrated amongst its elite athletes is swimming. As discussed in chapter 1, there is a substantial body of evidence demonstrating a high incidence of asthma, EIB, rhinitis and allergic disease in elite swimmers compared to the general population and other elite athletes (Helenius and Haahtela, 2000; Levai et al., 2016; Bougault et al., 2010; Valerie Bougault et al., 2009). This high prevalence is thought to arise due to the combined effects of the high ventilatory requirement of swimming and the noxious environment of the chlorinated indoor pools in which swimmers train and compete (Bougault et al. 2009). There is a paucity of studies looking at the management of these conditions in swimmers and none investigating the impact of a screening programme on health and wellbeing of swimmers or strategies to reduce the risk of exacerbations to optimally manage EIB.

A study by Levai *et al*., (2016) reported that 67% of elite British swimmers demonstrated objective evidence of EIB when screened using eucapnic voluntary hyperpnoea (EVH). Many competitive swimmers report symptoms arising from the upper airway tract. Indeed nasal obstruction, rhinorrhoea, sneezing, congestion and itching are reported by 74% of competitive elite swimmers (Bougault et al., 2010). The 'united airways disease' theory (Rimmer and Ruhno, 2006; Daabis, 2016) suggests that a single inflammatory process within the respiratory tract leads to manifestations in both the upper and lower airways and in the general population it is recognised that chronic rhinitis is a contributing factor to the

development of asthma and may affect its control (Pedersen and Weeke, 1983). A study of young competitive swimmers with rhinitis (Gelardi et al., 2012) found that in swimmers with non-allergic rhinitis, 63% had a predominant nasal neutrophilic inflammation. The mechanisms are still unclear, but it is thought that epithelial damage may occur as in lower airways (Bernard et al., 2003). As in the case of EIB, swimmer's rhinitic symptoms have been reported to attenuate and even disappear following a 2-week rest period (Bougault et al., 2010). Nasal obstruction can lead to sleep disturbance and fatigue, which has the potential for deleterious effect on swimming performance and athletes often report that exercise-induced rhinitis alters their ability to train and compete to their full potential (Silvers and Poole, 2006).

As well as affecting an athlete's quality of life, nasal obstruction and EIB may also contribute to an increased rate of upper respiratory tract infection (URTI). Nasal obstruction can lead to an increased reliance on mouth breathing, particularly during sleep and as such, swimmers can have an increased inhalation of dry, unfiltered air (Hellard et al., 2015). Helenius and Haahtela (2000) concluded that increased bronchial responsiveness and airway inflammation may predispose athletes to URTIs and in the general population asthma is associated with an increased incidence of pneumococcal disease and pneumonia, with a decreased risk when asthma is well controlled (O'Byrne et al., 2013).

The work of Levai *et al*., (2016) highlighted the potential for improvement in the management of respiratory health. A unique opportunity to work with British Swimming arose, to optimise respiratory health in swimmers with the long-term aim being to improve overall management of EIB to enhance health and thereby reduce the amount of training athletes miss, and consequently promoting optimal performance. This resulted in a three-part body of work:

Part One: The effect of screening and initiating treatment for EIB on health and wellbeing in elite swimmers.

Aim: To investigate the effect of screening and appropriate treatment for EIB on health and wellbeing in elite swimmers entering the British Swimming funded programme.

Part Two: Monitoring respiratory health with a change in training environment.

Aim: To assess the impact of a 7-week training camp in an outdoor pool on lung function, airway inflammation and respiratory symptoms in swimmers usually training indoors during the build-up to the Rio 2016 Olympic Games.

Part Three: A systematic approach to optimise total airway health prior to the 2016 Olympic games.

Aim: To report the findings of a systematic approach to evaluating the airway health of elite swimmers with a previous diagnosis of EIB prior to the Rio 2016 Olympics Games.

5.2 METHODS

Ethical approval for this series of studies was obtained from the School of Sport & Exercise Sciences Research Ethics Committee (Prop 007_2015_2016 and Prop 120_2015_2016) and all swimmers provided written consent prior to testing (appendix 5).

Part One: The effect of screening and initiating treatment for EIB on health and wellbeing in elite swimmers.

Study overview

Elite swimmers entering the GB Swimming funded programme were invited to participate in the study. None of the swimmers had previously participated in respiratory screening. The total study duration was one year, and the swimmers continued with their usual training and competition schedule throughout. On the 25th November 2015, swimmers entered the GB funded swim programme and began completing an online training and wellness diary on a daily basis. After 6-months on the programme they completed a detailed respiratory assessment. Following the assessment, swimmers were treated for EIB where necessary and previous medications were adjusted where appropriate. All swimmers continued completing the training and wellness diary daily for the remaining 6 months until 24th November 2016.

Study methods

Respiratory assessment

Prior to assessment swimmers who were already prescribed medication for asthma / EIB (n = 2) were asked to withhold treatment at the time of assessment in accordance with guideline recommendations (Crapo et al., 2000). All swimmers reported that they were free from illness in the two weeks prior to assessment. They were requested to avoid exercise and caffeine for 4 hrs before each visit and arrived for testing at least 2 hrs postprandial.

Swimmers initially completed a health questionnaire to determine their medical history and evaluate the presence of respiratory symptoms (appendix 1). Peak nasal inspiratory flow (PNIF) was measured as an index of nasal obstruction (Ottaviano and Fokkens, 2016), with a peak flow meter (In check, Clement-Clarke International Ltd, Harlow, Essex, UK). Airway inflammation was assessed by determining fraction of exhaled nitric oxide (FeNO) (NIOX VERO (NIOX, Aerocrine, Sweden) (Dweik et al., 2011) and lung function was assessed by maximal flow volume spirometry (Spiro-USB and MicroLab, CareFusion, Germany) (M. R. Miller et al., 2005).

An EVH challenge was then conducted, as described in chapter 2. A test was considered positive for EIB if FEV_1 fell by ≥ 10% from baseline at two consecutive time points. At this point 4 × 100 µg of inhaled salbutamol was administered, and maximal flow volume loops were recorded 15 minutes post inhalation. Severity of EIB was classified as mild, moderate or severe dependant on the fall in FEV_1 post EVH (≥10% to <25%, ≥25% to <40% and ≥40% respectively) (Anderson and Kippelen, 2013). Swimmers with a positive EVH challenge were then prescribed medication by their physician in accordance with international guideline recommendations (Parsons et al., 2013). They were also given a pocket spacing chamber through which to take their inhaler and education regarding optimal inhaler technique (van der Palen et al., 1995).

Swimmers who failed to demonstrate normal baseline lung function (FEV_1 < 80% predicted) carried out a reversibility challenge instead of an EVH challenge. These swimmers self-administered 4 × 100 µg of inhaled salbutamol and maximal flow volume loops were once again recorded 15 minutes post inhalation. A positive reversibility challenge was defined as an increase in FEV_1 of ≥ 12% (Fitch et al., 2008).

Online training and wellness diary

Swimmers completed an online daily wellness questionnaire (Apollo Wellbeing Score) (appendix 7). First, they recorded their training status as either 'full training', 'modified training due to illness', 'modified due to injury', 'off training due to injury', 'off training due to illness', 'travelling' or 'day off resting'. Then they scored the following items on a scale of 1-7: 'Energy', 'Sleep', 'Stress', 'Do you feel ill?'. If the item 'Do you feel ill?' was scored as 3 or below, additional symptom options then became available to rate: 'runny nose', 'blocked nose', 'sneezing', 'sore throat', 'cough', 'head congestion', 'chest congestion', 'fever and muscle ache'. Scoring was on a scale of 1 to 7 for all items (1 = low energy, very poor sleep, extremely stressed, severely ill, severe symptom through to 7 = high energy, extremely good sleep, no stress, not at all ill, no symptom).

Part Two: Monitoring respiratory health with a change in training environment

Study Overview

Twenty-one elite internationally competitive swimmers, 10 males and 11 females training ≥ 25 hours per week in an indoor pool gave informed consent for their data to be used as part of a research study. Respiratory health was monitored in ten swimmers prior to, during and following a 7-week warm weather training camp, where all training took place in an outdoor pool (figure 5.1). Swimmers continued with their usual training and competition schedule throughout. Once prior to departure, once midway through the training camp and once two weeks after they returned home, swimmers completed a respiratory assessment. This comprised: measurement of fraction of exhaled nitric oxide (FeNO), Peak Nasal Inspiratory Flow (PNIF) and maximal lung function measured by spirometry and completion of the Mini Asthma Quality of Life Questionnaire (MiniAQLQ) (Juniper et al., 1999) (appendix 8) and the Mini Rhinoconjuntivitis Quality of Life Questionnaire (MiniRQLQ) (Juniper et al., 2000) (appendix 9). Data from an online wellness diary which the swimmers complete daily as part of them being on the funded programme was collected for 2 weeks prior to the training camp, throughout the training camp and for an additional 2 weeks on their return home. Wellness diary data was also collected from the 11 swimmers who did not attend the warm weather training camp and continued training at home in the indoor pool. These swimmers were used as a control group, however did not complete the respiratory assessments.

All respiratory assessments were completed ≥ 4 hours after the swimmers completed a training session and taken any short acting β_2-agonists (SABA). Swimmers also abstained from caffeine in this time.

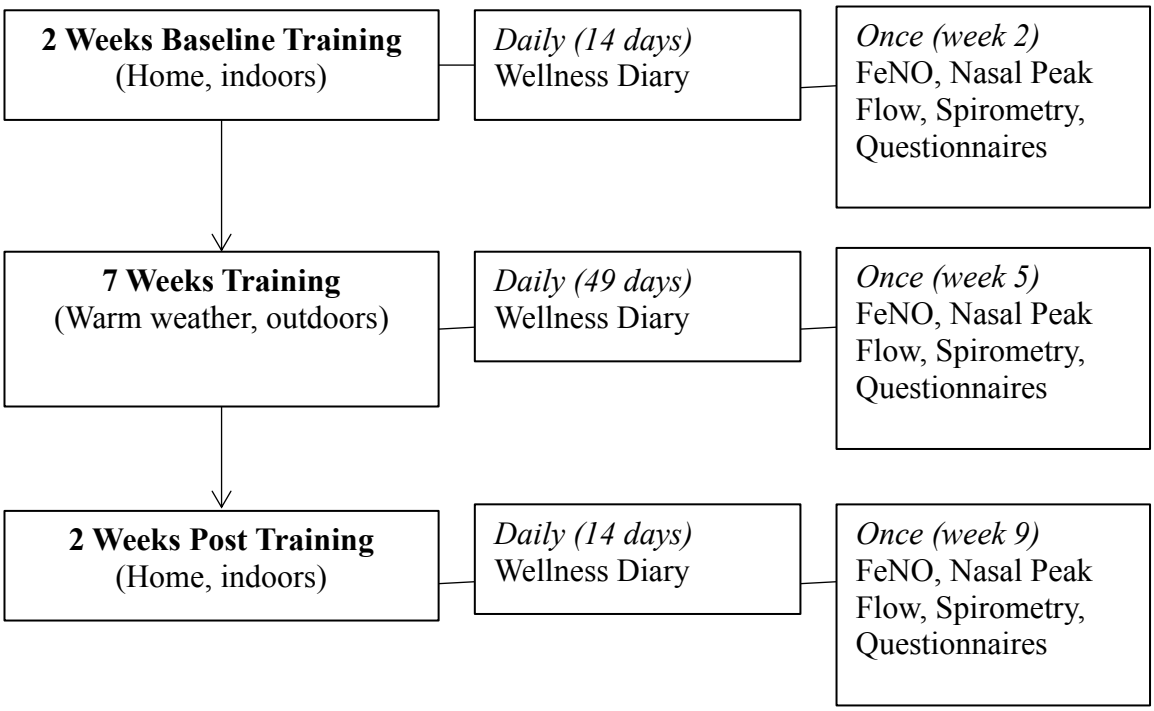

Figure 5.1. Study design

Study Methods

Respiratory assessment

Maximal resting lung function was assessed by spirometry and performed in accordance with the ATS/ERS recommendations (M. R. Miller et al., 2005) using digital spirometers (Spiro-USB and Microlab, Carefusion, Germany). Measurement was repeated until three acceptable attempts had been obtained. Forced expiratory volume in one second (FEV_1), peak expiratory flow (PEF), forced vital capacity (FVC) and FEV_1: FVC ratio (FEV_1/FVC) were recorded for each effort and the highest values used for analysis. Measurement of FeNO was performed in accordance with ATS/ERS recommendations (Dweik et al., 2011) using a NIOX analyser (NIOX VERO, (NIOX, Aerocrine, Sweden). Measurement was done in duplicate and the mean value used. Peak Nasal Inspiratory Flow (PNIF) was measured as an index of nasal obstruction (Ottaviano and Fokkens, 2016), with a peak flow meter (In check,

Clement-Clarke International Ltd, Harlow, Essex, UK). Three satisfactory maximal inspirations were obtained, and the highest value was used for analysis.

Wellness Diary

Swimmers completed their usual online wellness diary as described above in part 1.

Part Three: A systematic approach to optimise total airway health prior to the 2016 Olympics

Study Overview

Fifteen elite level swimmers (9 males, 6 females, age 22.2 ± 2.9 yrs) selected to compete at the 2016 Olympics provided written consent for their data to be used for research purposes. All swimmers had a prior diagnosis of EIB confirmed by EVH screening (Levai et al., 2016) and had previously been prescribed appropriate inhaler therapy and received education regarding inhaler technique.

Three months prior to the Olympic Games the swimmers underwent a systematic assessment of total airway health. At the assessment, maximal lung function, fraction of exhaled nitric oxide (FeNO) and peak nasal inspiratory flow (PNIF) were assessed. The swimmers also underwent an evaluation of total airway health, including co-morbidities with a respiratory consultant and an assessment of inhaler technique. Where appropriate, following the assessment the swimmer's treatment was changed to optimise their respiratory health.

Respiratory assessment

All respiratory assessments were completed ≥ 4 hours after the swimmers completed a training session. They were instructed to refrain from taking SABAs or consuming caffeine within this time. Nitrate supplementation was recorded and FeNO results of those swimmers using nitrate supplements were excluded.

Maximal lung function

Maximal lung function was assessed by spirometry and was performed in accordance with the ATS/ERS recommendations (M. R. Miller et al., 2005) using digital spirometers (Spiro-USB and Microlab, Carefusion, Germany). Measurement was repeated until three acceptable attempts had been obtained. FEV_1, PEF, FVC and FEV_1: FVC ratio were recorded for each effort and the highest values used for analysis.

Fraction of Exhaled Nitric Oxide (FeNO)

Measurement of FeNO was performed in accordance with ATS/ERS recommendations (Dweik et al., 2011) using a NIOX analyser (NIOX VERO, (NIOX, Aerocrine, Sweden). Measurements were carried out in duplicate and the mean value taken.

Peak Nasal Inspiratory Flow (PNIF)

PNIF was measured as an index of nasal obstruction (Ottaviano and Fokkens, 2016), with a peak flow meter (In check, Clement-Clarke International Ltd, Harlow, Essex, UK). Three satisfactory maximal inspirations were obtained, and the highest value was used for analysis.

Consultation

During the consultation with the respiratory consultant a systematic review of all symptoms was carried out along with a discussion of current medication and co morbidities.

Inhaler Technique

Inhaler technique was assessed first by asking swimmers to replicate the rate at which they inhaled through a flow meter to assess the inspiratory flow rate they typically generated. Then they were asked to take their inhaler as they would usually and were scored on the nine

items in Table 5.1 using ‘completed’ or ‘not completed’. Swimmers were given immediate feedback and had further instruction and practice where necessary.

Table 5.1. Items for inhaler check score.

Remove cap
Shake inhaler
Hold the inhaler upright
Exhale to residual volume
Keep head upright
Put mouthpiece in mouth
Inhale slowly and deeply (at less than 30L/min)
Actuate after starting to inhale
Hold breath for 5 seconds

Statistical analysis

Data are presented as Mean ± SD unless otherwise stated. Associations between gender and EVH result were assessed by Chi-square square analysis. In part 1 for the wellness questionnaire data swimmers were grouped according to whether there was an intervention in their treatment (INT) or not (NI). Data from the four weeks immediately after the respiratory screening were removed to account for treatment changes within this period. Data from ‘pre’ and ‘post’ respiratory screening were analysed as % of days to account for the difference in number of days in the Pre and Post time periods and were examined using 2-way mixed model ANOVA with repeated measures, or the non-parametric equivalent

Wilcoxon sign rank tests and Sign rank tests were used where appropriate. Symptom data was examined by Fisher's Exact test. In part 2 differences in data before, during and after the training camp were assessed using one-way repeated measures ANOVA. Where the assumptions of ANOVA were violated, Friedman tests were employed. Data from the wellness diaries were analysed using two-way mixed ANOVAs. In part 3 results were analysed using paired t-tests. All analyses throughout were conducted using SPSS software, V.23 (SPSS, IBM, Armonk, NY, USA) with significance accepted at $P < 0.05$.

5.3 RESULTS

Part One: The effect of screening and initiating treatment for EIB on reports of health and wellbeing in elite swimmers.

Subject characteristics

Fourteen swimmers completed the initial respiratory assessment; 7 males, 7 females, age 19 ± 1.5 yrs, height 177.2 ± 10.1cm, weight 68.9 ± 8.8kg. Swimmers had been training for 10.8 ± 2.5 years and were currently training 23.5 ± 2.6 hrs per week. Two swimmers had a history of asthma and both were currently taking preventative medication. One of these swimmers completed the respiratory screening having come off their medication as advised, the other with current asthma decided to complete the respiratory screening whilst on preventative medication.

Respiratory symptoms

Three swimmers did not complete the respiratory questionnaire. Six of the remaining 11 swimmers reported troublesome respiratory symptoms including cough, chest tightness, wheeze and dyspnoea at a frequency of 45, 36, 36 and 18% respectively.

Baseline spirometry & FeNO

All but one swimmer had normal resting lung function (FEV_1 > 80% predicted). This swimmer completed a reversibility challenge and demonstrated significant reversibility (an increase in FEV_1 of 19%). All remaining swimmers (n = 13) had resting lung function $\geq$

100% predicted (FEV_1: 119 ± 15%, FVC: 128 ± 15%). Five swimmers demonstrated an elevated FeNO (> 25 ppb). Only one swimmer demonstrated a high FeNO (>50 ppb) and this was the swimmer who demonstrated airway obstruction at rest.

Table 5.2. Respiratory assessment data.

		% Predicted
FeNO (ppb)	24 ± 13	-
PNIF (L/s)	183 ± 67	-
FEV_1 (L)	4.68 ± 0.83	115.5 ± 18.6
FVC (L)	6.00 ± 1.15	126.0 ± 16.7
PEF (L/min)	524 ± 112	98.9 ± 17.0
FEV_1/FVC (%)	78.1 ± 8.5	78.1 ± 8.5
%MVV during challenge	63.1 ± 13.8	-
Maximal % fall in FEV_1 from EVH	-14.6 ± 7.8	-

FeNO, fraction of exhaled nitric oxide; PNIF, peak nasal expiratory flow; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow; %MVV, percent of predicted maximal voluntary ventilation; EVH, eucapnic voluntary hyperpnoea.

EVH challenge

In addition to the swimmer who did not complete the EVH test due to resting FEV_1 being < 80% predicted, two additional swimmers were excluded from the EVH analysis due to not achieving an acceptable test, resulting in a borderline result (i.e. failed to achieve a $\dot{V}_E$ of ≥ 60% predicted MVV), this included the swimmer already on medication. A further two swimmers did not achieve a $\dot{V}_E$ of ≥ 60%, however they still demonstrated a positive test.

Eight out of the eleven swimmers (75%), demonstrated EVH positive (EVH+). The majority (n = 7) were classified as demonstrating mild EIB and one swimmer with moderate EIB, who was the swimmer with a previous history of asthma. There was no association between gender and EVH result (p = 0.31). All EVH+ swimmers demonstrated a subsequent improvement in FEV_1 following administration of 400 µg of inhaled salbutamol (FEV_1 = 19 ± 4%), with a range of 14-25%. Despite resting lung function being greater than predicted, 3 swimmers had reversibility to above baseline by 3 to 7%.

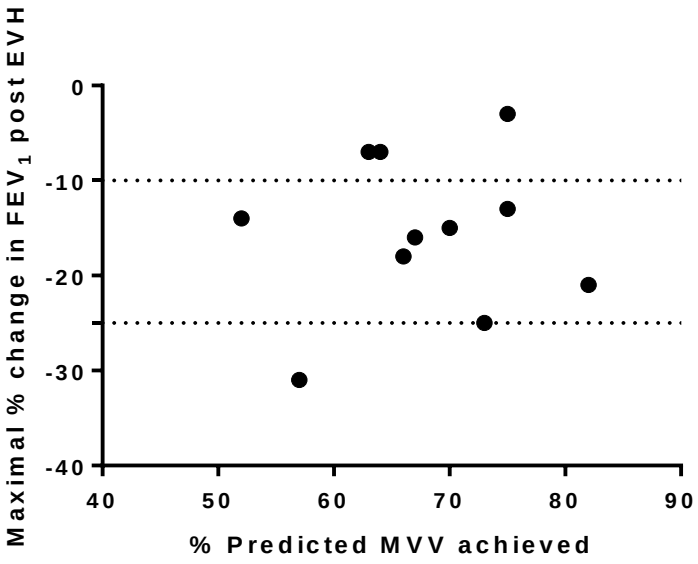

Figure 5.2. Percentage fall in FEV_1 after EVH (n = 11).

Vertical dashed line represents the threshold for mild EIB (>10 <25%) and moderate EIB (>25 <40%).

FEV_1, forced expiratory volume in 1 s; %MVV, percent of predicted maximal voluntary ventilation; EVH, eucapnic voluntary hyperpnoea.

Respiratory symptoms

There was a significant association between the presence of symptoms and likelihood of a positive EVH result as assessed by Fishers exact test in those who completed both the health questionnaire and EVH test ($n = 10$, $p = 0.05$).

PNIF

Two swimmers did not complete this part of the assessment ($n = 12$). Eleven swimmers demonstrated no evidence of nasal obstruction (PNIF > 120 L/min), one swimmer demonstrated moderate nasal obstruction (PNIF 50-80 L/min).

Wellness questionnaire data

Analysis for this section was carried out on $n = 10$, due to the remaining swimmers not completing the diary adequately. The total number of days included in the analysis were 183 days Pre-assessment (November to May) and 156 days post assessment (June – November). Prior to the respiratory screening 96% of wellness data was captured and following the respiratory screening 71% was captured. The majority of swimmers had a one-month break at some point during August or September during which the majority failed to enter data. Six swimmers formed the INT group; these were 5 newly diagnosed EVH+ and 1 previously diagnosed EVH+ whose medication had been increased. Four swimmers formed the NI group; these were 1 EVH-, 1 previously diagnosed asthmatic with no change in medication and 2 newly diagnosed EVH+ swimmers who didn't take any of the prescribed medication.

There was no difference in groups between the % of days for which they completed the online diary Pre (INT: 94.3 ± 5.3%, NI: 97.5 ± 1.7, p = 0.29). There was a difference post with the INT group completing fewer days than the NI group (INT: 68.2 ± 5.6, NI 78.0 ± 7.9%, p = 0.05).

There was no main effect (p = 0.94), group (p = 0.66), or interaction (p = 0.17) for time the percentage of time swimmers spent carrying out modified training.

Table 5.3. % of days in which training was either modified or lost due to illness.

		INT	**NI**	**Sig.**
% Days completed diary	Pre	94.3 ± 5.3	97.5 ± 1.7	0.29
	Post	68.2 ± 5.6	78.0 ± 7.9	
% Days modified training	Pre	1.8 ± 1.0	1.5 ± 1.3	0.17
	Post	1.0 ± 1.1	2.3 ± 3.3	
% Days lost to illness	Pre	0 ± 0	0 ± 0	
	Post	0.1 ± 0.3	0 ± 0	

INT, Intervention group; NI, No intervention group.

There was an increase in the % days rated as 6 or 7 over time (90.5 ± 6.9% to 93.7 ± 6.6%, p = 0.01) for all swimmers combined, but there was no difference between group (p = 0.78) or an interaction (p = 0.55). There were no differences in the median scores for energy (p = 1.00, p = 1.00), sleep (p = 1.00, p = 1.00) or stress (p = 0.50, p = 1.0) for over time for either the INT or NI group respectively. There were no significant main effects for time, group or

interactions for the % of days that the swimmers reported symptoms for (runny nose, blocked nose, sneezing, sore throat, cough or fever).

Part Two: Monitoring respiratory health with a change in training environment

Subject Characteristics

Ten swimmers attended the warm weather outdoor training camp. One swimmer was excluded from the data analysis as they were not available for the final respiratory assessment due to media commitments. The characteristics of the remaining swimmers (n = 9) comprised 5 females, 4 males, age 22.7 ± 2.9 years, height 178.9 ± 6.4 cm, weight 71.4 ± 9.0 kg. Six of the swimmers had a previous diagnosis of EIB confirmed by EVH, five of whom were taking preventative medication in form of ICS, one of whom was not taking any treatment. Two of the swimmers had demonstrated a negative result to EVH and one had not been previously screened by EVH but had no previous history, reported no symptoms or demonstrated any evidence of asthma in baseline spirometry.

The control group for the wellness data comprised 11 swimmers (6 females, 5 males, age 22.2 ± 4.5 years, height 179.0 ± 4.5 cm, weight 73.0 ± 11.8 kg). Eight of these swimmers had a diagnosis of EIB by EVH, 6 of whom were taking preventative medication. Two swimmers were EVH- and two had not had an EVH screen.

Table 5.4. Results from the respiratory assessments prior to, during and following the training camp.

	Pre	During	Post	Sig.
FEV_1 (L)	4.54 ± 0.58	4.55 ± 0.66	4.49 ± 0.63	0.41
FeNO (ppb)	21 (14)	15 (12)	14 (10)	0.12
PNIF (L/min)	170 (80)	180 (73)	180 (73)	0.67
MiniRQLQ score	2.2 (2.4)	1.0 (1.6)	1.4 (1.6)	0.28
MiniAQLQ score	6.5 (0.6)	6.5 (1.4)	6.3 (1.0)	0.50

Data mean ± SD. Data for FeNO, PNIF, RQLQ and AQLQ were not normally distributed and therefore analysed with Friedman tests and are presented as the median score (IQR).

FEV_1, forced expiratory volume in 1 s; FeNO, exhaled nitric oxide; PNIF, peak nasal inspiratory flow; MiniRQLQ, Mini rhinoconjuntivitis quality of life questionnaire; MiniAQLQ, mini asthma quality of life questionnaire.

No changes were seen in FEV_1 throughout the monitoring period ($p = 0.41$). Across the monitoring period, FeNO changed from Pre = 21.00 ppb, to during = 15.0 ppb, to Post = 13.5 ppb), however these differences were not statistically significant ($p = 0.12$). Nasal Peak Flow increased from Pre to During and stayed the same Post (170, 180 and 180 L/min respectively). These differences were not statistically significant ($p = 0.67$).

Over the monitoring period MiniRQLQ score decreased from Pre (2.20) to during the camp (1.00) and then increased again post camp (1.40). These differences were not significant (p

= 0.28). There was no difference (p = 0.50) in MiniAQLQ score across the monitoring period (Pre = 6.5, During = 6.5 and Post = 6.3).

Wellness Data

In the warm weather training camp group 11 days were spent performing 'modified training due to illness (2 during the camp and 4 on their return home) and 2 days were lost to illness (on the return home). In the control group, a total of seven days were spent performing 'modified training' (3 days during the camp and 4 when the other athletes returned) and three days training were lost due to illness (all whilst the other athletes were away). There was no significant interaction for the percentage of time spent by swimmers doing modified training (p = 0.45). There was also no significant main effect for group (p = 0.41). There was a main effect for time (p = 0.01) showing that the % of days spent doing modified training was 4% higher post training camp than pre-training camp. For the percentage of training days lost to illness there was no significant interaction (p = 0.45). There was also no significant main effect for group (p = 0.41). There was a main effect for time (p = 0.01), showing that the % of training days lost to illness was 4% higher post training camp than pre-training camp. There was no significant interaction of time or group for stress scores (p = 0.07). There was also no main effect for time (p = 0.60). There was however a main effect for group (p < 0.01) with the stress level of the training camp group 16% higher than the control group, showing the training camp group reported being more stressed than those who stayed at home. There was no significant interaction in energy scores (p = 0.45). There was also no main effect for time (p = 0.10) or group (p = 0.09).

Part Three: A systematic approach to optimise total airway health prior to the 2016 Olympic

Subject Characteristics

Fifteen elite level swimmers (9 males, 6 females, age 22.2 ± 2.9 yrs) selected to compete at the 2016 Olympics. All apart from one swimmer were regularly taking preventative medication for their asthma/ EIB; six in the form of an inhaled corticosteroid inhaler (ICS) and eight in the form of a combination inhaler (ICS with long acting β_2-agonist (LABA).

Maximal lung function

All swimmers demonstrated 'normal' spirometry (> 80% predicted, FEV_1/FVC >70%) (Table 5.5). FEV_1 at rest remained unchanged from their initial assessment (Pre: 4.79 ± 1.13, Post: 4.78 ± 1.10 (p = 0.78). Despite being prescribed treatment for EIB, three swimmers had an on-going airflow obstruction, recognised by a concave flow loop and bronchodilator reversibility of FEV_1 by 12.9 ± 7.7% above baseline (figure 5.3).

Table 5.5. Resting lung function

	Measured	**% Predicted**
FEV_1 (L)	4.78 ± 1.10	111.1 ± 16.6
FVC (L)	6.05 ± 1.33	121.4 ± 14.3
FEV_1 / FVC (%)	77.7 ± 6.2	93.47 ± 7.92
PEF L/min	498.07 ± 228.08	101.40 ± 12.54

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity

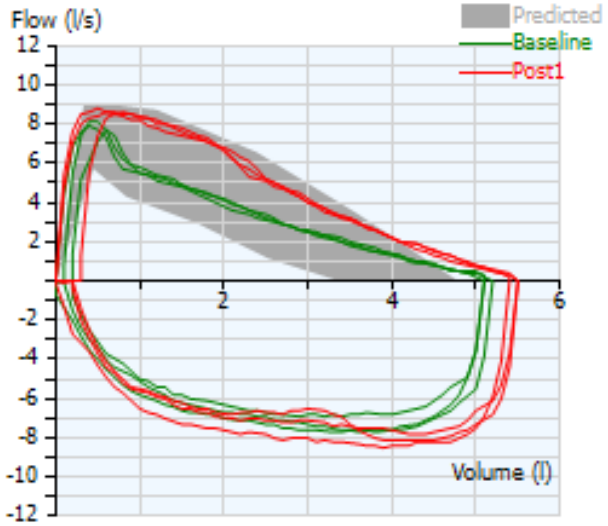

Figure 5.3. Example of flow volume loop of swimmer with airflow obstruction and subsequent reversibility with salbutamol.

FeNO

FeNO (n = 14 as one swimmer was removed due to nitrate supplementation) was significantly reduced compared to their initial screening visit (Pre: 28 ± 15 ppb, Post: 16 ± 7 ppb (p = 0.01) (Figure 5.4). Only two of the remaining 14 swimmers had an elevated FeNO (>25ppb) at the post measurement, however they both still showed improvement from their initial visit (26 ppb from 37 ppb and 29 ppb from 54 ppb).

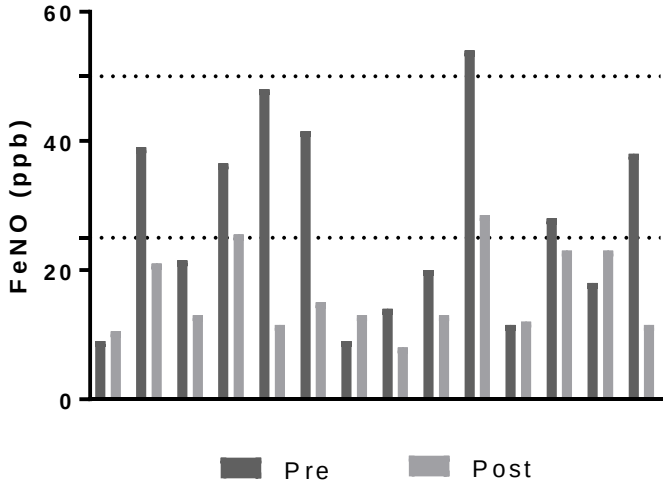

Figure 5.4. FeNO at Pre and Post. Dashed line indicates normal FeNO < 25 ppb and high FeNO > 50ppb.

FeNO, exhaled nitric oxide; Pre, initial EVH screening; Post, Pre-Olympic assessment.

PNIF

Three swimmers were found to have mild nasal obstruction (80 - 120L/min) and one had moderate obstruction (50-80L.min). There was no previous data for comparison.

Coexisting Conditions

All but one swimmer had at least one co-existing condition in addition to EIB including nasal disease, reflux, sensations of laryngeal closure, recurrent respiratory tract infection and abnormal breathing sensations (Figure 5.5). The highest of these comorbidities was nasal

disease (68%). 37% of the swimmers reported side effects from inhaler use (e.g. throat discomfort or voice disturbance).

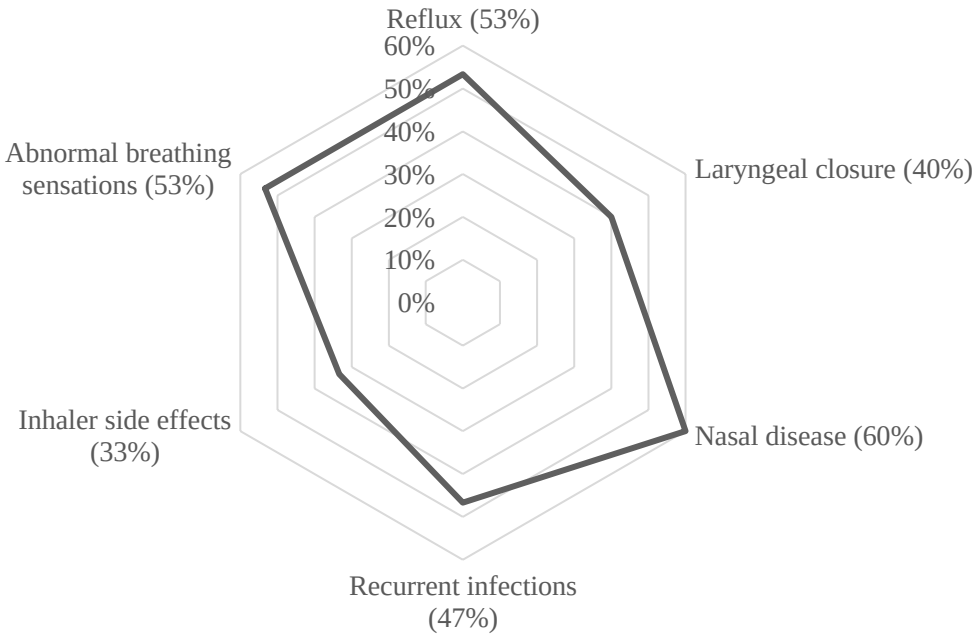

Figure 5.5. Coexisting conditions alongside EIB

Inhaler technique

All swimmers demonstrated an inhaler technique which was sub-optimal based on the rate of inhalation; flow rate = 348 ± 49 $L.min^{-1}$ (recommended 30 L/min for a standard metered dose inhaler (MDI)). Mean score for technique was 8 ± 1, out of 9 (Table 5.6), with the main fault in technique being the inhalation rate.

Table 5.6. Inhaler technique results

Inhaler Technique	**% of swimmers demonstrating technique correctly**
Remove Cap	100
Shake Inhaler	89
Hold Inhaler Upright	100
Exhale to Residual Volume	89
Keep Head Up	96
Mouth Piece in Mouth	96
Inhale Slowly	4
Actuate after inhale	92
Hold breath	92

5.4 DISCUSSION

Prevalence of EIB

In part one of this study, it was found that 75% of a cohort of elite swimmers entering the GB funded swim programme demonstrated evidence of airway hyper-responsiveness. Although it is widely acknowledged that there is a high prevalence of EIB amongst elite swimmers, this level is above what has previously been reported. Most recently Levai *et al*., (2016) found that upon screening 44 members of the GB swimming squad, 67% demonstrated objective evidence of EIB, however current results are in a small sample number (n = 14) and were not intended to assess the prevalence of EIB in swimming.

It is thought that swimmers are at particular risk of developing EIB due to their unique training environment. They are exposed to by-products of chlorine disinfection, in particular trichloramines which due to the nature of the sport they will inhale via oral breathing in high concentrations. Bougault and Boulet, (Bougault and Boulet, 2013) suggested that the by-products of chlorine which swimmers are inhaling have the potential to interact with airway epithelium resulting in oxidative stress and airway inflammation. Evidence of this inflammatory and remodelling processes have been observed in the bronchial mucosa of competitive swimmers (Bougault et al., 2012).

Respiratory symptoms

In contrast to the previous studies in this thesis and also in the literature, in the swimmers entering the GB programme, there was an association between respiratory symptoms and the incidence of EIB. Clearie *et al*., (2010) found that less than half of the elite swimmers they

assessed presenting with EIB reported symptoms that were suggestive of EIB. Similarly, none of the swimmers in the current study had reported them to the doctor previously and so it is likely that the athletes did not recognise them as troublesome. This is not unusual; it has been suggested that most competitive swimmers do not report their respiratory symptoms to a physician and in particular younger swimmers may consider nociceptive respiratory symptoms to be a 'normal phenomenon'.

Baseline lung function

Similar to prior reports in the literature (Rundell et al., 2001) the swimmers who presented EVH+ all had greater than predicted lung function at rest (FEV_1: 119 ± 15%, FVC: 128 ± 15%). Despite this, following administration of Salbutamol following EVH, FEV_1 still reversed to above baseline. As has been shown consistently, screening this group of elite athletes identified the presence of EIB in athletes with no previous history, in this study 67% of the swimmers who tested EVH+ had no history of asthma or EIB. Dickson *et al*., (2011) screened 228 elite athletes using EVH and of those who presented as EVH+ 73% had no previous history. They also (Dickinson et al., 2013) found that 66% of football players demonstrating EVH+ had no prior history and 67% of swimmers with objective evidence of EIB had no previous history (Levai et al., 2016).

Impact of treatment

What hasn't yet been determined in the literature is what effect treating EIB with standard asthma medications has upon athlete health. In chapter 4 we showed that treating EIB in footballers had a positive impact upon airway health and reduced the severity of EIB when assessed by response to an EVH challenge. In part one, one step further was attempted; to

investigate the impact this treatment had upon overall health and wellbeing and in doing so was the first study to attempt this. Unfortunately, due to time constraints of training and competition, permission was not given to carry out a follow up visit on the swimmers in part one to determine the effect of the treatment intervention on airway health and EIB severity. Looking at the health and wellbeing data collected from the daily online questionnaire, no significant differences were seen between the intervention and no intervention groups in the percentage of days lost to illness or, to the percentage of days spent carrying out modified training due to illness.

Whether or not uncontrolled EIB in athletes leads to an increased risk of illness and the potential mechanism behind this is still unknown. In the general population uncontrolled asthma appears to be linked to an increased risk of pneumococcal disease and pneumonia (O'Byrne et al., 2013), Helenius and Haahtela, (Helenius and Haahtela, 2000) have suggested that increased airway inflammation may predispose athletes to upper respiratory symptoms and it has been hypothesised that it may be a transient loss of control of this local inflammation which may account for some upper respiratory illness often seen in athletes (Bermon, 2007).

To collect data regarding the swimmers' wellness, the British Swimming online diary was used because swimmers were already required to complete this daily as part of being on the funded programme. Unfortunately, there were several limitations inherent to this approach. Firstly, swimmers complete this questionnaire first thing in the morning and as such we do not know if this accurately reflects what training was carried out that day. Also, the questionnaire was not designed for assessing control of respiratory symptoms and as such questions were not specific to this. No differences were found in the illness symptoms data, however, this may have been different if a questionnaire was completed after training which asked more specifically about symptoms of coughing, wheezing, dyspnoea etc. The

drawback with all these questionnaires is that it has been shown that athletes are often not able to perceive respiratory symptoms due to altered lung function (Simpson et al., 2015). Also, statistically this data is difficult to assess due to very small group sizes and small proportions of days reported as 'off training due to illness' or 'modified due to illness'. There was a higher proportion of days rated 6 or 7 in answer to 'Do you feel ill?' in the time period following the screening, however there was no difference between the two groups. This is most likely down to the timing of the screening which split the two periods of time as November to May and May to November. It is well known that illness risk is higher in winter than in summer and this has been confirmed by studies in athletes (He et al., 2013). The first time period in this study captured the entirety of the UK winter (November to March) where there is a surge of viral outbreaks. It would therefore have been beneficial for the monitoring period to continue until the following May so that two 6-month periods at the same time of year could be observed.

In part three, the significant reduction in FeNO demonstrates that the long-term treatment of EIB in swimmers, leads to an improvement in airway health. A greater reduction in FeNO was seen in those swimmers who had an elevated FeNO initially, with FeNO that was already low remaining unchanged. This confirms that inhaled corticosteroids (ICS) are beneficial in the treatment of EIB (Weiler et al., 2016).

Impact of training environment

The high prevalence of EIB in UK swimmers, even compared to other endurance athletes is thought to be due to the indoor pool environment in which chlorine is used as a disinfectant, often with inadequate control or ventilation. Indeed, one study showed a significant reduction in airway hyperresponsiveness in swimmers after an annual swimming pool clean (Simon-Rigaud et al., 1997).

The results from part two of this work showed no significant differences in respiratory health measured by resting FEV_1, FeNO, PNIF, MiniAQLQ and MiniRQLQ in a group of swimmers changing to training in an outdoor pool from their usual indoor pool environment. It is to be expected that no differences were seen in resting lung function measured by FEV_1. There was a trend however for FeNO, PNIF and MiniRQLQ score to improve whilst on training camp and training in an outdoor pool. It was hypothesised that removing the athletes from the indoor environment to train in an outdoor pool may remove the effect that chloramines may have on the airway; in an outdoor environment, there are no issues regarding poor ventilation and so there is no layer of chloramine on top of the pool where the swimmers breathe (Drobnic et al., 1996). Although the swimmers were taken out of the chlorine environment however, due to being in Australia and it was summer season rather than the UK, there were several anecdotal reports of swimmers reported suffering from hay fever symptoms.

Despite not being able to answer the initial question, monitoring spirometry, FeNO and NPIF; quick and easy measures of airway health whilst on training camp proved useful when used on an individual basis and treatment for allied conditions such as hay fever optimised as a result.

The wellness data in part two also showed no significant differences between the swimmers who were away on training camp and those training at home. There was however a significantly higher percentage of days spent completing modified training and of lost training due to illness on the return home from the training camp, compared to when the swimmers were away. There was also a significantly higher score for overall stress in the swimmers away on camp, than in the group who stayed at home. It is difficult to identify why these results were observed, however it highlights that although there may be some benefit of altering the swimmers training environment by travelling overseas to train

outdoors, there are many other factors to take into consideration to find an optimal balance of risk factors for overall health and wellbeing. The training camp environment increases the threat to an athlete's immunity in several ways; through the risk of long haul air travel (Schwellnus et al., 2012), an increase in training load, and an increase in other stressors such as decreased sleep, psychological stress, heat and potential changes in diet (Walsh, 2018).

There are several other ways in which the training environment can be improved for swimmers by reducing levels of trichloramines. Alternative methods for pool disinfection could be considered such as ozone, ultra violet and copper or silver (World Health Organisation, 2006). The chlorine concentration should be carefully monitored and adequate ventilation ensured (Bougault and Boulet, 2013). Other simpler measures can be taken by the swimmers themselves; it is the interaction between chlorine and nitrogen containing matter such as sweat and urine which results in the release of trichloramines and nitrogen chloride. By showering before swimming, using swimming costumes only for swimming and not urinating in the pool these interactions will lessen (Bougault and Boulet, 2012).

There were several limitations in the way part two was conducted, which made it difficult to establish if changing a swimmer's training environment leads to a change in airway health. It would have been ideal to conduct EVH tests on the swimmers at each of the measurement point to establish EIB control, however this was not possible due to the constraints of specialist equipment and trained staff to carry out these tests in Australia. It was also thought by the British Swimming medical staff that the swimmers would refuse repeat EVH testing due to the discomfort of the test.

In part two the respiratory assessment was conducted three weeks into the training camp. The original intention was that two assessments would be performed during the camp, however for logistical reasons this did not happen. Three weeks may have been too short a time for any significant changes in airway health to occur because of a change in

environment. There is evidence however that a period of as little as 15 days of light or no training normalises the response to EVH and methacholine in swimmers with previous evidence of EIB (Bougault et al., 2011).

The importance of follow up assessment

Despite being part of a screening programme and being prescribed treatment, in part three it was seen that in a final respiratory health check prior to the Olympics, some swimmers were still not taking their medication as prescribed and three swimmers demonstrated evidence of airway obstruction at rest. This was despite their lung function appearing 'normal' based on predicted values. It is common for athletes to demonstrate normal lung function at rest despite disease being present (Bonini et al., 2007) and so it would be beneficial for practitioners working with elite athletes to know what is optimal for individual athletes and to ensure the flow loops and reversibility are considered in any review of respiratory health. Over half of the cohort of swimmers assessed in part three also reported troublesome respiratory and allied symptoms including laryngeal dysfunction, reflux and nasal disease. This highlights the importance of including an in depth consultation as part of a screening programme as it is common for elite swimmers to suffer from these often coexisting conditions (Hull et al., 2017) and as discussed previously many swimmers do not recognise symptoms as abnormal and as such they go un reported. Nasal disease, in particular chronic rhinitis has been shown to be a contributing factor to the development of asthma and may also affect its control (Pedersen and Weeke, 1983). Nasal symptoms have also been shown to impair swimmer's quality of life and have the potential to worsen their performance (Bougault et al., 2010), however simple interventions such as swimmers using a nose clip during training may be beneficial in alleviating these symptoms (Gelardi et al., 2012). In addition to rhinitis, some swimmers in the current study demonstrated nasal obstruction.

Long term nasal obstruction has the ability to impair swimmers quality of life due to decreased sleep quality leading to chronic tiredness (Alaranta et al., 2005). There is also an increased risk of URTIs (Hellard et al., 2015) and as such its detection is important.

Inhaler technique

Inhaler technique amongst the swimmers was found to be sub-optimal and there were frequent reports of inhaler side-effects such as voice disturbance and sore throat. All swimmers had previous education in inhaler technique, so this emphasises the importance of reviewing athletes with a diagnosis of EIB regularly. Inhaler technique is particularly important in minimising laryngeal symptoms; when the inhalation rate is too high, the larynx will close, meaning the inhaler is deposited on the larynx increasing symptoms.

Summary and future directions

There were many limitations to this body of work, which have been discussed above. Unfortunately, usual scientific vigour could not be applied throughout due to working within the restrictions of elite sport. Nonetheless despite the lack of significant results this body of work serves as a useful pilot study and some important findings were made which will enable improved care of respiratory health within British swimming. These studies once again highlight the high prevalence of EIB and other allied respiratory conditions within elite sport, the majority of which were previously undiagnosed. This was the first study to attempt to look at the impact of a screening programme for EIB on athlete health. It would seem logical that the introduction of a screening programme has the potential to improve athlete wellness and to reduce the days spent carrying out modified training, however more robust ways to measure athlete wellness and training are required. Respiratory questionnaires, NPIF and

FeNO appear to be useful tools in monitoring respiratory health in athletes and future work should investigate their sensitivity. Alterations in training environment have the potential to improve swimmer's respiratory health, however these changes need to be managed carefully considering other aero allergens, travel and life stressors and as such improving indoor home training environment could produce a better benefit. It has also been shown that to ensure respiratory screening programmes are successful, regular in depth follow up and education must be included.

In conclusion it appears that respiratory health in elite swimmers can be optimised through systematic assessment of airway health and modification of training environment. Larger, well controlled studies are still required in this area and should investigate the impact of this approach upon performance as well as wellness.

Chapter 6. A Heat and Moisture Exchange Mask to Reduce Exercise Induced Bronchoconstriction

6.0 ABSTRACT

OBJECTIVES: To determine if a heat and moisture exchanger (HME) face mask can be effective in protecting against acute bronchoconstriction and post exercise cough in response to a cycle challenge in a cold, dry environment in asthmatic individuals. METHODS: Twenty-six participants with a clinician diagnosis of asthma (20 males, 6 females, age: 27.6 ± 9.2 yrs, height: 172.7 ± 7.3 cm, mass: 71.2 ± 12.8 kg, $\dot{V}O_2$peak: 42.75 ± 8.17 ml.kg.min^{-1}) gave informed consent and completed three standardised exercise challenges (EX) on a cycle ergometer in a randomised order. During EX participants wore either an HME mask (MASK), a sham mask (SHAM) with no HME, or no mask (CON). EXs were conducted at 8 ^{o}C and 24% RH. Following a 3-min set warm up participants completed 6-min cycling at 80% peak power output. Before and after each EX, maximal flow volume loops were recorded. Immediately post EX participants were fitted with a Leicester Cough Monitor (LCM) which they wore for 24-hours. Results were analysed using repeated measures ANOVA and Friedman's tests and data presented as the mean ± SD or median score. RESULTS: Eleven participants were removed from the analysis as they failed to demonstrate evidence of EIB. There were no differences in temperature ($p = 0.81$), humidity ($p = 0.25$), mean power ($p = 0.98$) or baseline FEV_1 ($p = 0.76$) between EX conditions. There was a difference in the % fall in FEV_1 following EX (MASK: -6.0, SHAM: -11.0, CON: -13.0%, $p < 0.01$), with the % fall following CON greater than that of MASK ($p < 0.01$). No differences were found between EX in cough count per hour over the 24-hour monitoring period or the number of coughs in the first hour post EX. CONCLUSION: HME masks can attenuate bronchoconstriction in asthmatic individuals when exercising in cold, dry environments.

6.1 INTRODUCTION

In chapter 4 of this thesis a high prevalence of EIB was found amongst footballers. In this cohort it was also observed that a high proportion of athletes were reluctant to take traditional standard asthma inhalers to control their EIB, particularly when the condition was mild. It is well known that environmental conditions play a large role in the development and exacerbation of EIB and in chapter 5 an attempt was made to investigate the impact that improving a training environment could have on respiratory health and EIB severity in athletes. Unfortunately, however there were many variables in the method in which this was carried out that were not able to be controlled. Another asthmogenic environment in which many athletes train in the UK is a cold, dry environment. It is not currently known however if reducing exposure to this environment during training and competition could lessen EIB severity providing an alternative non-pharmacological method of protecting athletes from EIB.

Inspiring relatively dry and cold air during moderate and vigorous physical activity is a significant trigger for bronchoconstriction. Bronchoconstriction following exercise is thought to be caused by dehydration of the airway surface liquid (ASL) which causes cell shrinkage and release of inflammatory mediators precipitating airway smooth muscle constriction (Anderson and Kippelen, 2005). This respiratory water loss and resultant airway surface mucosal drying may also lead to both physical and chemical activation of cough receptors (Banner et al., 1984). At rest, inspired air is warmed and humidified through heat exchange in the nasal cavity, however this mechanism is compromised during exercise, because when $\dot{V}_E$ exceeds approximately 35 L/min there is a switch from a nasal to oral predominant breathing pattern (Niinimaa et al., 1980). This alteration has a particular impact in cold weather, as the air temperature travelling through the trachea and bronchi has been shown to be as low as 20°C (McFadden et al., 1985). Repeated exercise in the cold is thought

to result in a continuous cycle of injury and repair, leading to chronically inflamed airways with the potential for cellular airway changes (Karjalainen et al., 2000). These modifications to airway structure and function may play a key role in the increased prevalence of EIB and cough observed in athletes who train and compete in cold and dry environments (Turmel, et al., 2011).

An increase in temperature and water content of inspired air has long been shown to prevent EIB in asthmatic subjects (Chen et al., 1979). More recently, Bolger *et al*., (2011) demonstrated that EIB in athletes was completely prevented by increasing the temperature and water content of an inspirate from 4°C, 37% RH to 25°C, 94% RH and that this warm, moist air also limited the disruption of the airway epithelium. Post exercise cough is also more likely in environmental conditions which promote airway heat and water loss from the airways (Banner et al., 1984).

One method of increasing the temperature of inspired air thereby potentially diminishing airway dehydration is to use a face mask which incorporates a heat and moisture exchanger (HME). The limited number of studies that have investigated the use of HME masks and have demonstrated a promising protective effect against EIB as measured by an attenuation in the fall in forced expiratory volume in one second (FEV_1), following exercise (Beuther and Martin, 2006; Brenner et al., 1980; Millqvist et al., 2000; Nisar et al., 1992). However, Parsons *et al*., (2013) suggest recommendations to use HME masks are weak based on the current availability of low-quality evidence. As well as having the ability to protect against EIB, HME masks may also have the potential to decrease the incidence of cough amongst athletes engaging in sports in cold dry environments through the warming and humidifying of inspirate.

In chapters 4 and 5 observations were made of athletes who were reluctant to take or did not take their prescribed medication, preferring to minimise pharmacological approaches to

treatment. It has also been shown that HME face masks may have an additive effect in controlling EIB in conjunction with prophylactic β_2-agonist (Millqvist et al., 2000). Additionally, despite EIB being attenuated by the administration of inhaled β_2-agonists, cough can remain a prominent symptom (Banner and Green, 1984). Therefore, the aim of this study was to determine if an HME face mask can be effective in protecting against acute bronchoconstriction and post exercise cough in response to a cycling exercise challenge in a cold, dry environment.

6.2 METHODS

Study population

Following approval from the Faculty of Sciences Research Ethics Advisory Group for Human Participants, University of Kent (0881516) thirty recreationally active participants exercising at least twice per week provided written informed consent to participate (appendix 6). All participants had a clinician-based diagnosis of asthma with experience of worsening symptoms consistent with EIB when exercising in cold dry environments. Exclusion criteria included daily use of oral corticosteroids, hospitalisation due to asthma in the six months prior to study commencement and resting FEV_1 <80% of predicted value. Furthermore, if participants didn't have a fall in FEV_1 of ≥10% at two consecutive time points following at least one of the exercise challenges (see below) they were not included in the analysis. All participants were free from illness in the two weeks prior to assessment. Participants were instructed to maintain their usual diet for the duration of the study, to avoid exercise and caffeine for 24 hrs and 4 hrs respectively before each visit and arrive at the laboratory at least 2 hrs postprandial.

Study design

All participants expressing an interest in taking part in the study initially completed a health questionnaire (appendix 1) and provided contact details for their general practitioner (GP), along with their consent for their GP to be notified that they intended to take part in the study (appendix 10). All GPs were notified by letter (appendix 11) and 14 days was allowed for any response to be received prior to the participant's first visit to the laboratory.

Once it was confirmed a participant met the inclusion criteria, they attended the laboratory on five occasions (figure 6.1). During visit one participants completed a $\dot{V}O_2$ peak test on a cycle ergometer, visit two was a familiarisation visit, and during visits three to five, participants completed a standardised cycle exercise challenge (EX) in a cold, dry environment. During these EXs, participants wore either an HME mask (MASK), a sham mask (SHAM) or no mask (CONT).

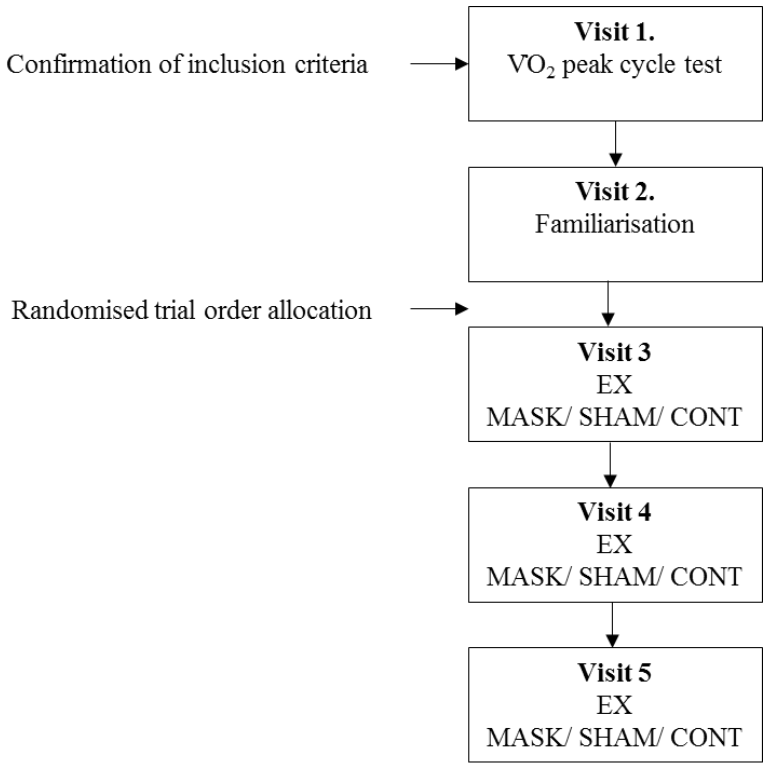

Figure 6.1. Flow chart of study design.

The HME MASK (ColdAvenger balaclava, USA) creates a micro-climate in front of the nose and mouth. A portion of each exhaled breath is retained in the ventilator cup and passively humidifies the next inhaled breath. The ventilator also manages extra moisture

away from the skin. The SHAM was the same HME mask with holes cut across the entire ventilator cup and the ventilator removed (figure 6.2).

Figure 6.2. A: Participant wearing the SHAM during an EX; B: MASK. C: SHAM.

The EXs were completed in a randomised order at the same time of day. The time between each visit was dependant on which medication a participant was currently taking. Participants previously prescribed inhaler medication for asthma / EIB ceased medication prior to each assessment (inhaled corticosteroids (ICS): 72 hours before; inhaled long-acting β_2-agonists (LABA): 48 hrs before; inhaled short-acting β_2-agonists (SABA): the day of the test) (Dickinson et al., 2011). Following each trial, participants had the same amount of time on their medication prior to once again stopping treatment before their next exercise challenge test. Participants who were not taking any medication, had a minimum of 48 hrs between trials.

Visit details

Visit One

Participants initially completed the Leicester cough questionnaire (LCQ) (Birring et al., 2003) (appendix 12). Anthropometric measures were taken, and they then performed a standardised incremental ramp test to volitional exhaustion to establish Peak Power on a cycle ergometer (Lode; Corival, Groningen, Netherlands) with simultaneous gas analysis (Cortex Metalyser 3b, CORTEX Biophysik GmbH, Germany). Initial power output was set at 50 Watts for 3 mins and thereafter the work rate increased by 20 Watts every 60 seconds. Participants were instructed to maintain their chosen cadence (between 70 & 100 rpm) for the duration of the test. The test was terminated upon volitional exhaustion or when the participant was no longer able to maintain their chosen cadence (i.e. a drop of >10 rpm) and $\dot{V}O_2$ peak was determined as the single highest 30 s average in $\dot{V}O_2$. Heart rate was recorded throughout (Polar RS400; Polar Electro Oy, Kempele, Finland) and Peak Power Output (PPO) was recorded.

Visit Two

Participants completed the EX challenge protocol as detailed below. Participants remained on prescribed asthma therapy for this visit as its purpose was familiarisation to the protocol and the EX was completed in a normal lab environment, without a mask. $\dot{V}_E$ was recorded for the final 6 mins of exercise via expired air passing directly through a dry gas meter using a mouth piece with a one-way valve. Spirometry was completed prior to and at 3, 5, 7, 10 and 15 mins after the challenge. Following the challenge, participants were given a cough

monitor which they were instructed to wear for the following 24-hour period (detailed below).

Visits Three to Five

Initially participants completed a cough 0-100mm visual analogue scale (VAS) (Spinou and Birring, 2014) (appendix 13). Airway inflammation was then assessed prior to each challenge by fraction of exhaled nitric oxide (FeNO) (NIOX VERO, NIOX, Aerocrine, Sweden) (Dweik et al., 2011). Resting lung function was measured by maximal flow volume spirometry (Spiro-USB and MicroLab, CareFusion, Germany) in accordance with international standards (Miller et al., 2005). Maximal flow-volume loops were subsequently measured in duplicate at 3, 5, 7, 10- and 15-mins post challenge, with the highest value at each time point used for analysis. If there was a >10% fall in FEV_1, 400 μg inhaled salbutamol was self-administered by the participant and maximal flow loops were repeated 15 minutes post administration to ensure FEV_1 had returned to within 10% of baseline. The EXs were conducted in an environmental chamber (TIS Services, Hampshire, UK) (8.6 ± 0.9 °C, 24.2 ± 4.2% RH) on a cycle ergometer (Lode; Corival, Groningen, Netherlands). A target power was set at 80% of peak power.

Details of the EX are described fully in chapter 2. In brief, participants first completed a 3-minute incremental warm up, cycling at work rate of 60, 75 and 90% of their target power for one minute at each stage. Participants then went straight into the EX cycling at their target power for 6 minutes. Heart rate was recorded throughout by a heart rate monitor (Polar RS400; Polar Electro Oy, Kempele, Finland).

Immediately after EX, participants were fitted with a Leicester Cough Monitor (LCM) (figure 6.3) (Matos et al., 2007) which they wore for a 24-hr period (see below for details).

Participants were asked to complete a paper diary in this period to detail sleep and wake time and any time in which they removed the monitor (appendix 14). They were also given an additional VAS to fill out on completion of the 24-hr period.

Leicester Cough Monitor

The LCM consists of an MP3 recording device and a lapel microphone (Sony, PX333). The monitor was fitted to the participants in a small unobtrusive running waist belt, with the microphone clipped to the participants clothing as close to the larynx as possible. Recording was initiated immediately on completion of each EX and the device was locked to ensure the participant could not stop the recording (see appendix 15 for full details). Participants were advised that the recording on the LCM would not be listened to at any point and were instructed to:

- Always wear the recorder and microphone unless having a shower or going to bed
- To keep microphone exposed and attached to a similar position on their clothing.
- To remove the recorder on going to bed and place it on a bedside table nearby.
- To remove the microphone and monitor if taking a bath or a shower, leaving it outside of the bathroom, re-attaching both when finished.

On completion of the 24 hr recording period participants were instructed to return the monitor as soon as possible. Data collected by the LCM was analysed by cough detection software based on the Hidden Markov model as described by Birring *et al*., (2008). This is a method used in speech recognition and automatically detects cough events whether occurring in isolation or in a bout. The LCM is a validated methodology (Birring et al., 2006, 2008) and has been used previously in patients with acute cough, chronic cough, and COPD (Lee et al., 2013; Yousaf et al., 2013).

Figure 6.3. Leicester Cough Monitor (LCM)

Statistical analysis

Data are presented as mean ± SD unless otherwise stated. Differences between the three EX conditions were examined using repeated measures ANOVA. Where data was not normally distributed, Friedman's test was used with post hoc pairwise comparisons where appropriate. All analysis was conducted using SPSS software, V.23 (SPSS, IBM, Armonk, NY, USA) with significance accepted at $P < 0.05$. Data presented as mean ± SD unless otherwise stated.

6.3 RESULTS

Thirty-four recreationally active participants initially enrolled in the study. Two dropped out following the $\dot{V}O_2$ peak trial, three following the familiarisation and two following the initial exercise trial. One subject was excluded due to poor resting lung function (FEV_1 <70% at baseline). Twenty-six participants (20 males, 6 females, age: 27.6 ± 9.2 yrs, height: 172.7 ± 7.3 cm, mass: 71.2 ± 12.8 kg, exercising: 5.8 ± 2.2 hours per week, $\dot{V}O_2$peak: 42.75 ± 8.17 ml.kg.min^{-1}) completed all exercise trials. All had a history of asthma/ EIB, 17 were currently using inhaled therapy for their asthma/ EIB; seven were using ICS, four a combined ICS and LABA and eight of whom were only using a SABA as needed.

Prior to analysis, flow volume loops from the exercise tests were inspected and those who failed to demonstrate a fall of FEV_1 of ≥10% from baseline measures following any of EX challenges were removed from subsequent analysis. These participants (n = 11) demonstrated either no fall, a very small <10% fall, or an increase in FEV_1 post exercise (range = +4 to -9% change in FEV_1 post EX), usually with a concomitant fall in FVC. Three of these participants had a history of EIB only, three were currently taking preventative ICS for their asthma, two a combination inhaler. Two were using SABA only and four were using no therapy.

Participant characteristics and baseline lung function

Characteristics of the fifteen remaining participants (12 males, 3 females) are shown in table 6.1. Lung function was normal in all participants at the familiarisation visit with no evidence of significant airflow obstruction (FEV_1 > 80% predicted). Three were currently not using any therapy, four were taking preventative ICS, two a combination inhaler and six were using inhaled SABA as required.

Table 6.1. Participant characteristics, $\dot{V}O_2$peak and baseline respiratory assessment data (Whilst using current medication) (n = 15).

	Measured	**% of predicted**
Age (years)	29.3 ± 9.2	-
Height (cm)	172.2 ± 8.1	-
Weight (kg)	73.4 ± 14.0	-
$\dot{V}O_2$peak	43.26 ± 8.11	-
Peak Power Output (W)	246.3 ± 42.7	-
FEV_1 (L)	3.57 ± 0.64	93.7 ± 9.0
FVC (L)	4.67 ± 0.87	103.7 ± 11.1
PEF (L/min)	536 ± 93	99.3 ± 11.0
FEV_1/FVC	76.7 ± 8.8	93.9 ± 10.2

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow.

During the familiarisation exercise trial, $\dot{V}_E$ was 68.50 ± 11.66 $L.min^{-1}$, which was 56.8 ± 9.1% of their maximum observed in the $\dot{V}O_2$ peak test, Mean HR was 165.2 ± 16.57 bpm, 92.7 ± 5.4% of their maximum HR from the $\dot{V}O_2$ peak test.

Environmental conditions

During the three exercise trials (MASK, SHAM, CONT), there were no differences in conditions in the environmental chamber (Table 6.2). There were also no differences in exercise intensity (Mean Power and Mean HR; Table 6.2).

Table 6.2. Chamber conditions and exercise performance between acute MASK, SHAM and CONT trials (n = 15).

	MASK	**SHAM**	**CONT**	**Sig.**
Temperature (°C)	8.4 (1.0)	8.3 (0.5)	8.6 (1.1)	0.81
Humidity (%)	24.8 ± 4.2	22.9 ± 3.8	24.3 ± 3.9	0.25
Mean Power (W)	172.3 ± 47.5	172.2 ± 46.5	172.3 ± 47.5	0.98
Mean HR (bpm)	165 ± 16	163 ± 14	165 ± 17	0.16

Data for temperature was not normally distributed and therefore analysed with Friedman's test and presented as the median score (IQR).

Exercise Trials

Results of spirometry and assessment of FeNO conducted prior to each exercise challenge showed no differences in resting lung function between trials (FeNO, FEV_1, FVC, PEF, FEV_1/FVC) (Table 6.3).

There was a significant difference ($p < 0.01$) in the maximal % change in FEV_1 following the exercise challenges, with the % fall following the CONT exercise trial being significantly greater than that of the fall following the MASK trial ($p < 0.01$), with all but one participant having a greater fall in FEV_1 following CONT than MASK (figure 6.4). Ten out of 15

participants also had a greater drop in SHAM than MASK (figure 6.4), however this was to a lesser extent and SHAM was not different to either the MASK (p = 0.17) or CONT (p = 0.51) trial.

Number needed to treat (NNT) analysis showed that in the MASK group, participants were 33% likely to have a ≥10% fall in FEV_1 post EX and in the CONT 87%. The absolute risk reduction of wearing an HME mask was therefore 0.54, meaning that the number of people who would need to wear an HME mask to avoid one participant having a ≥10% fall in FEV_1 post EX would be two people.

Table 6.3. Lung function pre and post challenge, n = 15.

	MASK	**SHAM**	**CONT**	**Sig.**
FeNO (ppb)	39 (33)	42 (33)	38 (51)	0.88
FEV_1 (L)	3.52 ± 0.58	3.51 ± 0.58	3.53 ± 0.61	0.76
FVC (L)	4.68 ± 0.81	4.65 ± 0.83	4.64 ± 0.80	0.62
PEF (L/min)	536 ± 81	531 ± 80	533 ± 89	0.57
FEV_1/FVC	75.60 ± 9.26	75.67 ± 8.82	76.33 ± 9.77	0.59
Maximal % change in FEV_1 post challenge	-6.0 (7.0) #	-11.0 (11.0)	-13.0 (9.0)	<0.01*

Data for maximal % change in FEV_1 and FeNO was not normally distributed and therefore analysed with Friedman's tests and presented as the median score (IQR). * indicates a

significant difference between exercise condition. # indicates significant difference to CONT.

FeNO, exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow.

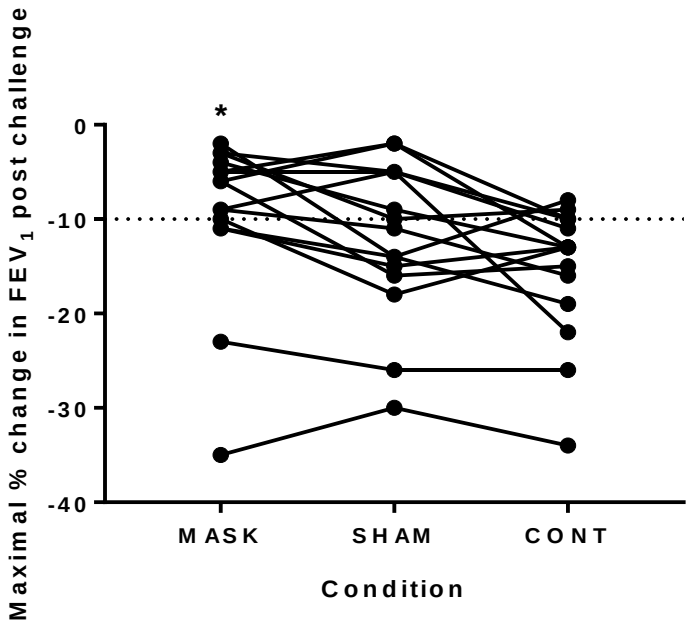

Figure 6.4. Maximum % fall from baseline in FEV_1 post exercise challenge. * indicates significant difference to CONT.

FEV_1, forced expiratory volume in 1 s. Dashed line represents the threshold for a positive challenge.

Cough

One participant could not participate in the cough monitoring due to their occupation. Four of the remaining 14 participants reported cough as a symptom they experienced following exercise.

Results from the LCQ showed all but one participant scored either a 6 = hardly any of the time or 7 = none of the time in all domains (physical, psychological and social) for the impact cough has upon the different aspects of their lives. The remaining participant scored 5 for all domains (a little of the time). At baseline (pre-familiarisation exercise trial) VAS score was 9 ± 7 mm out of 100 mm, with a range of 1 to 19 mm.

Four participants demonstrated a higher number of coughs per hour than considered normal in a 24-hour period (Normal = <5 females, <2 males) following all Ex trials. One of these participants demonstrated cough counts indicative of chronic cough > 20 coughs per hour. There were also 5 additional participants who demonstrated greater cough count per hour than normal following one or more EX trial (figure 6.5).

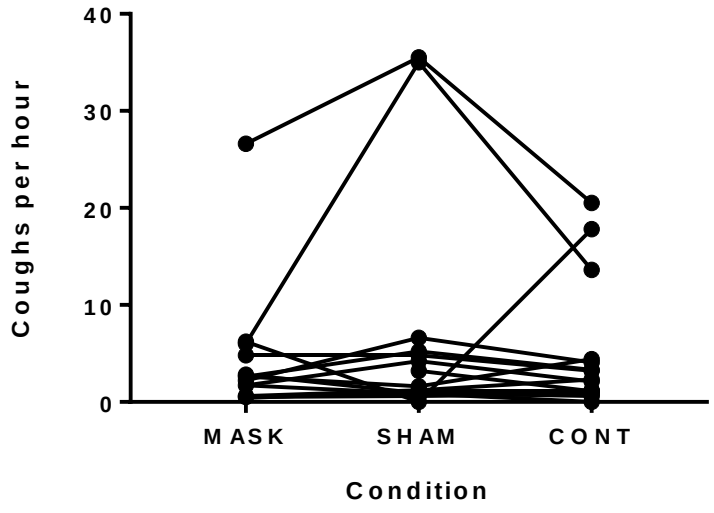

Figure 6.5. Cough per hour over 24-hour monitoring period.

No differences were seen between EX trials in cough count per hour over the 24-hours, number of coughs recorded in the first hour following Ex or participants self-report of cough 24-hours post EX trial using VAS (table 6.4).

Table 6.4. Cough Results n = 13

	MASK	**SHAM**	**CONT**	**Sig.**
Number of coughs per hour	3 (3)	2 (3)	3 (3)	0.06
Number of coughs in first hour post EX	17 (17)	15 (17)	8 (24)	0.92
VAS 24-hour post EX (mm) (n = 15)	7 (24)	14 (27)	10 (24)	0.52

Data was not normally distributed and therefore analysed with Friedman's test and presented as the median score (IQR).

The data was also analysed separating those who reported cough as a troublesome symptom (Yes Cough) and those who did not (No Cough), no difference was seen in LCQ total sore between the two groups (n = 15, Yes cough: 20 ± 1, No cough: 19 ± 2, p = 0.14). There was also no difference between these groups in the number of coughs in the first hour (n = 14, Yes cough: 18 ± 14, No cough: 13 ± 13, p = 0.56) or cough per hour in the 24-hour monitoring period (n = 14, Yes cough: 2 ± 2, No cough: 3 ± 2, p = 0.88), in the familiarisation visit or any of the experimental conditions.

No linear relationships were found between VAS score and cough per hour, % fall in FEV_1 and coughs per hour, % fall in FEV_1 and number of coughs in the hour after EX or LCQ score and cough count following the Familiarisation trial.

6.4 DISCUSSION

This study demonstrates that wearing an HME mask during a cycle exercise challenge (6 mins and 80% Peak power output) in a cold, dry (8.6 ± 0.9 ^{o} C and 24.2 ± 4.2% RH) environment results in an attenuation in % fall in FEV_1 post exercise, compared to not wearing a mask. Furthermore, we found that only 33% of participants with a clinician's diagnosis of asthma/ EIB demonstrated evidence of EIB (≥10% fall in FEV_1 post exercise) whilst wearing a mask, compared to 87% of participants who did so after EX with no mask, making the number needed to treat (NNT) two participants.

Results from our study are in agreement with earlier studies: Stewart *et al*., (1992) exercised asthma patients on either a cycle ergometer or a treadmill to 80% predicted HRmax wearing a mask in one trial and no mask in another. The author concluded that the mask which retained heat and moisture, effectively controlled EIB in most patients. They also noted that even whilst wearing the mask FEV_1 post exercise decreased by 9%, however this was similar to that produced by sodium cromoglycate which had been used for more than 20 years in treating EIB. The mask in our study was more effective in controlling EIB, however without the mask their patients exhibited a larger drop in FEV_1 of 18 to 52%. Similarly, in a study by Nisar et al., (1992) participants cycled for 6 mins whilst inhaling air from a cold air supply (-13°C). Participants completed both a mask and a control mask trial and results showed that with the control mask there was a 22% fall in FEV_1 post exercise, and this was reduced to -10% in the mask trial. This group also monitored temperature inside the two masks and discovered some warming of the air in the control mask to 10°C, whereas the active mask warmed the air to 19°C. In the present study our intention was to monitor both temperature and humidity inside the two mask trials, however in pilot testing we found that the commercially available probes were too large and therefore were highly affected by each expiration leaving us unable to achieve a reliable reading. Although we were not able to

measure temperature and humidity inside the used masks our results seem to indicate that some degree of warming and humidification of the inspired air took place. This was also in accordance with anecdotal reports of the subjective feedback of our participants. Two previous studies have also compared HME masks to pre-treatment with a bronchodilator and found that they were equally effective in attenuating a drop in lung function (Beuther and Martin, 2006; Millqvist et al., 2000). In Beuther and Martin's (2006) study, participants completed treadmill exercise tests, running for 10 min at 85% HR max, breathing medical grade air (-15 to -25°C). They found that FEV_1 fell 28% with a placebo mask, 6% with an active mask, and 11% with pre-treatment with albuterol. The authors concluded that the HME mask prevents the cold exercise induced decline in lung function at least as effectively as pre-treatment with albuterol. This study also showed that those with a <10% fall in FEV_1 also benefitted from wearing the active mask, which is what we found in the current study. Millqvist *et al*., (2000) showed that combining pre-treatment with β_2-agonists with a HME mask completely preserved lung function. Using an 18-minute exercise protocol of increasing load in an environment of around -10°C participants completed a no therapy trial, a β_2-agonist trial and a combined face mask and β_2-agonist trial and demonstrated mean falls of -27, -12 and -7% respectively. The authors noted that their results show that there may be various mechanisms underlying EIB; the combination of oedema and bronchoconstriction causing a fall in FEV_1. Whereas β_2-agonists cannot prevent possible oedema, a face mask probably can, on the other hand, results showed that the mask alone was not enough to prevent EIB entirely, so it seems likely that the exercise itself induces a certain amount of bronchoconstriction, probably via mediators. This may also be why we saw that those with a fall in FEV_1 of 25-40% was not completely effective.

In contrast to our study, these previous studies used asthmatic subjects who demonstrated larger drops in FEV_1 and utilised subfreezing conditions but did not control for humidity. We decided not to use such cold conditions, but looked at cold, dry conditions which athletes

training outdoors in the UK are more likely to encounter. We aimed to recruit participants with more severe asthma, however despite all participants having a physician's diagnosis of asthma, and the majority taking prescribed medication, 9 out of 27 (33%) demonstrated no evidence of EIB. This is an important issue by itself and is in support of the study in which Aaron *et al*., (2017) who found that in 33.1% of adults with physician-diagnosed asthma, evidence of asthma could not be established. This highlights that objective testing should be utilised more frequently in the diagnosis and follow up of asthma patients.

Athletes, particularly winter sport athletes are at increased risk of developing EIB and this could possibly be due to inspiring large amounts of cold and dry air during training and competition, leading to drying of and injury to the mucosa, ultimately inducing EIB (Anderson and Kippelen, 2008). It has been suggested that the use of β_2-agonists may make it possible for more cold, dry air to reach the lower airways and although reduce bronchoconstriction, may in the end cause more injury to the mucosa (Millqvist et al., 2000). This combined with the effectiveness of the HME mask suggests that there may be value in encouraging all athletes exercising in cold, dry environments who are susceptible to EIB, to wear an HME mask where practical, whether they are using medication or not. Another potential benefit of using an HME for the prevention of EIB is that it has the potential to reduce the amount of β_2-agonists that athletes use prophylactically, which would be beneficial as there is the potential for the development of tachyphylaxis and a potential desensitisation of repeat dosing (Anderson et al., 2006).

Many of the earlier studies only include the use of either a sham or a no mask trial, it was felt important to include of both trials. We found that although not significant, there was an attenuation of the fall in FEV_1 in the SHAM condition, this is in support of the AsthmaUK 'scarfie' campaign and suggests that it may be beneficial for athletes who may not want to wear an actual mask to cover their nose and mouth with a scarf. Previous support of this idea

was shown in an earlier study where portable surgeons paper masks produced a slight attenuation of EIB (Brenner et al., 1980).

None of the aforementioned studies looked at symptoms related to EIB and whether these could also be attenuated by wearing an HME. Recently, the Leicester Cough monitor has proven useful in objectively assessing cough in asthma management (Fukuhara et al., 2016) and an alternative automated device has also been found capable of increasing patient awareness of the patterns of cough for early detection of worsening asthma (Rhee et al., 2015). These studies show that cough monitoring has great potential for assessing the response to asthma therapy. This was the first study to attempt to measure objective and subjective measures of cough following exercise in participants with EIB. Banner *et al*., (1984) showed that cough was most likely following hyperpnoea in environments which promoted respiratory water loss, as the resultant airway surface mucosal drying can lead to both physical and chemical activation of the cough receptors. Results from the present study however showed no differences in cough frequency following exercise between the MASK, SHAM or CONT trials for either cough per hour over the 24-hour monitoring period, or more acutely in the hour post EX. In addition, no differences were seen in the participant′s perception of cough as indicated by VAS. This however is unsurprising because the relationship between objective cough frequency and subjective measures of cough such as VAS has been shown to be mild to moderate (Birring et al., 2006). Only six participants in the present study reported cough as a troublesome symptom following exercise and although 11 participants demonstrated a level of cough which would be considered higher than normal (≥5 females, ≥2 males) in one or more trial, only two demonstrated levels usually observed by patients with chronic cough. It may be that cough frequency in the current cohort was too small to observe any change. Low levels of cough were unexpected given that cough is the most commonly reported respiratory symptom by athletes (Hull et al., 2017) and in previous

chapters this was a commonly reported symptom. Participants in this study were recreational athletes rather than elite so this may go some way to explain this observation.

A limitation of this study as previously mentioned, is compared to earlier studies, participants in this study group in the main demonstrated mild EIB. It would be beneficial to see if the HME mask could attenuate the fall in FEV_1 in participants with more severe EIB. As seen in figure 6.2 to wear the mask we used it needs to be attached to a balaclava. Participants reported that they became very hot even in a short exercise bout, which would potentially be a barrier to athletes wearing the mask during training. Future investigations may look to develop a heat and moisture mask that is more practical to wear in UK weather. Additional areas for future study may be looking at the efficacy of an HME mask compared to an everyday scarf which athletes may be more likely to utilise. Also, looking at the effects of wearing an HME long term during training to see if protecting the airway from injury reduces severity of EIB would be beneficial as this could be an alternative form of treatment.

In conclusion, HME masks can attenuate bronchoconstriction in individuals with asthma/ EIB when exercising in cold, dry environments. HME Masks may be helpful in the management of athletes who do not wish to take prophylactic drug treatment.

Chapter 7. Discussion

DISCUSSION

7.0 Key Research Study Findings

- Chapter three demonstrated that a standardised exercise challenge (EX) in a dry (26% RH) environment was found less provocative than eucapnic voluntary hyperpnoea (EVH) and a positive EVH challenge was found not predictive of a positive response to EX.
- Chapter four revealed that 29% of a cohort of elite football players demonstrated evidence of exercise induced bronchoconstriction (EIB) when screened using EVH. Treatment of these EVH positive (EVH+) players with standard asthma therapy resulted in improved airway inflammation and reduced airway-hyper-responsiveness, and this was associated with a potential beneficial impact on performance.
- Chapter five highlighted that 75% of elite swimmers entering the GB funded programme appear to have EIB when screened for respiratory dysfunction. No significant differences between the health and wellbeing of swimmers who received a treatment intervention following a screening for EIB compared to those who didn't were found. There were also no significant differences in respiratory health or general health and wellbeing in swimmers training outdoors at a warm weather training camp compared to those remaining at home training indoors. The findings of a systematic approach to evaluating total airway health in elite swimmers with EIB prior to the 2016 Olympics was also reported, concluding that respiratory health in elite swimmers can be optimised through a systematic assessment of airway health.
- Chapter six showed that a heat and moisture exchange (HME) face mask can be effective in protecting against acute EIB. This however was not found to have an impact on the incidence of cough.

7.1 High prevalence of EIB in athletes

Data from this thesis adds to the already well-established body of evidence that there is a high prevalence of EIB amongst elite athletes both in the UK and worldwide. Previously prevalence has been reported to vary between 20 and 68% in British summer Olympic sports (Dickinson et al., 2005; Levai et al., 2016), and similar levels have been reported by numerous authors across the world in athletes competing in both summer and winter sports (Bougault et al. 2009; Helenius et al. 1998; Larsson et al. 1993; Mannix et al. 1996; Mannix et al., 1999; Wilber et al. 2000). The two sports which were investigated in this thesis, namely football and swimming are two of the most popular participation sports in the UK (Jones et al., 2011) and in the case of football, the world (Kunz, 2007). In chapter 4 the largest ever cohort of elite footballers to date were screened for EIB using EVH and 29% of players across the three top divisions in England were found to have objective evidence of EIB, which concurs with previous small scale studies of footballers in the UK (Dickinson et al., 2013). A recent study has since found a prevalence of only 7% in football players living in a humid tropical region (Mousinho Gomes et al., 2018). It is difficult to discern whether this is the true prevalence of players in these areas due to the differing environments in which they train and play, or due to the difference in methods of diagnosis. Mousinho and colleagues' chosen bronchoprovocation method was a field running test in environmental conditions of 30°C and 82% RH, whereas recommended guidelines when using an exercise test are to do so in ambient conditions of 20-25°C and RH of ≤ 50%. There is the potential for EIB prevalence in footballers to vary around the world due to the differing environments in which they play, however certainly results for this thesis could be applied to other countries in Europe, whose football players train outside all year round and are exposed to cold climates, dry air and high pollen levels. As such, it is thought that this condition would be of interest to football governing bodies such as UEFA, who as part of their core mission aim to protect the health of their athletes.

It is well documented that swimmers have a high prevalence of EIB (Helenius et al. 1998; Pedersen et al. 2008; Bougault et al. 2009; Stadelmann et al., 2011; Levai et al. 2016), perhaps the highest of any sport and data collected as part of chapter 5 concurred with these previous findings showing that 75% of swimmers entering the GB funded programme demonstrated evidence of EIB when objectively assessed using EVH.

There is limited literature regarding gender differences in prevalence of EIB. Although not a primary objective of this thesis, it was found that across all studies which determined prevalence recruiting both genders, there was a 35 and 22 % prevalence of EIB in females and males respectively when assessed by EVH and exercise challenges. This is in support of Norqvist *et al*., (2015) who demonstrated female athletes have a higher prevalence of EIB than their male counterparts. No associations were found however between gender and result of either EVH or Exercise challenges in any of the studies in this thesis.

7.2 Diagnosis of EIB

There is still some debate amongst the literature regarding the optimal bronchoprovocation challenge to identify EIB in athletes. The favoured bronchoprovocation challenge of the IOC-MC is EVH (Anderson et al., 2001) and as such EVH is often referred to as the 'gold standard' test. Recently however this has been challenged (Hull et al., 2016) and it would seem logical to use exercise as the provocative stimulus to detect an exercise induced condition. Results of chapter 3 showed that EVH demonstrated greater sensitivity than a standardised exercise challenge in the diagnosis of EIB. These results were in agreement with previous studies comparing both lab and field exercise challenges to EVH (Dickinson et al. 2006; Rundell et al. 2004; Mannix et al., 1999). The reduced sensitivity of exercise compared to EVH is thought to be due to the differences in the two main contributors to the airway response; inspired air water content (Evans et al., 2005) and $\dot{V}_E$ (Carlsen et al., 2000).

Despite attempting to standardise these using an environmental chamber with additional dehumidifiers to reach a relative humidity of 26% and an algorithm using individual heart rate response to guide exercise intensity, it remained impossible to match the low humidity of the medical grade air of EVH and the high ventilation which can be achieved by hyperventilation. Inclusion of an additional visit to the laboratory for a $\dot{V}O_2$max test prior to the exercise challenge did not lead to a higher $\dot{V}_E$ during the exercise challenge. Using the heart rate based algorithm (Ansley et al., 2010), a mean $\dot{V}_E$ of 77 ± 11% of predicted MVV was achieved. However, by using 80% of peak power, $\dot{V}_E$ was lower (57 ± 9% MVV) meaning that in chapter 3 only four participants out of 31 did not achieve the 60% MVV required for an acceptable test, whereas 10 out of 14 did not meet the 60% threshold in chapter 6. In both of these chapters the recommended HR target of 80-90% of predicted maximal heart rate (Bonini and Palange, 2015) was met 89 ± 4.8% (chapter 3) 87.70 ±6.4% (chapter 6).

During chapter three, it was found that although it would seem logical to use exercise in the diagnosis of an exercise induced condition, there are still many limitations to using an exercise challenge, many of which would be particularly restrictive when attempting to carry out a squad screening. Firstly, as discussed above, it was difficult to ensure an adequate $\dot{V}_E$. Also, a specialist environmental chamber is required, which needs several hours to achieve the desired environment, can only be used by one athlete at a time to ensure control over the environmental conditions and needs time between each participant for environmental conditions to return to the correct level. The equipment for EVH testing on the other hand can be portable, numerous athletes can be tested back to back, there is no variation in environmental conditions and the test does not interfere with an athlete's training in the same way as a six minute near maximal exercise test might. In addition, $\dot{V}_E$ is easy to standardise ensuring athletes to achieve ≥ 60% MVV; in chapter three, all twenty-seven participants

achieved this level, chapter 4 only four out of ninety-five football players didn't reach the target and in chapter 5 only four out of thirteen swimmers did not.

There is some debate in the literature regarding the appropriate threshold for a positive EVH test indicative of EIB. The widely accepted 10% fall in FEV_1 post EVH was recently challenged by Price *et al*., (2017) who suggested that a more appropriate threshold in an athletic population may be 15%. This was based upon analysis of 224 healthy asymptomatic athletes, taking an abnormal response as a mean +2 SD fall from baseline. By using only asymptomatic athletes in this analysis however there is a bias to the data. The authors suggest that using the 10% threshold, 20% of these athletes would provide a positive test and evidence for EIB. This level of prevalence would however be expected in an athletic population (Dickinson et al., 2005). The authors suggest that based on their previous work there is poor reproducibility of EVH suggestive of mild EIB (Price et al., 2015). Williams *et al*., (2015) however showed good reproducibility at all severities both long and short term. During chapter 4, the two players who performed a repeat EVH challenge despite not taking their recommended medication demonstrated almost identical falls in FEV_1 at a level indicative of mild EIB (-14% to -14% and -10% to -11%). Chapter 3 provided no evidence to support a change in criteria to a 15% fall in EVH; in this chapter it was seen that applying the 15% threshold resulted in 5 participants no longer being identified as EVH+ one of whom demonstrated EX+, highlighting that a change in the criteria may increase the risk of producing false negatives.

A change in the criteria for a positive exercise challenge to a fall in FEV_1 of $\geq$ 6% has also been suggested by some authors (Helenius et al., 1998; Rundell et al. 2000). This lower threshold to exercise would appear logical given 'normal' response to exercise is mild bronchodilation (Todaro, 1996), whereas EVH is more provocative and a 'normal' response to EVH in non-asthmatic subjects has been found to be around 4% (Hurwitz et al., 1995). In

chapter 3 if the criteria had been changed to a fall in FEV_1 of $\geq$ 6%, this would have led to one further participant being identified as having evidence of EIB through the exercise challenge. This participant however was not EVH positive and show this change in criteria may lead to false positive diagnoses.

Although many authors have established that EVH is more sensitive than exercise challenges (Dickinson et al. 2006; Rundell et al. 2004; Rundell et al. 2000; Mannix et al., 1999) as Anderson and Kippelen (Anderson and Kippelen, 2013) suggest, ultimately the appropriate cut off will be determined by the investigators need to either be more sensitive or more specific. For the purposes of this thesis, where EVH was predominantly used in a screening scenario of elite athletes, it was decided that using the criteria of two consecutive falls in FEV_1 of 10% was most appropriate. In these circumstances, sports only have a short time frame to assess large numbers of athletes and therefore athletes are only able to perform a one-off test. Although EVH has been cited as potentially being overly sensitive (Price et al. 2016), if negative, then EIB is able to be ruled out. If a mild positive test is found i.e. a fall in FEV_1 of 10-15% caution can be applied by ensuring that there is no concomitant fall in FVC and the flow loops demonstrate a concaving shape. Also, by using other evidence from the respiratory assessment such as FeNO, nasal peak flow and atopy status the physician is can initiate a trial of treatment. The criteria for two consecutive falls in FEV_1 also protects against results being influence by fatigue or poor technique. In chapter 4 all four players with very mild EIB (falls in FEV_1 post EVH of 10-14%) showed a reduction of EIB severity following a nine-week trial of standard asthma inhaler therapy. In addition, two of these players had a high FeNO (>50ppb) at baseline which was subsequently within normal limits at follow up. Two of these players were also included in the analysis of performance results and following nine-weeks of treatment $\dot{V}O_2$ max was also found improved.

7.3 Respiratory symptoms and EIB

Literature has consistently shown a poor correlation between respiratory symptoms during and after exercise and objective evidence of airway narrowing (Rundell et al., 2001; Dickinson et al., 2005), with some athletes reporting symptoms despite airway obstruction being blunted by medication (Simpson et al., 2015) whereas only a minority of asthmatic athletes reporting any respiratory symptoms (Turcotte et al. 2003). This thesis supports this previous research and provides further evidence to support that objective testing is essential to establish a diagnosis of asthma and EIB (Parsons et al., 2013). Chapters 3 and 4 show that respiratory symptoms were not predictive of a positive EVH or exercise challenge. Also, although not reported in chapter 6, when all participants were included, respiratory symptoms were not predictive of a positive exercise challenge (p = 0.93). Result from chapter 5 do not support this finding as respiratory symptoms in this group of swimmers were predictive of EVH test result. Despite this however, these swimmers had not reported them previously as troublesome to the team physician.

Previous studies have found that cough is the most commonly reported respiratory symptom in elite athletes (Dickinson et al., 2005, 2006; Rundell et al., 2001). A high prevalence of cough was seen in all studies in this thesis; it was the most frequently reported symptom in chapters 3 and 5 and the second most reported symptom by footballers in chapter 4. The exception to this is that it was the third most commonly reported symptom in chapter 6 behind chest tightness and wheezing. This could potentially be due to the different population used in this study; rather than elite athletes, this group were exercising asthmatics. As athletes do not often recognise respiratory symptoms, it would be beneficial to have a tool which could objectively measure symptoms such as cough in this population. Chapter 6 showed that the Leicester cough monitor (LCM) was an easy to use tool in a recreationally active group. In line with research suggesting that athletes do not recognise respiratory

symptoms, there was no relationship between the objective measure of cough and self-report of cough using a VAS. The different exercise conditions (HME mask, sham and no mask condition) had no effect on the level of cough in this study, however cough frequency was low and only a few participants reported cough as a troublesome symptom. The device however was simple to use and unobtrusive and has the potential to be used as a monitoring tool for objective monitoring of cough and interventions in this population. This would likely be beneficial in groups such as cold weather athletes (Turcotte, J B Langdeau, et al., 2003) who report that coughing becomes more prominent and problematic over the course of their competitive season (Turmel et al., 2012).

7.4 Misdiagnosis of EIB

Throughout this thesis, by using EVH and exercise challenges as objective assessment methods for EIB, a high level of both under and over diagnosis of EIB and asthma has been found. In chapter 3, 7% of participants were found to have no objective evidence of EIB despite a prior diagnosis and taking medication for this condition, whereas 11% of participants demonstrated evidence with no prior diagnosis. Ten percent of footballers demonstrated a fall in FEV_1 in keeping with a diagnosis of EIB despite no previous history and 3% of players were on medication for asthma/ EIB including daily ICS despite providing no objective evidence for this condition when assessed. In the population of swimmers screened for EIB on entry to the GB funded programme, 50% were found to have previously undiagnosed EIB. Furthermore, in chapter 6, 44% of a population of recreational active individuals with a diagnosis of asthma or EIB showed no evidence of either condition when objective testing in the form of spirometry and exercise challenge testing in a cold, dry environment was performed. Four of these individuals were not taking any medication for their diagnosis of asthma/ EIB, however the remaining participants were regularly using

inhaled therapies including, SABA, ICS and even combination ICS and LABA inhaler therapy. Combining the data from this thesis showed that in total, 8% of individuals may be taking medication unnecessarily and even more have a current diagnosis for a condition which they most likely do not have.

This alarming result it not exclusive to an athletic population; Aaron *et al.,* (2017) found no evidence of asthma could be found in 33% adults with physician-diagnosed asthma, highlighting that objective testing should be utilised more frequently in the diagnosis and follow up of asthma patients as well as in athletes. Worryingly when questioned, quarter of family practitioners said that they would initiate treatment on clinical information alone when presented with adults with exercise-related respiratory symptoms (Hull et al., 2009). There are health risks associated with unnecessary asthma medication; the long term use of SABAs has been associated with degenerative changes in lung function (Bonini et al., 2013) and improper use of ICS has been seen to lead to adrenal failure and growth suppression in children and mucosal immuno- suppression and an increased risk of respiratory infections in adults (Sabroe et al., 2013). The cost of inappropriate treatment for asthma and EIB is also likely not trivial. Mukherjee *et al*., (2016) reported that the costs to the NHS associated with asthma are estimated to be at least £1.1 billion, with 74% of these costs for provision of primary care services (60% prescribing, 14% consultations). With improved initial diagnostics, the initial cost of objective tests could be offset with reductions in unnecessary prescriptions and follow up appointments.

Given the high prevalence of EIB in athletes, detection and appropriate treatment of EIB is essential. The high number of athletes demonstrating objective evidence of EIB despite not having a history of asthma/ EIB or reporting symptoms highlights the potential need for screening in this population. Failure to implement appropriate treatment presents a potential for deterioration and exacerbation of EIB. Undetected and uncontrolled EIB can lead to fatal

consequences (Becker et al., 2004) and when left untreated EIB severity may increase due to airway injury (Anderson and Kippelen, 2008). Untreated, EIB also has the potential to have deleterious effects of performance (Stensrud et al., 2007). The long-term effects of leaving EIB untreated in susceptible athletes is unknown however and is an important area for future study.

7.5 Treating EIB in athletes

Current guidelines for treatment of EIB in athletes is chiefly based upon guidelines for standard asthma care and expert opinion due to the lack of research in athletes. Chapter 4 looked to start to fill this void and the impact of standard asthma therapy on EIB control in elite footballers was investigated. This study found that nine-weeks of appropriate inhaler therapy based on EIB severity successfully provided adequate control of EIB and a reduction of airway inflammation. Unfortunately, although treatment was recommended for all twenty-seven EVH+ footballers, only seven took the medication as prescribed, resulting in a large limitation of the study. This study also attempted to go one step further and be the first to investigate the effect of treating EIB on performance in this elite population. Once again, the high attrition rate meant that a small sample size was left for the final analysis, so the results had to be interpreted with caution. Despite not being statistically significant, results were however promising; EVH+ players demonstrated a larger mean increase in $\dot{V}O_2$ peak higher than that of the EVH- players, bringing the mean $\dot{V}O_2$ peak of the two groups in line with each other. Further work with an adequately powered study is required to follow this up. Similar findings have also been reported in sports with similar demands; Brukner *et al*., (2007) found that Australian Rules football players with newly diagnosed EIB had a significant improvement in $\dot{V}O_2$ max following six weeks of treatment compared to controls

and although again not statistically significant Spiteri *et al*., (2014) demonstrated that appropriately medicating elite rugby players with previously undiagnosed EIB improved their performance in a rugby specific aerobic exercise. It has been hypothesised that this impact on performance may be due in part to the attenuation of bronchoconstriction during and following exercise which results in reduced alveolar ventilation and efficiency of alveolar-to-arterial blood O_2 exchange (Haverkamp et al., 2007). It may also simply be due to the athletes being able to complete higher quality training sessions whilst on treatment as their recovery between sets is not compromised due to airway narrowing. It was hoped that further evidence of the effects of appropriately treating screen detected EIB in athletes would be obtained in chapter 5 where the impacted of screening in swimmers was investigated. Unfortunately, due to time constraints in the swimmer's programme, a follow up assessment was not able to be made. In chapter 5 however, the impact of screening and appropriately treating EIB on general health and wellbeing was investigated. No significant differences were seen in in the number of days lost to illness or spent carrying out modified training between the group who were given an intervention (either an initiation of treatment or a change in their previous medication) and the group who did not receive an intervention (EVH- swimmers and those swimmers who although EVH+ did not take their treatment. There were many limitations to results in chapter 5 as discussed within that chapter and future studies could investigate this area by using a larger group of athletes with EIB initiating treatment, looking at the impact treatment has over a certain time frame by using a more specific wellness monitoring tool. This is an important area of investigation as association between uncontrolled asthma/ EIB has been suggested to predispose athletes to upper respiratory tract infections (URTIs) (Helenius and Haahtela, 2000) and in the general population an increased risk of pneumococcal disease and pneumonia (O'Byrne et al., 2013). With the leading cause of time lost to training in elite sport being due to respiratory illness,

by treating known underlying illness may reduce an athlete's risk of respiratory illness and therefore lead to less time of training lost and therefore improved performance.

Chapter 6 demonstrated that as well as pharmacological treatment of EIB, alternative methods of reducing EIB may be available to athletes as an HME mask was found successful in attenuating the fall in FEV_1 after an exercise challenge in recreationally active asthmatics. This result was in keeping with previous research (Stewart et al., 1992; Nisar et al., 1992; Beuther and Martin, 2006) and could be a useful strategy in the treatment of in mild cases of EIB because as highlighted in chapters 4 and 5, there are a number of athletes who although demonstrate EVH+ were particularly reluctant to take any standard asthma medication. Clearly a mask would not be feasible to wear in a sport such as swimming, however during training for outdoor sports particularly during the winter months a mask could worn by athletes which can acutely control EIB exacerbations. There is also the potential that if worn regularly, these masks may also have the potential to limit the development of EIB in cold weather athletes by interrupting the continuous cycle of injury and repair, which ultimately leads to chronically inflamed airways with the potential for cellular airway changes (Karjalainen et al., 2000). This is yet to be studied.

7.6 Translation into practice

As already discussed, there is a high prevalence of EIB which can lead to suboptimal health and performance in athletes. Appropriate strategies need to be put in place in elite sport to optimise management of EIB in athletes. There is some debate as to whether screening for EIB should be carried out in athletic populations; some authors have called for screening for EIB to be implemented (Dickinson et al., 2005; Holzer and Brukner, 2004; Vakali et al., 2017), however others want to ensure that before widespread screening is implemented the prevalence, the ability to detect the condition of interest and an understanding of the impact

in the population of interest is demonstrated (Wilson and Jungner 1968; Ansley et al., 2013; Hull et al., 2007; Hull and Rawlins 2016). Results of this thesis add to the growing body of evidence which shows a high prevalence in an athletic population. It shows that EIB can be successfully detected by EVH testing and it begins to highlight the positive impact that treating screen detected athletes with EIB can have. Gerald *et al*., (2007) suggested that despite a lack of evidence for the adoption of screening programmes, limited case detection programmes could be appropriate where there is a high prevalence of undiagnosed asthma and where newly identified patients have access to quality care. This would certainly apply to an athletic population and data from this thesis would support the implementation of case detection programmes in elite sport. If sports are to adopt this practice however it is vital that programmes are structured to include monitoring and education of athletes demonstrating evidence of EIB. The work involving the elite footballers highlighted that despite support from staff, many players did not to take the inhalers, and were confused about what they should be taking and when. This showed that for a screening programme to be effective in elite sport, time needs to be invested in educating players about EIB and its treatment and it is vital to have the cooperation of the athletes from the start. Miller *et al*. (2005) supports this by highlighting the importance of educating coaching staff in the management of asthma. Whilst working with the elite swimmers, although only anecdotal evidence, swimmers attending the pre-Olympic assessment were attending for their third or fourth respiratory assessment and despite having received education at each visit, they were only just beginning to realise the importance of taking their preventative treatment regularly. Chapter 5 also demonstrated the importance of follow up testing and monitoring of athletes once on treatment for EIB as despite apparently taking regular preventative treatment some swimmers still demonstrated obstruction at rest, highlighting that either their compliance or inhaler technique needed improving or their medication needed to be stepped up.

It is recommended that repeat testing of athletes is carried out at intervals during an athletes career in those initially demonstrating EVH- because EIB has been shown to develop over the course on an athletic career (Knöpfli et al., 2007). The frequency at which repeat testing should be carried out has not yet been investigated.

A key factor in the development of EIB in athletes is the interaction between the ventilatory demand of their sport and the environment in which it is performed, leading one group to consider whether in fact airway dysfunction in athletes should be considered an occupational lung disease (Price et al., 2013). In chapter 5, no difference was seen in airway inflammation, wellbeing or nasal peak flow after changing the indoor environment in which the swimmers usually train to the outdoor environment in Australia. One reason for this might have been that although the swimmers were no longer being exposed to the noxious trichloramines, many were instead suffering from hay fever through exposure to airborne allergens.

Sports have a duty of care to their athletes and as such, in sports in which there is a high prevalence of EIB, everything possible should be done to minimise the detrimental effects. As well as appropriate screening and monitoring of respiratory health, in swimming for example as discussed in chapter 5, measures can be taken such as using alternative methods of pool disinfectant, ensuring adequate ventilation and establishing pool hygiene regulations. In outdoor sports as previously discussed an HME mask could be a viable method of removing some of the environmental impact. Additionally measures such as trying to ensure training is not scheduled outside in low humidity or during times of high pollen or pollution and avoiding carrying out sessions close to major roads or during rush hour would also be advisable where possible (Dickinson *et al*., 2018).

It is not only the elite population to which the results of this thesis are relevant. A high level of misdiagnosis of asthma and EIB were uncovered in participants of all levels of athletic ability. This can be translated into general practice to add to the growing body of literature

which recommends objective testing for the diagnosis of asthma and EIB (Aaron et al., 2017). This may save valuable NHS resources on unnecessary prescriptions and protect patients from needless medications and enable them to obtain differential diagnoses for their symptoms. The importance of education would also be equally, if not more important in this group who do not have staff on hand to remind them to take medication.

7.7 Overall Limitations

The limitations of this thesis have been discussed in the current chapter and as part of the individual studies. The main limitations of this thesis have largely been due to its applied nature. Research within elite sport is notoriously difficult to control due to the high-pressure, fast-moving nature, limited access to the athletes and many demands on their time. Very few studies have been carried out in elite athletes, particularly regarding the impact of treatment on EIB, wellbeing and performance and as such despite several limitations this thesis adds value to a scarce body of literature. The other big limitation was, ironically despite such a high prevalence at the very elite level, the difficulty of recruiting participants with EIB for the laboratory-based studies. This in itself was an interesting finding however, uncovering the high level of misdiagnosis amongst recreationally active individuals.

7.8 Areas for future study

A key area which requires investigation is the effect that treating screen detected EIB athletes has on their performance. This was attempted during chapter 4 but due to large numbers of dropout was not adequately powered. Similarly, the long-term impact of EIB on athlete health and wellbeing requires further investigation, reporting on the impact of treatment in optimising performance and investigating the link between asthma/ EIB and frequency of

respiratory tract infection. The effect of improvements in training environments, making them less noxious environments should also be investigated and may be an important area in athlete welfare and the long-term effects of wearing an HME mask during training upon EIB control and development could be a way of investigating this.

7.9 Conclusions

Data from this thesis demonstrates a high prevalence of EIB in elite athletes, specifically football players and swimmers. It was found that respiratory symptoms are not predictive of EIB and objective assessment is required. EVH was found to be the most sensitive diagnostic method for EIB in an applied sporting environment and the original 10% threshold for a cut off for fall in FEV_1 post EVH seems appropriate in these circumstances. Standard asthma treatment appears to be successful in the elite athletic population in improving airway health and reducing EIB severity. Furthermore, it may have the potential to improve athletic performance in these athletes, enabling them to compete on a level playing field with their non-EIB counterparts. Further investigation is required to determine if screening and treating athletes for EIB where appropriate may also improve overall health and wellbeing. HME masks could be a viable alternative method of treatment for EIB. No impact on changing the training environment in swimmers from an indoor to an outdoor pool was found and as such the impact of improving training environment requires further investigation.

Overall using evidence from this thesis, it would be recommended that case detection programmes are established for athletes in sports which put them at a greater risk of developing EIB. As part of this appropriate follow up, monitoring and education is required, and training environments should be optimised in relation to reducing exposure to asthmogenic environments.

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APPENDICES

Appendix 1

University of Kent

Health Questionnaire

Name: _____ Age:_____ Date: _____

SECTION 1. – General Health

PART 1.

	Yes	No
1. In the last 4 weeks have you suffered from or been treated for chest infection?	☐	☐
2. In the last 2 weeks have you suffered from or been treated for any other illnesses?	☐	☐
3. Do you currently smoke? If yes, how many cigarettes per day? _____	☐	☐
4. Are you pregnant or have you had a baby in the last 6 months?	☐	☐
5. Do you have any injury or condition that limits your mobility? If yes, please explain: _____	☐	☐
6. Are you currently taking any medications? If yes, please provide details: _____	☐	☐
7. Have you to your knowledge had any adverse/allergic reaction to any medication? If yes, please provide details: _____	☐	☐

PART 2

	Yes	No
1. Has your doctor ever said that you have a heart condition OR high blood pressure?	☐	☐
2. Have you ever experienced wheeze/cough or felt pain in your chest at rest or when you do physical activity?	☐	☐
3. Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4. Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
5. Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
6. Do you have a bone or joint problem that limits your physical activity? (e.g. knee, ankle, shoulder, elbow or other)	☐	☐
7. Has your doctor ever said that you should only do medically supervised physical activity or is there any other condition why you shouldn't participate in physical activity?	☐	☐

If you answered **NO** to all of the questions above, please go to **SECTION 3** on Page 5.

If you answered YES to one or more of the questions in Part 2 above, please go to **SECTION 2** on

SECTION 2. – Chronic Medical Conditions

	Yes	No
1. **Do you have cancer of any kind?**	☐	☐
a. Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head and neck?	☐	☐
b. Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
2. **Do you have Heart Disease or Cardiovascular Disease?** *This includes coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm.*	☐	☐
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
b. Do you have an irregular heartbeat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	☐	☐
c. Do you have chronic heart failure?	☐	☐
d. Do you have a resting blood pressure equal to or greater than 140/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure.)	☐	☐
e. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
3. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event.*	☐	☐
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
b. Do you have any impairment of walking or mobility?	☐	☐
c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐

	Yes	No
4. Do you have any Metabolic Conditions? *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes.*	☐	☐
a. Is your blood sugar often above 13.0 mmol/L?	☐	☐
b. Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys and the sensation in your toes and feet?	☐	☐
5. Do you have other Metabolic Conditions such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease or liver problems?	☐	☐
6. Do you have a Respiratory Disease? *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure.*	☐	☐
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
c. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs.	☐	☐
d. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week) or have you used your rescue medication more than twice in the last week?	☐	☐
7. Do you have any Mental Health Problems or Learning Difficulties? *This includes Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome etc.*	☐	☐

	Yes	No
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
8. Do you have Arthritis, Osteoporosis or Back Problems?		
a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments.)	☐	☐
b. Do you have joint problems causing pain, a recent fracture or facture caused by osteoporosis or cancer, displaced vertebra (e.g. spondylolisthesis) and/or spondylosis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
c. Have you had steroid injections or taken tablets regularly for more than 3 months?	☐	☐
9. Do you have any other medical condition not listed above or do you live with two chronic conditions?	☐	☐
a. Have you experienced blackout, fainted or lost consciousness as a result of a head injury within the last 12 months?	☐	☐
b. Do you have a medical condition that is not listed, such as epilepsy, neurological conditions, kidney problems etc.?	☐	☐
c. Do you currently live with two chronic conditions?	☐	☐
10. Are there any other relevant conditions/injuries/illnesses that you are aware that you have which have not been covered in this questionnaire?	☐	☐

If yes to questions 9 or 10 please provide details:

_____

Please go to **SECTION 3** on Page 5.

University of Kent

SECTION 3. – Sport Information

1. **How many hours of exercise do you do per week?** _____
2. **What is your main sport?** _____
3. **How long have you been taking part in your main sport?** _____
4. **How often do you train for your main sport?** _____
5. **How often do you compete in your main sport?** _____
6. **At what level of competition do you compete?**

   Recreational ☐ Local ☐ County ☐

   National ☐ International ☐
7. **During or after training or competition do you experience any of the following?** Please circle as many as appropriate.

   Coughing | Excess Mucus Production | Chest Tightness

   Wheezing | Difficulty in Breathing (Dyspnoea) | **NONE** of above

   Other_____
8. **During training or competition what environmental conditions seem to make your breathing worse?** Please circle as many as appropriate.

   Cold Climate | Dry Air | High Pollen Content

   High Pollution | Altitude | **NONE** of above

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

SECTION 4. – Respiratory Health

	Yes	No
1. **Have you ever suffered from asthma?**	☐	☐

2. Did you use asthma medication in the past?
3. **Are you currently diagnosed asthmatic?** ☐ ☐
4. Are you currently using medication for your asthma? ☐ ☐
5. **Do you suffer from exercise-induced asthma (EIA)?** ☐ ☐
6. Are you currently using medication for your exercise-induced asthma (EIA)? ☐ ☐
7. **Have you ever had a Eucapnic Voluntary Hyperventilation Challenge (EVH) test?** ☐ ☐

   If yes, the test was a. Positive ☐ b. Negative ☐ c. Don't know/Can't remember ☐

If you have answered **YES** to either or all of 'Questions 2, 4 and 6' please complete table below.

Asthma Medication Table Example:

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

For further guidance, please go to Appendix 1 on Page 7.

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

Appendix 1. Glossary of Asthma Medications

Reliever (Blue inhaler)	Preventer (Brown, red or orange inhaler)		Combination (Purple inhaler)	Other
SALBUTAMOL	**BECLOMETASONE**	**SALMETEROL**	**FORMOTEROL + BECLOMETASONE**	Montelukast
Airomir®	Asmabec®	Serevent®	Fostair®	Pranlukast
Asmasal®	Beclazone®	**FORMOTEROL**	**SALMETEROL + FLUTICASONE**	Zafirlukast
Salamol®	Becodisks®	Atimos®	Seretide®	
Salbulin®	Clenil Modulite®	Foradil®	**FORMOTEROL + BUDENOSIDE**	
Salbutamol®	Pulvinal Beclomethasone®	Oxis®	Symbicort®	
Pulvinal®	Qvar®	**CROMOGLYCATE**		
Ventolin®	**BUDENOSIDE**	Intal®		
TERBUTALINE	Easyhaler Budenoside®	**NEDOCROMIL**		
Bricanyl®	Novolizer Budenoside®	Tilade®		
	Pulmicort®			
	CICLESONIDE			
	Alvesco®			
	FLUTICASONE			
	Flixotide®			
	MOMETASONE			
	Asmanex Twisthaler®			

Appendix 2

Pre EVH Questionnaire

Name: ..... **DOB:**.....

Height: ..... **Weight:**.....

General Health Questionnaire

	YES	NO
In the last 4 weeks have you suffered from or been treated for chest infection?		
In the last 2 weeks have you suffered from or been treated for any other illnesses?		
Have you consumed any caffeine within the past 4 hours?		
Do you have an injury or condition which means you cannot train full time?		
Do you have any cardiovascular conditions *Including coronary artery disease, High blood pressure, Heart failure, Abnormality of heart rhythm.*		
Do you have any Metabolic Conditions? *Including Type 1 Diabetes, Type 2 Diabetes, and Pre-Diabetes.*		
Do you have a Respiratory Disease? *Including Chronic Obstructive Pulmonary Disease (COPD) and Pulmonary High Blood Pressure.*		
Do you have any other medical condition not listed above		
Are you currently taking any medications?		
Have you to your knowledge had any adverse/allergic reaction to any medication?		
If you answered 'Yes' to any of the questions above, please provide details below:		

1. **During or after training or competition do you experience any of the following?** Please circle as many as appropriate.

Coughing	Excess Mucus Production	Chest Tightness
Wheezing	Difficulty in Breathing (Dyspnoea)	**NONE** of above

Other_____

2. **During training or competition what environmental conditions seem to make your breathing worse?** Please circle as many as appropriate.

Cold Climate	Dry Air	High Pollen Content
High Pollution	Altitude	**NONE** of above

Other_____

3. **Do you use any form of therapy/training to aid your breathing?**

Respiratory Health

	Yes	No
1. Have you ever suffered from asthma?		
2. Did you use asthma medication in the past?		
3. Are you currently diagnosed asthmatic?		
4. Are you currently using medication for your asthma?		
5. Do you suffer from exercise-induced asthma?		
6. Are you currently using medication for your exercise-induced asthma?		
7. Have you ever had an EVH test?		
8. If yes, was the result a. Positive b. Negative c. Don't know		

If you have answered YES to either or all of 'Questions 2, 4 and 6' please complete table below.

For help, see Appendix 1

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

Appendix 1: Glossary of Asthma Medications

Reliever (Blue inhaler)	Preventer (Brown, red or orange inhaler)		Combination (Purple inhaler)	Other
SALBUTAMOL	BECLOMETASONE	SALMETEROL	FORMOTEROL + BECLOMETASONE	Montelukast
Airomir®	Asmabec®	Serevent®	Fostair®	Pranlukast
Asmasal®	Beclazone®	FORMOTEROL	SALMETEROL + FLUTICASONE	Zafirlukast
Salamol®	Becodisks®	Atimos®	Seretide®	
Salbulin®	Clenil Modulite®	Foradil®	FORMOTEROL + BUDENOSIDE	
Salbutamol®	Pulvinal Beclomethasone®	Oxis®	Symbicort®	
Pulvinal®	Qvar®	CROMOGLYCATE		
Ventolin®	BUDENOSIDE	Intal®		
TERBUTALINE	Easyhaler Budenoside®	NEDOCROMIL		
Bricanyl®	Novolizer Budenoside®	Tilade®		
	Pulmicort®			
	CICLESONIDE			
	Alvesco®			
	FLUTICASONE			
	Flixotide®			
	MOMETASONE			
	Asmanex Twisthaler®			

Appendix 3.

CONSENT FORM

University of Kent

Title of project: Diagnosing EIB in athletes

Name of investigator: Anna Jackson, Dr John Dickinson

Participant Identification Number for this project: _____

Please initial box

1. I confirm I have read and understand the information sheet dated 12.10.15 (Version4) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason.
(If you have any questions, please send an email to arj20@kent.ac.uk. ☐

3. I understand that my responses will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised responses. ☐

4. I agree to take part in the above research project. ☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent *(if different from lead researcher)* *To be signed and dated in presence of the participant*	Date	Signature
_____	_____	_____
Lead researcher	Date	Signature

Copies:

When completed: 1 for participant; 1 for researcher

Appendix 4.

CONSENT FORM

University of Kent

Title of project: Screening for Exercise-Induced Bronchoconstriction in team sports: The impact of appropriate therapy on airway health and performance

Name of investigator: Anna Jackson, Dr John Dickinson

Participant Identification Number for this project: _____

Please initial box

1. I confirm I have read and understand the information sheet dated 12.05.15 (Version1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. *(If you have any questions, please send an email to arj20@kent.ac.uk.* ☐

3. I understand that my responses will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised responses. ☐

4. I consent to providing urine samples as part of this research study. I understand that these will be used for research purposes as outlined in the participant information sheet. I understand that samples will be anonymised prior to storage and analysis. ☐

5. I agree to take part in the above research project.

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent *(if different from lead researcher)* *To be signed and dated in presence of the participant*	Date	Signature
_____	_____	_____
Lead researcher	Date	Signature

Copies:

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in main file

Appendix 5.

CONSENT FORM

University of Kent

Title of project:

Monitoring respiratory health in elite swimmers

Name of investigators:

Anna Jackson, Dr John Dickinson, Dr John Greenwell, Dr Ben Hollis

Participant Identification Number for this project: _____

Please initial box

1. I confirm I have read and understand the information sheet dated 15.12.15 (Version3) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason.
(If you have any questions, please send an email to arj20@kent.ac.uk. ☐

3. I understand that my responses will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised responses. ☐

4. I agree to take part in the above research project. ☐

Name of participant	Date	Signature
Name of person taking consent *(if different from lead researcher)*	Date	Signature
Lead researcher	Date	Signature

To be signed and dated in presence of the participant

Copies: *When completed: 1 for participant; 1 for researcher site file*

Appendix 6.

CONSENT FORM

University of Kent

Title of project: The Use of a Heat and Moisture Exchange Mask to Reduce Exercise Induced Bronchoconstriction Severity and Improve the Airway Health of Individuals with Asthma

Name of investigators: Dr John Dickinson, Dr. James Hopker, Dr John Molphy, Oliver Schumacher and Anna Jackson

Participant Identification Number for this project: _____

Please initial box

1. I confirm I have read and understand the information sheet dated 12/10/1616 (Version 3) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. ☐
3. I understand that my data will be anonymised before analysis. I give permission for members of the research team to have access to my anonymised responses. ☐
4. I agree to take part in the above research project. ☐
5. Would you like to receive a report of the study outcomes and your individual responses once the study complete? ☐

You can contact the principal researcher listed below for questions specifically related to this study. ***Dr. John Dickinson***, at the School of Sport and Exercise Sciences, University of Kent (email: J.W.Dickinson@kent.ac.uk).

Name of participant	Date	Signature
Name of person taking consent *(if different from lead researcher)*	Date	Signature

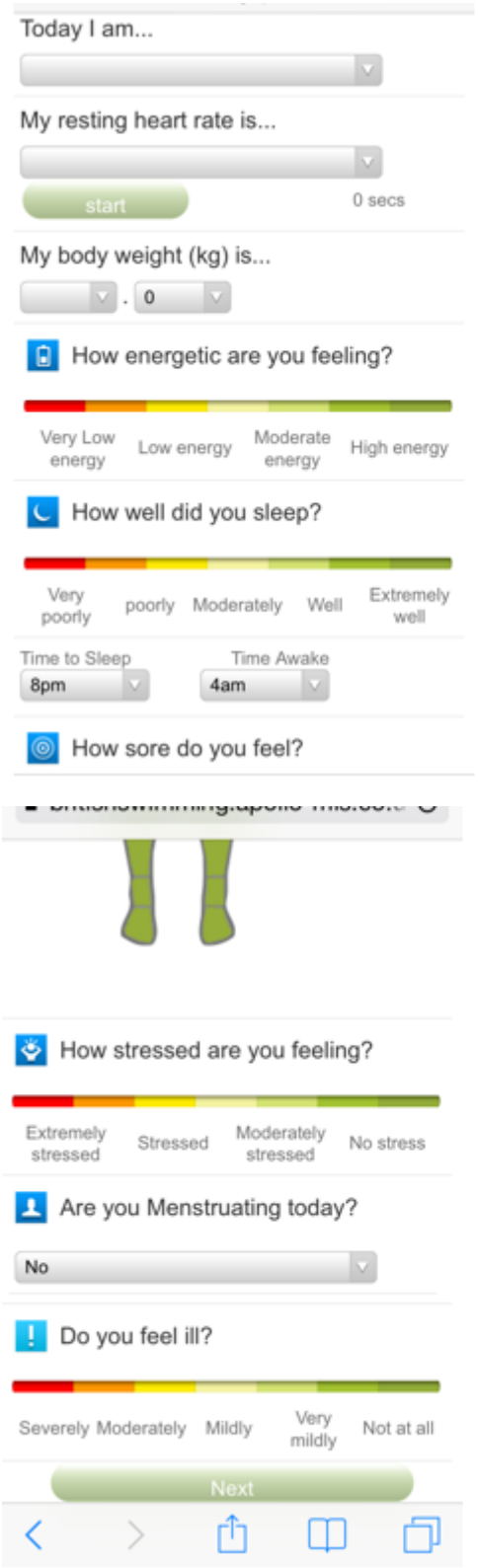
To be signed and dated in presence of the participant

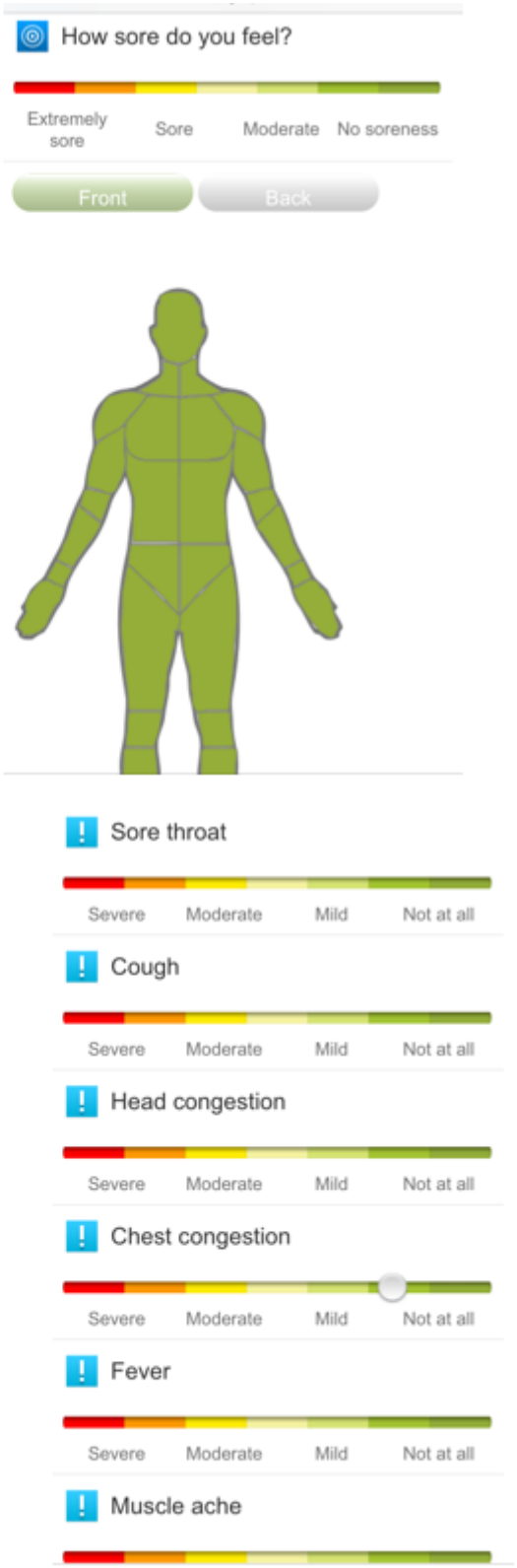
Lead researcher	Date	Signature

Copies: *When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in main file*

Appendix 7.

Screen shots from the daily online wellness diary





Appendix 8.

MINI ASTHMA QUALITY OF LIFE QUESTIONNAIRE (UNITED KINGDOM)
SELF-ADMINISTERED

PATIENT ID _____

DATE _____

Please complete **all** questions by circling the number that best describes how you have been during the **last 2 weeks as a result of your asthma.**

IN GENERAL, HOW MUCH OF THE TIME **DURING THE LAST 2 WEEKS** DID YOU:

		All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
1.	Feel SHORT OF BREATH as a result of your asthma?	1	2	3	4	5	6	7
2.	Feel bothered by or have to avoid DUST in the environment?	1	2	3	4	5	6	7
3.	Feel FRUSTRATED as a result of your asthma?	1	2	3	4	5	6	7
4.	Feel bothered by COUGHING?	1	2	3	4	5	6	7
5.	Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?	1	2	3	4	5	6	7
6.	Experience a feeling of CHEST TIGHTNESS or CHEST HEAVINESS?	1	2	3	4	5	6	7
7.	Feel bothered by or have to avoid CIGARETTE SMOKE in the environment?	1	2	3	4	5	6	7
8.	Have DIFFICULTY GETTING A GOOD NIGHT'S SLEEP as a result of your asthma?	1	2	3	4	5	6	7
9.	Feel CONCERNED ABOUT HAVING ASTHMA?	1	2	3	4	5	6	7
10.	Experience a WHEEZE in your chest?	1	2	3	4	5	6	7

IN GENERAL, HOW MUCH OF THE TIME **DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
11. Feel bothered by or have to avoid going outside because of WEATHER OR AIR POLLUTION?	1	2	3	4	5	6	7

HOW **LIMITED** HAVE YOU BEEN **DURING THE LAST 2 WEEKS** DOING THESE ACTIVITIES **AS A RESULT OF YOUR ASTHMA**?

	Totally Limited	Extremely Limited	Very Limited	Moderate Limitation	Some Limitation	A Little Limitation	Not at all Limited
12. STRENUOUS ACTIVITIES (such as hurrying, exercising, running up stairs, sports)	1	2	3	4	5	6	7
13. MODERATE ACTIVITIES (such as walking, housework, gardening, shopping, climbing stairs)	1	2	3	4	5	6	7
14. SOCIAL ACTIVITIES (such as talking, playing with pets/children, visiting friends/relatives)	1	2	3	4	5	6	7
15. WORK-RELATED ACTIVITIES* (tasks you have to do at work)	1	2	3	4	5	6	7

*If you are not employed or self-employed, these should be tasks you have to do most days.

DOMAIN CODE:

Symptoms: 1, 4, 6, 8, 10
Activity Limitation: 12, 13, 14, 15
Emotional Function: 3, 5, 9
Environmental Stimuli: 2, 7, 11

Appendix 9.

MINI RHINOCONJUNCTIVITIS QUALITY OF LIFE QUESTIONNAIRE

(ENGLISH FOR UK VERSION)

SELF-ADMINISTERED

PATIENT ID_____

DATE_____

Please complete **all** questions by circling the number that best describes how **troubled** you have been during the **last week as a result of your nose/eye symptoms.**

		Not troubled	Hardly troubled at all	Somewhat troubled	Moderately troubled	Quite a bit troubled	Very troubled	Extremely troubled
ACTIVITIES								
1.	REGULAR ACTIVITIES AT HOME AND AT WORK (your occupation or tasks that you have to do regularly around your home and/or garden)	0	1	2	3	4	5	6
2.	RECREATIONAL ACTIVITIES (indoor and outdoor activities with friends and family, sports, social activities, hobbies)	0	1	2	3	4	5	6
3.	SLEEP (difficulties getting a good nights sleep and/or getting to sleep at night)	0	1	2	3	4	5	6
PRACTICAL PROBLEMS								
4.	NEED TO RUB NOSE/ EYES	0	1	2	3	4	5	6
5.	NEED TO BLOW NOSE REPEATEDLY	0	1	2	3	4	5	6

MINI RHINOCONJUNCTIVITIS QUALITY OF LIFE QUESTIONNAIRE

(ENGLISH FOR UK VERSION)

SELF-ADMINISTERED

PATIENT ID_____

DATE_____

How troubled have you been during the last week as a result of these symptoms?

	Not troubled	Hardly troubled at all	Somewhat troubled	Moderately troubled	Quite a bit troubled	Very troubled	Extremely troubled
NOSE SYMPTOMS							
6. SNEEZING	0	1	2	3	4	5	6
7. STUFFY/BLOCKED NOSE	0	1	2	3	4	5	6
8. RUNNY NOSE	0	1	2	3	4	5	6
EYE SYMPTOMS							
9. ITCHY EYES	0	1	2	3	4	5	6
10. SORE EYES	0	1	2	3	4	5	6
11. WATERING EYES	0	1	2	3	4	5	6
OTHER SYMPTOMS							
12. TIREDNESS AND/OR FATIGUE	0	1	2	3	4	5	6
13. THIRST	0	1	2	3	4	5	6
14. FEELING IRRITABLE	0	1	2	3	4	5	6

Appendix 10.

Permission to contact your GP

Before you are able to take part in this study, we must write to your GP to let them know of your intention to participate and give them 14 days to respond. If we do not hear back from them, we will assume that there is no problem with you taking part. On the next page there is a copy of the letter we will send them.

Please complete the form below to provide us with your GPs contact details.

Your name: _____
D.O.B: _____

GP Name: _____
Practice Name: _____
Address: _____
Postcode: _____
Telephone: _____
Email: _____

Any additional information: _____

Appendix 11.

Date

GP Name
GP Address

Dear Dr *GP name*,

RE: ***Participants Name and Date of Birth***

Participants name has informed us you are their GP. I am writing to inform you *Participants name* has volunteered to take part in a study investigating the use of a heat and moisture exchange mask to reduce exercise induced bronchoconstriction severity and improve the airway health of individuals with asthma
I have attached the participant information sheet which provides an outline to the study. On request I can supply the full protocol.

If you wish to express any concerns over the participant taking part in this study please contact me within 14 days of receiving this letter. If I don't hear from you I assume you have no objection to *participants name* participating in the study.

Kind regards,

Dr. John Dickinson

School of Sport and Exercise Sciences
University of Kent

Medway Building,
Chatham Maritime,
Kent ME4 4AG

Office: 01634 202998
Mob: 07912038415
Email: j.w.dickinson@kent.ac.uk

Appendix 12.

University Hospitals of Leicester NHS
NHS Trust

Leicester Cough Questionnaire (LCQ)

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by TICKING the response that best applies to you. Please answer ALL questions, as honestly as you can.

This questionnaire will remain confidential.

Name

Date

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

1.	All of the time	☐
2.	Most of the time	☐
3.	A good bit of the time	☐
4.	Some of the time	☐
5.	A little of the time	☐
6.	Hardly any of the time	☐
7.	None of the time	☐

2. In the last 2 weeks, have you been bothered by sputum (phlegm) production when you cough?

1.	Every time	☐
2.	Most times	☐
3.	Several times	☐
4.	Some times	☐
5.	Occasionally	☐
6.	Rarely	☐
7.	Never	☐

3. In the last 2 weeks, have you been tired because of your cough?

1.	All of the time	☐
2.	Most of the time	☐
3.	A good bit of the time	☐
4.	Some of the time	☐
5.	A little of the time	☐
6.	Hardly any of the time	☐
7.	None of the time	☐

4. In the last 2 weeks, have you felt in control of your cough?

1.	None of the time	☐
2.	Hardly any of the time	☐
3.	A little of the time	☐
4.	Some of the time	☐
5.	A good bit of the time	☐
6.	Most of the time	☐
7.	All of the time	☐

5. How often during the last 2 weeks have you felt embarrassed by your coughing?

1.	All of the time	☐
2.	Most of the time	☐
3.	A good bit of the time	☐
4.	Some of the time	☐
5.	A little of the time	☐
6.	Hardly any of the time	☐
7.	None of the time	☐

6. In the last 2 weeks, my cough has made me feel anxious.

1.	All of the time	☐
2.	Most of the time	☐
3.	A good bit of the time	☐
4.	Some of the time	☐
5.	A little of the time	☐
6.	Hardly any of the time	☐
7.	None of the time	☐

7. **In the last 2 weeks, my cough has interfered with my job, or other daily tasks.**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

8. **In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life.**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

9. **In the last 2 weeks, exposure to paints or fumes has made me cough.**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

10. **In the last 2 weeks, has your cough disturbed your sleep?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

11. **In the last 2 weeks, how many times a day have you had coughing bouts?**

1. All the time (continuously) ☐
2. Most times of during the day ☐
3. Several times during the day ☐
4. Some times during the day ☐
5. Occasionally through the day ☐
6. Rarely ☐
7. None ☐

12. **In the last 2 weeks, my cough has made me feel frustrated.**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

13. **In the last 2 weeks, my cough has made me feel fed up.**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

14. **In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

15. **In the last 2 weeks, have you had a lot of energy?**

1. None of the time ☐
2. Hardly any of the time ☐
3. A little of the time ☐
4. Some of the time ☐
5. A good bit of the time ☐
6. Most of the time ☐
7. All of the time ☐

16. **In the last 2 weeks, have you worried that your cough may indicate a serious illness?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

17. **In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?**

1. All of the time ☐
2. Most of the time ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

18. **In the last 2 weeks, my cough interrupted conversation or telephone calls.**

1. Every time ☐
2. Most times ☐
3. A good bit of the time ☐
4. Some of the time ☐
5. A little of the time ☐
6. Hardly any of the time ☐
7. None of the time ☐

19. **In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends.**

1. Every time I cough ☐
2. Most times when I cough ☐
3. Several times when I cough ☐
4. Some times when I cough ☐
5. Occasionally when I cough ☐
6. Rarely ☐
7. Never ☐

Thank you for completing this questionnaire.

Designed by MEDICAL ILLUSTRATION
at LEICESTER ROYAL INFIRMARY
Birring/RESPIRATORY MEDICINE/11.02/18447/Y

Appendix 13.

Cough Visual Analogue Score

Subject ID: _____ Date: _____ Trial: _____

Please put a cross on the line to indicate where on the scale your cough has been in the last 24 hours whilst you have been wearing the cough monitor.

No cough |_____| Worst cough ever

Appendix 14.

Cough Monitor Diary

Subject ID:_____ Date: _____Trial:_____

Recording Start Time:_____

You have been asked to wear the cough monitor for a 24-hour period. The monitor will record all sound over the 24-hour period but this will be analysed automatically by a computer program and is not played back. All information recorded is kept strictly confidential.

Please read this information sheet and use the diary to record meal and bedtimes. Please also write down any comments, suggestions or problems you experience with the monitor.

Please ensure you return the monitor to us when you come in for your next visit. You do not need to stop the recording.

Advice

Showering: If you want to take a shower or bath, take off the microphone and leave it in your room with the monitor bag. Put the bag and microphone back on when you finish. You may also do the same if you wish to take it off whilst you go to the toilet.

Sleeping: Place the monitor bag on a bedside table when you go to bed. If you feel uncomfortable to sleep with the microphone around your neck, take it off and place it on your bedside table together with the monitor bag. Put the microphone back on when you get up in the morning.

Diaries

Please record the time you started and finished your main meals

Main Meals	**Start**	**Finish**
e.g. Breakfast	*07:30*	*07:40*

Please record the time that you went to bed, and got up in the morning

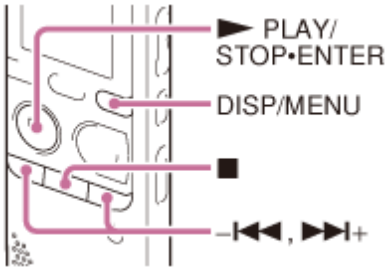
Bed time:	To Bed	Get up
	e.g. 23:00	*07:00*

Please record any comments, suggestions or problems you experienced with the cough monitor:

Appendix 15.

Cough Monitoring Instructions

Cough Monitor Initial Setup



Menu options are set as follows:

- Add/overwrite OFF
- VOR OFF
- DPC (speed con) OFF
- Easy search OFF
- Alarm OFF
- AVLS OFF
- Protect OFF
- Divide Leave blank as it is, move on to next option.
- Move and copy Leave blank as it is, move on to next option.
- Delete Leave blank as it is, move on to next option.
- Select memory Built in memory

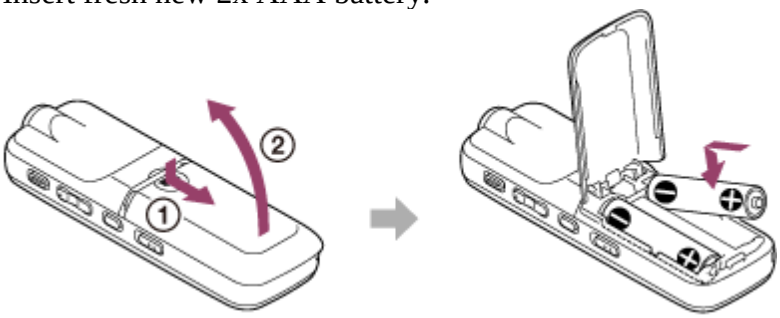
Detail memory This option contains submenus that need to be checked. Press Enter TO CHECK HIDDEN SUBMENU.

- REC Mode appears: Press Enter to enter.
- Find 48kbps with forward. Press Enter to select 48kbps.
- Press forward to show next menu options, see below.
- REC Mode 48kbps
- Mic Sensitivity LOW
- LCF (Low Cut) ON
- Select Input MIC IN
- Continuous Play OFF
- Noise Cut Level Maximum
- LED OFF
- Language English
- Date &Time (See date and time section)
- Time display 24-Hour
- Beep OFF

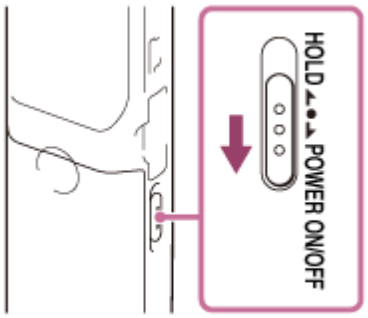
- Auto Power OFF 5min
- Format Leave blank
- [Back] Leave blank
- Press stop button to return to main display.

Preparing the Cough Monitor for a Participant

Insert fresh new 2x AAA battery.



Turn ON the monitor by sliding and holding the "HOLD・POWER ON/OFF" switch in the direction of "POWER ON/OFF" for more than 1 second, see figure below.



Check Time and Date (should already be set). Note date and time will be lost 1 minute after removal of batteries if they are not replaced.

- Press and hold the DISP/MENU button for 1 second to display menu options.
- Press button to go to Detail menu.
- Press Enter button
- Use forward button to move to Date & Time.
- Press Enter button to choose manual.
- Press Enter button to show date and time.

If date/time is incorrect, reset using following instructions:

- Press and hold the DISP/MENU button for 1 second to display menu options.
- Press button to go to Detail menu.
- Press Enter button
- Use forward button to move to Date & Time.
- Press Enter button to choose manual.
- Press Enter button and year that is set will be highlighted.
- To change year press or button until correct year is displayed.
- Press Enter button to confirm and to move on to the next parameter.

- Repeat process using or to change date/time and the Enter button to confirm until date and time set correctly.
- 24H should be selected
- When you set the minute by pressing Enter button, ensure "executing" message appears to confirm Date & Time are set.
- Press stop button to return to main display.

Check microphone wire is plugged into the microphone port (NOT Headphone socket).

Check screen display shows the following:

- Display should show Folder01 - - h- - m - -s at the top of screen.
- Display should show 00/00 in centre of screen.
- Record speed should be SP.
- "LCF" displayed bottom left of screen.
- The battery is full.

If these settings are incorrect, ensure all old recordings have been uploaded and deleted and see initial setup to ensure settings are correct. If you are not sure use a different recorder instead.

Starting a recording

When you need to start a 24-hour recording

- Press record button firmly.
- Check screen display shows REC; the recording clock is counting upwards and folder 01/01 is displayed.
- Slide the hold button on the side of the monitor upwards to LOCK the controls. *Sliding into HOLD function is critical, otherwise the recorder may accidentally switch off and lose data.*
- Test HOLD function by pressing DISP button. HOLD message should appear, then move on to next step. If HOLD is not set, keep pressing DISP button to return to counting timer clock display, then slide HOLD button on the side upwards and repeat HOLD test again.
- Immediately, the Investigator should talk into the microphone (at a distance 30 cm) and say the following clearly and reasonably loudly:

  "Asthma UK Subject number…Date… Start time… Trial…"
- Write in subject number into accompanying paperwork (participant instructions/ diary and VAS).
- Give the participant the following instructions:
  - Always wear the recorder and microphone unless having a shower or going to bed.
  - Keep microphone exposed and attach to similar position if changing clothing.

- When you go to bed, you must remove the microphone and place it on a bedside table close to you so that the microphone wire is not in danger of getting tangled around you. Place the recorder also on the bedside table.
- If you are going to have shower/bath, then remove the microphone and monitor and leave it outside of the bathroom. Re-attach them both once you have finished showering.
- Do not remove the recorder from bag.
- Avoid putting pressure on the bag that may press buttons on the recorder and accidentally terminate recording.
- Do not pull microphone wire as it may become disconnected from recorder inside bag. If microphone wire becomes disconnected, reconnect, record time and duration disconnected in the sleep diary and inform us.
- Fill in the diary to record sleep and awake time and any comments. Returned this with the cough monitor.
- Ensure the VAS form is completed at the end of the 24-hour period.

- Record the cough monitor number along with participant ID and date.

Attach the cough monitor to subject

- Attach the microphone clip to the patients clothing so that the microphone is as close to the larynx as possible.
- Position the bag/holder over the subject's waist and place the monitor inside the bag so that it is protected.
- Use cable tie to secure the two zips on the bag (if available) to prevent subjects from opening the bag.

Return of the monitor from the participant

- Participants should remove the monitor after 24 hours, without stopping the recording.
- They should be instructed to return the monitor as soon after the 24 hours as they can, or on their next visit along the VAS and the diary.
- When the monitor is returned. Stop the recording. DO NOT PLAY BACK OR LISTEN.
- Upload the files via USB to be sent for analysis ASAP.