Predicting Steroid Responsiveness using Exhaled Nitric Oxide

Laura Rawcliffe

A thesis submitted for the degree of Bachelor of Medical Science with Honours at the University of Otago, Dunedin, New Zealand
Abstract

Background:
In susceptible individuals, exercise can be a potent trigger of bronchoconstriction resulting in symptoms which are commonly diagnosed as exercise-induced asthma (EIA). Empiric trials of inhaled corticosteroids (ICS) are often employed as treatment in preventing EIA symptoms, yet there is marked variability in treatment response. This heterogeneity may be explained by the differing pathological mechanisms which predispose to exercise-induced bronchoconstriction (EIB). These include airway inflammation, which itself is heterogeneous. Patients with eosinophilic airway inflammation, compared to non-eosinophilic, demonstrate greater protection against EIB with regular ICS therapy. Additionally, the degree of sputum eosinophilia correlates with the severity of EIB. Exhaled nitric oxide (F_{E}NO) is a non-invasive surrogate biomarker for eosinophilic airway inflammation. Increased levels of F_{E}NO are associated with the presence and severity of EIB, and in patients with non-specific respiratory symptoms can predict steroid responsiveness. Investigating the potential ability of F_{E}NO measurements to identify patients with EIA symptoms likely to have a favourable response to ICS would reduce empiric prescribing, and is therefore clinically important.

Hypothesis:
Patients with EIA symptoms and high F_{E}NO are more likely to respond to ICS treatment, compared to those with low F_{E}NO

Aims:
1. Calculate the predictive utility of F_{E}NO measurements in patients with EIA symptoms and airway hyper-responsiveness (AHR) for response to ICS
2. Compare the effectiveness of ICS in the management of patients with EIA symptoms with low versus high F_{E}NO
3. Confirm that pre-treatment measurement of F_{E}NO is an important way to approach the management of patients with EIA symptoms.
Methods:
Patients with EIA symptoms and AHR to mannitol and/or exercise challenge were enrolled. A randomised, crossover, placebo-controlled trial of budesonide 800µg b.d was undertaken. Each treatment period was one month in duration, with an intervening two week washout. Patients were allocated to a low or high FE<sub>No</sub> group based on their pre-treatment off-steroid measurement, using 45ppb as the cut-point. The following endpoints were measured at baseline and after each treatment arm: FE<sub>No</sub>, spirometry, AHR to mannitol and exercise challenges, Asthma Control Questionnaire score, and Borg Dyspnoea Score.

Results:
Forty five symptomatic patients were screened and seventeen fulfilled the eligibility criteria. FE<sub>No</sub> had a high predictive utility for steroid responsiveness (ROC AUC=0.833). The optimum cut-point for FE<sub>No</sub> to predict steroid responsiveness in this population was 41.0ppb with corresponding sensitivity, specificity, positive and negative predictive values of 78.6%, 66.7%, 91.7% and 40.0% respectively.

Analyses by FE<sub>No</sub> stratification revealed there were no significant improvements in any of the measured endpoints following budesonide in the low FE<sub>No</sub> group, except for FE<sub>No</sub> itself (33.4ppb vs. 17.6ppb; p=0.006). In contrast, patients with a high baseline FE<sub>No</sub>, demonstrated a reduced FE<sub>No</sub> (76.5ppb vs. 36.1ppb; p=0.007) and reduced AHR to mannitol (PD<sub>15</sub> to mannitol increased; 193mg vs. 443mg; p=0.010). Improvements in asthma control score and AHR to exercise challenge approached, but did not reach, significance (p=0.071 and 0.063 respectively).

Conclusions:
Patients with a high pre-treatment FE<sub>No</sub> demonstrated clinical improvements following treatment with budesonide, whereas those with a low FE<sub>No</sub> showed no improvement. Pre-treatment FE<sub>No</sub> measurements may be used to predict whether patients with EIA respiratory symptoms will respond to ICS treatment. These data support the use of this simple test to aid clinical decisions in the management EIA.
Acknowledgements

Firstly, I would like to acknowledge my supervisor, Professor Robin Taylor for his excellent guidance this past year. You have generously given your time to teach me both within the research and clinical settings, sparking a passion for Respiratory Medicine. I am ever grateful for the opportunity to have experienced clinical research and for this I thank you.

The support from Jan Cowan, Senior Technician and Research Manager within the Unit has been extraordinary. Thank you for dedicating your time, knowledge and eye for detail to all aspects of this research. Without you this year surely would not have been possible and I cannot put to words how much I appreciate all you have done.

Thirdly, I thank Dr. Ben Brockway, Respiratory Physician, for his valuable contributions to the clinical aspects of my research. You were always willing to teach and have given me the opportunity to develop my medical knowledge and skills. Thank you for all you have done this year.

Thanks also to Rochelle Palmay, Respiratory Technician, for her assistance through-out the duration of this research. I greatly appreciate all the support you have given me.

Thank you to Dr. Samuel Lucas for his devoted technical assistance with the exercise challenges and for use of his laboratory space, without which this study would not have been possible. Many thanks also to Carissa Murrell and Luke Wilson for your valued involvement with the exercise challenges and your willingness to help.

Thanks to Dr. Kate Jones and Dr. Leon Chang, Respiratory Registrars, for their readiness to provide medical cover during exercise challenges.

Professor Peter Herbison provided valuable statistical advice for the analyses contained in this thesis. I thank you for the all your input and dedicated time, it is very much appreciated.
Thank you to the Faculty of Medicine for their scholarship which has supported me this past year.

Above all I would like to thank the patients who so willingly gave their time and volunteered for this research. Without your generosity there simply would be no study. I am grateful for your perseverance through times of uncomfortable procedures and sincerely thank you.

Finally, to my family and my James, thank you for all your love and support.
# Table of Contents

Abstract ...................................................................................................................................... i
Acknowledgements ................................................................................................................ iii
Table of Contents ..................................................................................................................... v
List of Tables ........................................................................................................................ viii
List of Figures ........................................................................................................................ ix
List of Abbreviations ................................................................................................................ x
Chapter One: Exercise-Induced Asthma .................................................................................. 1
  1.1 Epidemiology .................................................................................................................. 1
  1.2 Disease Burden ................................................................................................................ 2
  1.3 Mechanisms .................................................................................................................... 3
  1.4 Diagnosis ......................................................................................................................... 5
    1.4.1 Symptoms .................................................................................................................. 5
    1.4.2 Objective evidence .................................................................................................... 6
      1.4.2.1 Spirometry .......................................................................................................... 7
      1.4.2.2 Reversibility ....................................................................................................... 7
      1.4.2.3 Peak Expiratory Flow .......................................................................................... 7
      1.4.2.4 Bronchoprovocation tests .................................................................................. 9
        1.4.2.4.1 Exercise Challenge ...................................................................................... 10
        1.4.2.4.2 Eucapnic Voluntary Hyperventilation ......................................................... 11
        1.4.2.4.3 Mannitol Challenge .................................................................................... 12
      1.4.2.5 Approach by Physicians ................................................................................. 12
Chapter Two: Current Therapeutic options for managing Exercise-Induced Asthma .......... 14
  2.1 Beta\textsubscript{2} Agonists ............................................................................................... 14
  2.2 Inhaled Corticosteroids ................................................................................................. 15
  2.3 Oral Leukotriene Antagonists ....................................................................................... 15
  2.4 Mast cell stabilisers ....................................................................................................... 16
  2.5 Empiric trials versus pre-selection ................................................................................ 17
Chapter Three: Exhaled Nitric Oxide ..................................................................................... 29
  3.1 Measurement .................................................................................................................. 29
  3.2 Clinical Applications ..................................................................................................... 29
    3.2.1 FE\textsubscript{NO} and Asthma .................................................................................. 29
3.2.2 $F_E$NO and EIA ................................................................. 30
3.2.3 $F_E$NO and Steroid responsiveness ............................................. 30

Chapter Four: Detailed Methods ............................................................................................ 32

4.1 Laboratory Testing................................................................................................. 32
  4.1.1 Fraction of Exhaled Nitric Oxide ($F_E$NO) .................................................. 32
  4.1.2 Skin Prick Testing ....................................................................................... 32
  4.1.3 Spirometry and Assessment of Reversibility ............................................ 32
  4.1.4 Peak Expiratory Flow Rate ................................................................. 33

4.2 Bronchial Challenges ............................................................................................ 33
  4.2.1 Mannitol Challenge ................................................................................. 34
  4.2.2 Exercise Challenge .................................................................................. 34

4.3 Qualitative methods ............................................................................................ 36
  4.3.1 Borg Scale ............................................................................................... 36
  4.3.2 Visual Analogue Scale ........................................................................... 36
  4.3.3 Asthma Control Questionnaire ............................................................. 36

4.4 Additional methods and considerations ......................................................... 39
  4.4.1 Inhaled Corticosteroid treatment withdrawal .......................................... 39
  4.4.2 “Loss of Control” Criteria for ICS withdrawal ........................................ 42
  4.4.3 Randomisation technique .................................................................... 43
  4.4.4 Blinding of treatments ........................................................................... 43

Chapter Five: Methods .................................................................................................. 44

5.1 Patients ............................................................................................................. 44

5.2 Study Design .................................................................................................... 44
  5.2.1 Phase One: Screening ........................................................................... 45
  5.2.2 Phase Two: Trial of treatment ............................................................. 46

5.3 Statistical Analyses ......................................................................................... 46

Chapter Six: Results ...................................................................................................... 49

6.1 Primary Analyses ............................................................................................. 49
  6.1.1 Baseline Characteristics ........................................................................ 49
  6.1.2 Comparison of results after placebo and budesonide for all patients ....... 49
  6.1.3 Comparison of results after placebo and budesonide by $F_E$NO stratification 54
  6.1.4 Proportion of responders in each $F_E$NO group .................................... 54
  6.1.5 Relationship between baseline $F_E$NO and the change in AHR following treatment with budesonide .................................................. 58
6.1.6 Utility of FeNO for predicting steroid responsiveness.........................61
6.2 Secondary Analyses .................................................................................................70
6.2.1 Relationship between FeNO and AHR in patients presenting with exercise symptoms suggestive of asthma ...........................................................70
Chapter Seven: Discussion ...............................................................................................74
Chapter Eight: Summary and Conclusions ........................................................................80
References ..............................................................................................................................81
List of Tables

Table 2.1: Summary of previous 20 years literature on effects of medication used to treat exercise-induced asthma ................................................................. 18

Table 4.1: Asthma Symptom Score ................................................................................................................. 40

Table 4.2: Loss of Control Criteria ............................................................................................................... 42

Table 6.1: Baseline Characteristics of Eligible vs. Ineligible Populations ....................................... 51

Table 6.2: Baseline Characteristics of low vs. high $F_{E\text{NO}}$ groups ................................................. 52

Table 6.3: Comparison of post-placebo vs. budesonide results in all patients .......................... 53

Table 6.4: Comparison of post-placebo vs. budesonide results in low $F_{E\text{NO}}$ group ........ 55

Table 6.5: Comparison of post-placebo vs. budesonide results in high $F_{E\text{NO}}$ group .......... 56

Table 6.6: Proportion of responders to budesonide in each $F_{E\text{NO}}$ group ................................ 57

Table 6.7: Sensitivities and specificities of different $F_{E\text{NO}}$ cut points for steroid responsiveness .................................................................................................................. 63

Table 6.8: Sensitivities and specificities of different $F_{E\text{NO}}$ cut-points for PD$_{15}$ mannitol increase of ≥1 doubling dose ........................................................................................................ 65

Table 6.9: Sensitivities and specificities of different $F_{E\text{NO}}$ cut-points for Asthma Control Questionnaire reduction ≥0.5 ........................................................................................................ 67

Table 6.10: Sensitivities and specificities of different $F_{E\text{NO}}$ cut-points for complete protection from exercise-induced bronchoconstriction .......................................................... 69

Table 6.11: Sensitivities and specificities of different $F_{E\text{NO}}$ cut points for predicting hyper-responsiveness to mannitol and/or exercise-induced bronchoconstriction ..................................... 73
List of Figures

Figure 1.1: Flow Volume Loop Curve – no functional abnormality (normal) ...................... 8

Figure 1.2: Flow Volume Loop Curve – obstructive defect .................................................. 8

Figure 4.1: The Borg Scale .................................................................................................. 37

Figure 4.2: Asthma Control Questionnaire ......................................................................... 38

Figure 4.3: Example page from patient diary ...................................................................... 41

Figure 5.1: CONSORT diagram of participant flow through study ................................. 48

Figure 6.1: The correlation between the change in \( PD_{15} \) to mannitol with ICS compared to placebo and baseline \( FE_NO \) (n=17) ................................................................. 59

Figure 6.2: The correlation between the change in \( PD_{15} \) to mannitol with ICS compared to placebo and baseline \( FE_NO \) (n=16) .................................................................................................................. 60

Figure 6.3: Receiver Operating Characteristic curve of different \( FE_NO \) cut points for steroid responsiveness .......................................................................................................................... 62

Figure 6.4: Receiver Operating Characteristic curve of different \( FE_NO \) cut-points for \( PD_{15} \) mannitol increase of \( \geq 1 \) doubling dose ............................................................. 64

Figure 6.5: Receiver Operating Characteristic curve of different \( FE_NO \) cut-points for Asthma Control Questionnaire reduction \( \geq 0.5 \) ................................................................................. 66

Figure 6.6: Receiver Operating Characteristic curve of different \( FE_NO \) cut-points for complete protection from exercise-induced bronchoconstriction ......................................................................... 68

Figure 6.7: Baseline \( FE_NO \) and relationship to baseline \( PD_{15} \) to mannitol .................. 71

Figure 6.8: Receiver Operating Characteristic curve of different \( FE_NO \) cut points for predicting airway hyper-responsiveness to mannitol and/or exercise-induced bronchoconstriction ........................................................................ 72
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>Micrograms</td>
</tr>
<tr>
<td>µmol</td>
<td>Micromoles</td>
</tr>
<tr>
<td>ACQ</td>
<td>Asthma Control Questionnaire</td>
</tr>
<tr>
<td>ACQ-5</td>
<td>Shortened Asthma Control Questionnaire with 5 questions</td>
</tr>
<tr>
<td>ACQ-6</td>
<td>Shortened Asthma Control Questionnaire with 6 questions</td>
</tr>
<tr>
<td>AHR</td>
<td>Airway Hyper-Responsiveness</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>b.d</td>
<td>To be taken twice a day</td>
</tr>
<tr>
<td>BDP</td>
<td>Beclomethasone Dipropionate</td>
</tr>
<tr>
<td>BMedSc (Hons)</td>
<td>Bachelor of Medical Science with Honours</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetres</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>conc.</td>
<td>Concentration</td>
</tr>
<tr>
<td>CONSORT</td>
<td>Consolidated Standards of Reporting Trials</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ECRHS</td>
<td>European Community Respiratory Health Survey</td>
</tr>
<tr>
<td>EIA</td>
<td>Exercise-Induced Asthma</td>
</tr>
<tr>
<td>EIB</td>
<td>Exercise-Induced Bronchoconstriction</td>
</tr>
<tr>
<td>EIW</td>
<td>Exercise-Induced Wheeze</td>
</tr>
<tr>
<td>ERS</td>
<td>European Respiratory Society</td>
</tr>
<tr>
<td>EVH</td>
<td>Eucapnic Voluntary Hyperventilation</td>
</tr>
<tr>
<td>FEF₂₅-₇₅</td>
<td>Forced Expiratory Flow between 25 to 75% of Forced Vital Capacity</td>
</tr>
<tr>
<td>FENO</td>
<td>Fraction of Exhaled Nitric Oxide</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced Expiratory Volume in one second</td>
</tr>
<tr>
<td>FP</td>
<td>Fluticasone Propionate</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced Vital Capacity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled Corticosteroid</td>
</tr>
<tr>
<td>IOC-MC</td>
<td>International Olympic Committee- Medical Commission</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile Range</td>
</tr>
<tr>
<td>ISAAC</td>
<td>International Study of Asthma and Allergies in Childhood</td>
</tr>
<tr>
<td>LOC</td>
<td>Loss of Control</td>
</tr>
<tr>
<td>Mane</td>
<td>To be taken in the morning</td>
</tr>
<tr>
<td>MDI</td>
<td>Metered Dose Inhaler</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams</td>
</tr>
<tr>
<td>mins</td>
<td>Minutes</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitres</td>
</tr>
<tr>
<td>mmHg</td>
<td>Millimetres of mercury</td>
</tr>
<tr>
<td>MVV</td>
<td>Maximum Voluntary Ventilation</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predicted Value</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PC₂₀</td>
<td>Provocative concentration causing a 20% reduction in FEV₁</td>
</tr>
<tr>
<td>PD₁₅</td>
<td>Provocative dose causing a 15% reduction in FEV₁</td>
</tr>
<tr>
<td>PD₂₀</td>
<td>Provocative dose causing a 20% reduction in FEV₁</td>
</tr>
<tr>
<td>PEFR</td>
<td>Peak Expiratory Flow Rate</td>
</tr>
<tr>
<td>PETCO₂</td>
<td>Partial Pressure of End Tidal Carbon Dioxide</td>
</tr>
<tr>
<td>PETO₂</td>
<td>Partial Pressure of End Tidal Oxygen</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts Per Billion</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predicted Value</td>
</tr>
<tr>
<td>PV₂₀</td>
<td>Provocative volume causing a 20% reduction in FEV₁</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operating Characteristic</td>
</tr>
<tr>
<td>SCOTT</td>
<td>Standing Committee on Therapeutic Trials</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SE%</td>
<td>Sputum Eosinophil Percentage</td>
</tr>
<tr>
<td>sec</td>
<td>Second</td>
</tr>
<tr>
<td>SPT</td>
<td>Skin Prick Test</td>
</tr>
<tr>
<td>yrs</td>
<td>Years</td>
</tr>
</tbody>
</table>
Chapter One: Exercise-Induced Asthma

Asthma is a respiratory disorder that is characterised by airway inflammation, airways hyper-responsiveness, reversible airway obstruction and airway remodelling (1). Many different stimuli, such as allergens, cold air, exercise and viral infections, can trigger acute bronchoconstriction. These stimuli act on the airways, through many mechanisms, consequently leading to increased resistance with resultant symptoms. This thesis explores exercise-induced asthma (EIA), the airways disease defined by the presence of bronchial hyper-responsiveness with or without the presence of inflammation, and airflow obstruction induced by exercise.

1.1 Epidemiology

The global prevalence of asthma and asthma symptoms has a highly variable geographic distribution. The European Community Respiratory Health Survey (ECRHS) collected information on the prevalence of respiratory symptoms, self reported asthma attacks, and use of asthma medication from 48 centres located in 22 countries (2). Participating centres were mostly in Western Europe but also included Australasia, the British Isles and the USA. During 1994, the reported prevalence of wheeze was high in Dublin, Ireland (32.0%), Caerphilly, United Kingdom (29.8%), Huelva, Spain (29.2%), Melbourne, Australia (28.8%), Dundee, United Kingdom (28.4%), and Wellington, New Zealand (27.3%). The ECRHS also undertook airway challenge testing for hyper-responsiveness in 35 centres, 16 countries, via a standardised methacholine test (3). Data on the degree of airways hyper-responsiveness was collected from 13,161 men and women, defined by the provocative dose of methacholine causing a 20% reduction in Forced Expiratory Volume in one second (FEV$_1$). The results regarding airway responsiveness reflected the prevalence data on self reported respiratory symptoms in the British Isles and Australasia.

Symptoms of asthma can often be present in childhood, with some countries having a particularly high prevalence. During 2000-2003, the International Study of Asthma and Allergies in Childhood (ISAAC) undertook a survey of 798,685 children aged 13-14 years from 233 centres in 94 countries to measure the global prevalence and severity of asthma
symptoms in children (4). The prevalence of current wheeze, defined as wheezing in the previous 12 months, varied greatly between centres. Current wheeze in 13-14 year olds was high in Wellington, New Zealand (32.6%) and significantly lower in Tibet, China (0.8%).

Additionally, exercise-induced symptoms suggestive of asthma are common. A large cross-sectional study of 8,571 adolescents in Sweden reported 13% had experienced exercise-induced wheeze (EIW) within the past 12 months (5). Furthermore, the prevalence of exercise-induced bronchoconstriction (EIB) may be as much as five times greater in elite, olympic athletes compared to the general population (6-7). It has been proposed that the increased prevalence of EIB found in elite endurance athletes may be related to high sustained ventilation rates causing airway injury (8). Additionally, this effect may be amplified when the athlete is training and competing in environmental conditions that place further stress on the airways, such as with swimmers exposed to chlorine in swimming pools and winter athletes exposed to cold air (9).

In summary, there are substantial variations in the prevalence of asthma and more particularly, EIA symptoms. Respiratory symptoms are common in most western countries and reported by both adults and children. Further to this, elite athletes may be disproportionately affected due to increased exposure to airway stressors during training and competition.

1.2 Disease Burden

The burden of disease associated with any chronic condition may have both social and economic impacts.

“Quality of life is defined as individuals' perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns”

World Health Organisation (10)
The socio-economic burden of asthma was explored in the large European Community Respiratory Health Survey (11). The disease burden, defined in that study as frequency of symptoms affecting daily activities and the need for hospital services, was directly related to a reduction in quality of life. Uncontrolled asthma and thus high disease burden correlated with an increased frequency of doctor visits and a higher number of spirometry tests as compared to asthmatics with few respiratory symptoms. Asthmatics reported economic and other losses through work days lost (16.4%), reduced leisure time (13.2%), emergency department visits (7.1%) and hospitalisations (2.0%) due to breathing problems in the past twelve months. This clearly illustrates the importance of achieving and maintaining good asthma control for asthmatic patients.

In paediatric populations the consequence of having exercise-induced asthma can further impair the quality of daily life. A health survey of children in Japan revealed that in addition to the reduced quality of life reported by asthmatic children compared to non-asthmatic children, asthma symptoms that were exercise-induced resulted in further reductions in life quality (12).

Taken together, these findings emphasise not only the health-related impacts of asthma on an individual’s daily activities and quality of life, but also the health resources used to monitor (spirometry) and treat (emergency department visits and tertiary care hospitalisation) this common disease.

1.3 Mechanisms

Understanding of the underlying pathophysiological mechanisms of EIA has been progressively increasing since the first hypothesis was proposed in 1979: “the airway cooling theory”. This explanation for the observed bronchoconstriction occurring after exercise was proposed because EIB is associated with heat loss from the airways (13). It was concluded that respiratory heat exchange was the primary stimulus for EIB with the magnitude of airflow limitation being directly proportional to the thermal load on the airways.

As further research emerged “the thermal hypothesis” was proposed in 1986 (14). This hypothesis incorporated not only cooling of the airways during periods of hyperpnoea but
also rapid rewarming that follows in the post-exertional period as mechanisms for EIB. It was proposed that during exercise, prolonged hyperpnoea resulted in airway cooling which in turn stimulates bronchial vasoconstriction and reactive hyperaemia. This consequently would lead to vascular leakage and subsequent airway oedema (14-15). However, this theory did not account for involvement of the bronchial smooth muscle or inflammatory mediators, leaving the explanation somewhat incomplete. Further to this, evidence for “the osmotic hypothesis” began to emerge following observations that bronchoconstriction occurred whilst patients inhaled hot dry air during exercise (16). This hypothesis proposed that airway cooling was not essential in the pathogenesis of EIB, but that water loss was the primary stimulus.

“The osmotic hypothesis” first emerged in 1977 when it was concluded that “the water content of inspired air is an important variable in the development of exercise-induced asthma” (17). In 1984, Hahn et al. examined the effect of varying the temperature of inspired air during exercise whilst maintaining a constant humidity (18). It was shown that the severity of EIB did not vary with changing of the inspired air temperature when the water content was kept the same. These findings were supported by the findings of Ingenito et al. in 1988 when a series of cold gas inhalation challenges was conducted and examination of the temperature-gradient and evaporative energy losses undertaken (19). Hyperventilation challenges using gases with the same water carrying capacity but significantly different volume heat capacities were used. They concluded that respiratory heat loss was not the primary stimulus but that osmotic effects in the airways were likely to be of greater importance in the pathogenesis of EIB.

The underlying pathophysiology associated with “the osmotic hypothesis” was evident once it was possible to calculate the volume of airway surface liquid and the evaporative loss required to condition the inhaled air (20). The calculated volume of surface liquid was found to be less than 1mL in the first ten generations of airways and thus it was likely that conditioning the inspired air could subsequently lead to increased osmolarity of the surface liquid (21). Evidence of the mucosal surface becoming dehydrated and unable to maintain an iso-osmolar fluid under conditions of high ventilation with dry air was established by Tabka et al. (22). It was proposed that in susceptible individuals, as the airways become hyperosmolar, fluid would move from the surrounding mucosal epithelial cells to restore the fluid balance. This would transiently lead to cell shrinkage. As the cell volume is
restored there is release of mediators such as histamine, prostaglandins and leukotrienes. These mediators can act on the bronchial and vascular smooth muscle to cause constriction of the airways and increased vascular permeability.

Therefore, in combination, these theories indicate mechanisms by which in susceptible individuals, conditioning inspired air during exercise can result in hyper-osmolar mucosal surface fluid, triggering bronchoconstriction through mediator release. This primary stimulus is further amplified by heat loss whereby increased vascular permeability consequently causes airway oedema and thus increased obstruction.

1.4 Diagnosis

As previously stated, asthma can affect both young and adult populations. Respiratory symptoms, suggestive of asthma, that present in early childhood can either persist into adult life or spontaneously resolve. Late onset asthma is also increasingly recognised and can become evident in the later years of life. The variable and episodic nature of this disease can make diagnosis somewhat problematic. EIA can be additionally difficult to diagnose, especially in the primary care setting, as respiratory symptoms associated with exercise are non-specific.

1.4.1 Symptoms

The diagnosis of asthma in a child is often based on the parent or guardian’s report of symptoms to the physician. A typical pattern of respiratory symptoms is often diagnostic. The diagnosis of asthma must be considered when more than one of the following symptoms are present: cough, wheeze, difficulty breathing and chest tightness (1). It is known that increased frequency and severity of wheezy episodes in children are positively associated with an increased risk for developing chronic persistent asthma (23-26). Viral upper respiratory tract infections in childhood commonly present with cough and wheeze (27-28). Viral infection in atopic infants increase the odds of subsequent asthma development (29).

It is sometimes possible to associate the timing of childhood wheezy episodes with stimuli such as exercise, changes in temperature and allergen exposure if the child is sensitised.
These features support a diagnosis of asthma (1). Similarly, adults presenting with cough, wheeze, difficulty breathing and/or chest tightness should also have asthma considered as a potential diagnosis. Late-onset asthma can emerge as a new health issue at any time from age twenty.

When symptoms suggestive of asthma are solely associated with exercise, history alone is not sufficiently reliable to confirm true EIB. Rundell et al. demonstrated the inability of self reported symptoms in elite athletes to correlate with the diagnosis of EIA (30). In a further study, exercise challenges were undertaken to establish the presence of EIA. In this elite athlete population, although 91% of those with objective EIA reported at least one symptom of EIA, 48% of those without objective EIA reported symptoms (31).

The difficulties in correctly diagnosing EIA also exist for paediatric populations. Among fifty two children in Vancouver diagnosed with EIA on the basis of respiratory symptoms alone, there were marked inconsistencies. Only eight (15.4%) met objective diagnostic criteria for EIA when exercise testing was undertaken. Whereas fourteen (26.9%) had vocal cord dysfunction/ sigh dyspnoea, twelve (23.1%) were unfit, seven (13.5%) had a habitual cough and the remaining eleven (21.1%) had no abnormalities on clinical or laboratory testing (32). Limited access to, and utilisation of testing facilities, can therefore affect the accuracy of diagnosis, as self reported symptoms alone are unreliable markers of disease presence. Therefore objective evidence for and against the presence of EIA is essential if an accurate diagnosis and management plan are going to be achieved.

1.4.2 Objective evidence

There is variable access to tests used in the diagnostic assessment and management of EIA. Some larger specialised centres have these tests available on site, whereas others require referral through to a secondary care service. Additionally some tests are currently still only available in the research setting. Understanding the diagnostic utility and limitations of the currently available tests for diagnosing and managing patients suspected of EIA is important to ensure best practices.
1.4.2.1 Spirometry

Current guidelines advocate the use of pulmonary function testing, including spirometry, in the clinical work-up of a patient with suspected asthma (1). In practice however, there is under utilisation and some uncertainty in the interpretation of spirometric results by family physicians and paediatricians (33). Lack of access to a spirometer and inexperience in conducting test training are recognised barriers by primary care physicians in the assessment of asthmatic patients (34).

Although spirometry is the gold standard for detecting airflow obstruction it is important to note that the test is relatively insensitive. Normal spirometry, recorded when the patient is asymptomatic, does not exclude the presence of asthma (1). This is especially important to consider in patients with EIB who, without the triggering stimulus of exercise, may have normal or supra-normal resting airways function.

1.4.2.2 Reversibility

The presence of airflow obstruction that is reversible can be determined by repeating spirometry after the administration of a bronchodilator. In accordance with the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines, an FEV₁ increase of 200mL and 12%, fifteen minutes after administration of a bronchodilator, usually 400µg salbutamol via a spacer, is required to define the presence of reversibility (35-36). This can be helpful in the diagnosis of asthma, but is more likely to be demonstrable when obstruction is present on spirometry. The test is therefore not sensitive in patients with exclusively EIB.

1.4.2.3 Peak Expiratory Flow

Peak expiratory flow is the “maximum flow achieved during an expiration delivered with maximal force starting from the level of maximal lung inflation” (37). Portable hand held peak flow meters are relatively inexpensive and allow recordings of peak flow to be undertaken easily at home or in the field. It is recommended that peak flows should be recorded as the best of three manoeuvres, with the two highest being within 40L/min of each other (37). Peak expiratory flow is best used to assess airflow variability over a period
Figure 1.1: Flow Volume Loop Curve – no functional abnormality (normal)

Figure 1.2: Flow Volume Loop Curve – obstructive defect
of at least two weeks and increased frequency of recordings will result in a more accurate estimate (38). Regular self peak expiratory flow monitoring by asthmatic patients is frequently used to guide treatment decisions and develop objective based action plans. It has been shown that peak flow based action plans are effective and protect asthmatic patients from severe exacerbations (39). Understanding the role of peak flow measurement for EIA is important. Peak flow variation is correlated, although weakly, to bronchial hyper-responsiveness and therefore may provide some value in assessing persons suspected of EIA (40). However in a group of asthmatic patients whose peak flows had been maintained between 80-100% personal best for at least three months, 20.5% were still positive for EIB (41).

Assessment of EIB presence can be undertaken using pre- and post-exercise PEFR. However, the sensitivity and specificity of change in PEFR >15%, to detect individuals with positive exercise challenges (change in FEV$_1$ >15%), in a study by Giannini et al. was 0.18 and 0.95 respectively (42). In this setting, exercise PEFR is poorly sensitive. Further, the diagnostic utility of PEFR measurements for screening large groups of school children for EIB has been shown to be poor due to the large number of false positives obtained (43). Therefore, the role of peak expiratory flow recordings for diagnosing EIA are somewhat limited and are best used to assess and monitor asthma control.

### 1.4.2.4 Bronchoprovocation tests

Provocation tests have the capacity to provide strong evidence for the diagnosis of EIA by demonstrating the presence of bronchial hyper-responsiveness. There are two classes of challenges used to quantify airway responsiveness; direct and indirect. Direct challenges involve agents, such as histamine and methacholine, that provoke bronchoconstriction by directly stimulating the contraction of smooth muscle cells (44). Indirect challenges such as exercise, eucapnic voluntary hyperventilation (EVH), hypertonic saline and mannitol, induce bronchoconstriction through pathways other than directly stimulating muscle cells. These indirect challenges exert their effects through inflammatory cells, epithelial cells and bronchial nerves which, when stimulated, subsequently release mediators or neurotransmitters that result in bronchial myocyte contraction (45). It has been proposed that indirect challenges may reflect the degree of bronchial hyper-responsiveness due to airway inflammation in EIA more accurately (45).
1.4.2.4.1  Exercise Challenge

Standardised protocols for exercise challenge testing advocate a $\geq 10\%$ reduction in $\text{FEV}_1$ post exercise as diagnostic of EIB (46). This is based on exceeding two standard deviations from the mean baseline $\text{FEV}_1$ seen in a healthy population, as recommended by the ATS (47) and ERS (8). Protocols advocate the use of either a treadmill or stationary cycle ergometer as the principal exercise apparatus. Environmental conditions such as temperature and humidity have been well documented to influence the severity of EIB and therefore should be controlled during challenge tests. The sensitivity and specificity of an indoor cycle ergometer exercise challenge under ambient conditions was evaluated by Eliasson et al. (48). The change in $\text{FEV}_1$ that permitted clear distinction between the EIB positive group and healthy controls, test specificity 100%, was an $\text{FEV}_1$ fall of 9%. However the sensitivity was a low 10%. Therefore, in this setting, the ability of the exercise test to detect EIB was poor and was not recommended as a screening challenge to evaluate exercise-induced symptoms suggestive of asthma.

When cold dry air was used during exercise tests in children, the sensitivity of the test to distinguish EIB due to asthma from other chronic lung diseases increased, without a decrease in specificity, in comparison to exercise tests with room air (49). Under these conditions the maximum sensitivity and specificity of a cold, dry air exercise test was 72% and 72% respectively. The cut point for the required fall in $\text{FEV}_1$ was 10.2%. This protocol allows for better detection and investigation of EIB.

The assessment of elite athletes using exercise challenges may be inadequate to effectively detect EIB. Dickinson et al. compared the diagnostic utility of three bronchial challenges to screen elite winter athletes for EIA (50). Laboratory based exercise challenges with room air, sport-specific field-based exercise tests, and EVH were compared. Of the fourteen elite athletes tested, none had demonstrable EIB with the laboratory based exercise challenge. In comparison, when sport-specific exercise challenges were conducted, 21% had a positive response and furthermore, EVH identified 71% with bronchial hyper-responsiveness. This is in keeping with other evidence in the literature suggesting, especially for elite athletes, that EVH is a more sensitive and appropriate test in the diagnosis of EIA.
1.4.2.4.2 Eucapnic Voluntary Hyperventilation

This provocation challenge was first described in 1984 (51) and developed as a surrogate for exercise testing in the assessment of EIB (52). The recommended protocol involves the patient seated, breathing dry air which contains increased carbon dioxide (~5% CO₂, 21% oxygen, balance nitrogen). Voluntary hyperpnoea is maintained for six minutes at a target ventilation rate of 30 x FEV₁. When FEV₁ is greater than 1.5L and carbon dioxide in the inspired air is between 4.9-5.0%, the end-tidal carbon dioxide is usually maintained at eucapnic levels, 38-42mmHg, for ventilation ranges between 40-105L/min (52). Additionally, when the patient has reduced lung function or is known to have moderate or severe asthma, an incremental progressive protocol described by Brannan et al. can be employed (53).

This bronchial challenge is modelled to provide high ventilation rates, as experienced during exercise, requiring conditioning and humidification by the airway mucosa and increased osmolarity of the airway surface liquid. As previously described, it is the change in osmolarity and consequent mucosal cell shrinkage/re-expansion that stimulates mediator release and ultimately bronchoconstriction (54). In contrast to exercise challenges, EVH is a more sensitive test. Hurwitz et al. have reported modest sensitivity (63.3%) and high specificity (90%) for detecting EIB among 120 subjects (55). The diagnostic thresholds for the fall in FEV₁ was 10% change or greater after EVH.

Assessing EIB in athletes using standard exercise tests can be difficult (54). It may be that these standard protocols do not adequately mimic the intensity athletes experience during training and competition. Due to the techniques involved in EVH, whereby the subject increases their ventilation voluntarily without the need for a provoking stimulus like exercise, this challenge has proven highly appropriate in elite athlete assessment. The sensitivity is high. When challenges were undertaken by elite swimmers, Castricum et al. concluded that “the EVH challenge is a highly sensitive challenge for identifying EIB in elite swimmers, in contrast to the laboratory and field based exercise challenge tests, which significantly under diagnose the condition” (56).

Furthermore, elite athletes are required to provide documentation of either asthma, EIB or airways hyper-responsiveness (AHR) in order to use beta₂ agonists at top level competition.
such as the Olympic Games (57). The International Olympic Committee Medical Commission (IOC-MC) accepts the results of an EVH challenge as evidence for EIB.

1.4.2.4.3 Mannitol Challenge

Bronchoprovocative challenges that alter the osmolarity of the airway surface liquid, such as exercise and EVH, had previously been shown to be appropriate in the assessment of EIB. Based on this premise, a bronchial challenge was developed in 1997, using inhalation of a dry powder of mannitol (58). This simple challenge involves the inhalation of progressively increasing doses of encapsulated mannitol with subsequent spirometric manoeuvres 1 minute after each dose. The challenge is stopped when either a 15% reduction in FEV\textsubscript{1} from baseline is observed or until a total of 635mg of mannitol has been administered.

When directly compared to EVH in the assessment of EIB in a population of elite athletes, mannitol had a sensitivity of 96% and a specificity of 92% for identifying those with a positive EVH test (59). Results from mannitol challenges are now regarded as acceptable evidence of AHR, and are used to justify beta\textsubscript{2} agonist use by the IOC-MC.

1.4.2.5 Approach by Physicians

A recent study in the United Kingdom, which surveyed how exercise symptoms suggestive of asthma in athletes are managed by primary care physicians, showed that 71% of physicians would employ the use of objective measures in their initial work-up of patients with possible EIB (60). However, the tests they most commonly selected were exercise peak expiratory flow rate (44%) and spirometry with bronchodilator (35%). These have previously been shown to have poor diagnostic utility.

A similar survey was conducted in the United States. This explored how family care physicians and respiratory specialists approached the diagnosis and treatment of EIA (61). In this study, primary care physicians were less likely (18.3%) to request bronchoprovocation testing in a newly presenting patient with suspected EIB compared to specialists (50.9%). After adjustment for the number of patients and children with asthma seen per month, specialists had higher odds of ordering bronchial challenges than primary
care physicians (OR=6.80). The reasons for these observed differences in physician approaches are unclear, but may be due to differences in access to and familiarity with the different objective tests available.
Chapter Two: Current Therapeutic options for managing Exercise-Induced Asthma

Once a diagnosis of EIA has been confirmed, the physician will then need to evaluate the need for treatment and the type of medications to prescribe. Determining which therapeutic agent to use is not always a clear cut decision and often empiric trials are undertaken.

2.1 Beta₂ Agonists

Inhaled beta₂ agonists, such as salbutamol and salmeterol, act specifically on the beta₂ adrenoceptors in bronchial smooth muscle. The differing chemical structure of these two molecules account for their differing pharmacokinetics (62). Short-acting beta₂ agonists, such as salbutamol, are predominately hydrophilic molecules and stimulate the beta₂ adrenoceptor directly from the extracellular compartment. This leads to a rapid onset of bronchodilation through smooth muscle relaxation. Salbutamol remains at the active site for only a relatively short period of time. Clinically therefore, this medication is used for the short term, immediate relief of asthma symptoms. Short-acting beta₂ agonists also act as functional antagonists, preventing mast cell degranulation and hence smooth muscle contraction. When administered 15 minutes before exercise, salbutamol provides protection from EIB for up to one hour (63-64).

Long-acting beta₂ agonists, such as salmeterol, are lipophilic molecules and exert their action more slowly. When they approach the target cell they are taken up into the cell membrane and activate the beta₂ adrenoceptor in a steady sustained manner (65). The pharmacokinetic properties of long-acting beta₂ agonists result in a slower onset and longer duration of action. Salmeterol is not immediately effective against EIB, but regular treatment affords protection from EIB for up to 9 hours (63-64, 66) and bronchodilatation for up to 12 hours (63).

There are problems related to the use of both short- and long-acting beta₂ agonists for prevention and symptomatic relief of asthma symptoms. All beta₂ agonists are associated with tachyphylaxis (66-69) resulting in impaired response to rescue bronchodilator (67) observed with regular dosing. For these reasons short-acting beta₂ agonists, such as
salbutamol, are prescribed for as required use only. Long-acting beta\textsubscript{2} agonists should not be prescribed as a sole therapy but rather used as an adjunct with inhaled corticosteroids (ICS) in difficult to control asthma.

### 2.2 Inhaled Corticosteroids

Inhaled corticosteroids reduce bronchial inflammation (70), EIB (70-74) and AHR (71, 74) when taken regularly and are frequently prescribed for the management of EIA if the symptoms are frequent and thought to be associated with airway inflammation. However the magnitude of therapeutic response is varied. Waalkens \textit{et al.} treated children with EIA with long-term ICS and this resulted in a 33\% reduction in the prevalence of EIA and a 50\% reduction in severity (75). Clearly this indicates that only a proportion of patients with EIA are responsive to ICS treatment.

Differing pathophysiology within the airways of patients with EIA may explain the observed differences in clinical benefits associated with ICS. Classification of asthma using induced sputum to identify inflammatory cells has allowed for classification of subtypes. There are two major phenotypes: eosinophilic asthma and non-eosinophilic asthma. Eosinophilic asthma is defined as greater than 1.9\% of eosinophils in a representative sputum sample, from steroid naïve airways (76). Non-eosinophilic asthma is a combination of various other identified cellular subtypes including neutrophilic and paucigranulocytic inflammation. The presence of sputum eosinophilia has been shown to predict protection from EIB by high dose ICS (70). Additionally non-eosinophilic asthmatics have a poor response to ICS therapy, with no change in AHR or improvement in quality of life, compared to those with eosinophilic airway inflammation who demonstrate marked improvements with treatment (77). Therefore, the observed inter-person variability to ICS may be due to heterogeneity in inflammatory subtypes, including exercise-induced asthmatic populations and should be an important consideration when selecting treatment and assessing treatment responses.

### 2.3 Oral Leukotriene Antagonists

Cellular constituents present in the airway, such as eosinophils (78), neutrophils (79), mast cells (80), and macrophages (81), are associated with pro-inflammatory cysteiny
leukotrienes synthesised from arachidonic acid through the 5-lipoxygenase pathway. Cysteinyl leukotrienes are potent bronchoconstrictors and are 100-1,000 fold more potent than histamine (82). They further contribute to the pathogenesis of EIA by increasing bronchial blood flow and epithelial permeability. This results in airway oedema restricting airflow and hence gas exchange. Leukotrienes also impede bronchial airflow by increasing mucus secretion and inhibiting muco-ciliary clearance (83). A comparison of urinary leukotriene concentrations between children with EIA and healthy controls revealed similar baseline levels but significantly increased post-exercise concentrations in those who demonstrated EIB (84).

Leukotriene receptor antagonists can provide significant protection against EIB (85-87). The role of leukotriene receptor antagonists, such as montelukast, for EIA is significant. There has been no reported tachyphylaxis associated with long term use or rebound worsening of symptoms following withdrawal (88). The addition of montelukast to ICS as a controller therapy is more effective in protecting against EIB compared to the addition of the long-acting beta₂ agonist salmeterol (87, 89-91).

### 2.4 Mast cell stabilisers

Mast cells located in the airway release mediators such as histamine (92), prostaglandin D₂ (93), and cysteinyl leukotrienes (80). During hyperosmolar provocation, such as may occur in the airways during exercise, mast cells become activated and release these mediators (94-95). Subsequently in subjects with hyper-responsive airways, EIB ensues (94, 96).

In twenty four subjects with demonstrable AHR to mannitol, nedocromil sodium greatly attenuated AHR, and in twelve (50%), provided complete protection (97). A comparison of montelukast, formoterol, and cromoglycate following two weeks treatment showed a significant reduction in AHR to mannitol, 47%, 61% and 67% respectively, and protection from EIB in 50%, 83% and 67% (98). When cromoglycate is used in combination with other anti-asthma medications there is additional protective effect from EIB (99-100).
2.5 Empiric trials versus pre-selection

Often empiric trials of the different treatments are employed with little prior evidence as to which will be most beneficial. Currently, inhaled beta$_2$ agonists are the first line treatment for intermittent EIB, and ICS are introduced into treatment regimens if symptoms become persistent or are deemed inadequately controlled (1). However, as stated, treatment responses to ICS are heterogenous and thus may not prove the most appropriate treatment for all patients with EIA symptoms. A clinical tool with the ability to stratify patients into those likely to benefit from ICS therapy from those unlikely to show clinical or physiological improvements, could potentially eliminate the need for time-consuming and costly empiric trials and allow effective treatment initiation.
Table 2.1: Summary of previous 20 years literature on effects of medication used to treat exercise-induced asthma

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Design</th>
<th>Treatment</th>
<th>Method</th>
<th>Results</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beta₂ Agonists</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richter 2002 (101)</td>
<td>25 asthmatics with EIB</td>
<td>Randomised, double-blinded, double dummy, placebo controlled, 4 period crossover trial</td>
<td>Turbuhaler: 1. Formoterol 12µg 2. Terbutaline 500µg Diskus: 3. Salmeterol 50µg</td>
<td>Single dose then ex tests on 12 separate days at 5, 30 or 60min after inhalation</td>
<td>EIB did not differ between active treatments at 5, 30 or 60min after inhalation and the onset of bronchodilation was slowest with salmeterol</td>
<td>Protective potency between these long and short acting beta₂ agonists similar</td>
</tr>
<tr>
<td>Ferrari 2000 (102)</td>
<td>14 athletes with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>Formoterol 12µg</td>
<td>Ex tests 15min and 4hrs after treatment</td>
<td>Formoterol increased pre-test FEV₁ and reduced EIB at 15min and 4hr tests</td>
<td>Short term administration provides rapid protective effect against EIB</td>
</tr>
<tr>
<td>Simons 1997 (66)</td>
<td>16 asthmatics, 12-16yrs old with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>Addition of salmeterol (50µg 1x daily) to regular concurrent ICS</td>
<td>Once daily addition of salmeterol to regular beclomethasone for 28 days with ex test 1 &amp; 9hrs after salmeterol on day 1 &amp; day 28</td>
<td>Salmeterol provided protection on day 1 from EIB at both 1 &amp; 9hrs after dosing but on day 28 protection was only sustained at the test 1hr after dosing</td>
<td>Long-term administration of salmeterol showed reduced duration of protection despite being used in combination with ICS &amp; low frequency</td>
</tr>
<tr>
<td>de Benedictis 1996 (63)</td>
<td>12 asthmatic children, 7-14yrs old with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>1. Salmeterol 50µg 2. Salmeterol 25µg</td>
<td>Each treatment arm with ex test 1 &amp; 12hrs after administration &amp; 2-10 day washout between treatments</td>
<td>Both doses provided significant bronchodilation for up to 12hrs &amp; protection from EIB at 1hr</td>
<td>25µg may be a suitable dose for children in the acute protection from EIB compared to 50µg</td>
</tr>
</tbody>
</table>

EIB: Exercise-Induced Bronchoconstriction
<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Design</th>
<th>Treatment</th>
<th>Method</th>
<th>Results</th>
<th>Conclusions</th>
</tr>
</thead>
</table>
| Newnham 1993 (64) | 12 asthmatics with EIB | Randomised, double-blinded, placebo controlled, crossover trial | 1. Salmeterol 50µg  
2. Salbutamol 200µg | Single dose then ex tests at 1, 6 & 12hrs after inhalation | At hr post dose there was significant protection from EIB with both active treatments & only salmeterol continued to protect at the 6 & 12hr tests | Salmeterol offers up to 12hrs protection from EIB when administered as single dose compared to salbutamol which protects for up to 1hr |
| Henriksen 1992 (103) | 12 boys, 8-15yrs old with perennial asthma & EIB | Randomised, double-blinded, placebo controlled, crossover trial | 1. Formoterol 12µg  
2. Salbutamol 200µg | Single dose then ex test 30mins & 3hrs after. If protection still seen further test done at 5.5hrs and 8hrs | Salbutamol offered significant protection 30mins after inhalation but not thereafter. Formoterol demonstrated a significant effect in protecting from EIB in most children for up to 8hrs | Formoterol was more effective in protecting against EIB and had a longer duration of action |
| Anderson 1991 (104) | 18 asthmatics with EIB | Randomised, double-blinded, placebo controlled, crossover trial | 1. Salmeterol 50µg  
2. Salbutamol 200µg | Day 1- familiarisation ex test. Day 2/3- single dose salmeterol or salbutamol (double blind), 30min rest then 4 ex tests 2hrs apart. Day 4- single placebo dose (single blind), 30min rest, then 2 ex tests 2hrs apart | Similar protection from EIB at 0.5hr after inhalation. Salmeterol provided greater protection at 2.5, 4.5 & 6.5hrs after administration compared to salbutamol. Similarly salmeterol also had a more prolonged period of bronchodilation than salbutamol | The extent of bronchodilation & protection from EIB afforded by salbutamol & salmeterol is similar, however salmeterol has a longer duration of action |
<p>| Beta Agonists-tachyphylaxis | Hancox 2002 (67) | Randomised, double-blinded, placebo controlled, crossover trial | Salbutamol 100µg 2 puffs, 4x daily (daily dose= 800µg) | 6-10 days of treatment then withheld for 8hrs before subsequent ex test | Greater post-exercise FEV₁ fall in the pre-treated salbutamol group and FEV₁ remained lower in this group after rescue salbutamol administered | Regular short acting β₂ agonist treatment leads to increased EIB and suboptimal bronchodilator response to β₂ agonist |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Design</th>
<th>Treatment</th>
<th>Method</th>
<th>Results</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcia 2001</td>
<td>19 patients with EIB</td>
<td>Randomised, double-blinded, placebo controlled, parallel group trial</td>
<td>Formoterol</td>
<td>Treatment for 28 days with 2 ex tests on days 1, 14 &amp; 28 separated by 3hrs</td>
<td>Tachyphylaxis developed at day 14 to the protective effect of formoterol but did not progress further by day 28</td>
<td>Formoterol should be recommended only as PRN in EIB due to tachyphylaxis development</td>
</tr>
<tr>
<td>Nelson 1998</td>
<td>20 patients with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>Salmeterol</td>
<td>1 month each treatment arm with 1 week washout. Ex tests on day 1, 14 &amp; 29, 30mins and 9hrs after morning dose of each arm</td>
<td>Salmeterol attenuated EIB at all times but on day 14 and 29 there was a greater decrease in evening FEV\textsubscript{1} compared to morning FEV\textsubscript{1}</td>
<td>Protection of EIB is maintained with long-term administration of salmeterol but its duration of action decreases</td>
</tr>
<tr>
<td>Kippelen 2010</td>
<td>7 athletes with EIB &amp; 8 untrained subjects with mild asthma</td>
<td>Randomised, single-blinded, placebo controlled trial</td>
<td>Beclomethasone dipropionate (BDP) MDI</td>
<td>1. Placebo 10min before EVH 2. BDP 1500µg 4hrs before EVH</td>
<td>BDP inhibited the EVH-induced bronchoconstriction in both population groups</td>
<td>Single high dose of BDP has an acute protective effect via blunting mast cell activation</td>
</tr>
<tr>
<td>Duong 2008</td>
<td>26 steroid naïve asthmatics with EIB</td>
<td>Randomised double-blinded, 2 parallel arms, double dummy, crossover trial</td>
<td>Ciclesonide MDI 1. 40 and 160µg 2. 80 and 320µg</td>
<td>Treatment with ICS for 3/52 with ex test &amp; sputum induction at weekly intervals, 3-8/52 washout then 2\textsuperscript{nd} arm</td>
<td>Only high dose ICS attenuated sputum eosinophil % (SE%). SE% was correlated to severity of EIB &amp; also predicted the magnitude of response to high dose ICS, but not to low dose ICS</td>
<td>Sputum eosinophilia may be useful in predicting responders to ICS in asthmatic patients with EIB</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Design</td>
<td>Treatment</td>
<td>Method</td>
<td>Results</td>
<td>Conclusions</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------------------------</td>
<td>-------------------------------------------</td>
<td>---------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Subbarao 2006 (72)</td>
<td>26 steroid naïve asthmatics with EIB</td>
<td>Randomised double blinded, 2 parallel arms, double dummy, crossover trial</td>
<td>Ciclesonide MDI 1. 40 and 160µg 2. 80 and 320µg</td>
<td>Treatment with ICS for 3/52 with ex test at weekly intervals, 3/52 washout then 2nd arm</td>
<td>EIB improved with all doses, 160 &amp; 320µg showed continuing improvement in FEV₁ with no plateau</td>
<td>Max attenuation in exercise response still increasing after 3 weeks high dose (&gt;200µg) ICS</td>
</tr>
<tr>
<td>Thio 2001(106)</td>
<td>9 asthmatic children, 8-16yrs old with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>Fluticasone Propionate 1,000µg (FP)</td>
<td>FP (or placebo) then spirometry every hour for 4 hrs then an ex test. 7-14 days washout</td>
<td>FEV₁ significantly increased after 1hr inhalation and FP provided clinical protection in 5 out of 9 children</td>
<td>Single high dose of FP had an acute protective effective over EIB in 5 out of 9 asthmatic children</td>
</tr>
<tr>
<td>Hofstra 2000 (71)</td>
<td>37 children, 6-14yrs old, with EIB</td>
<td>Randomised, double-blinded, placebo controlled, parallel group trial</td>
<td>Fluticasone Propionate (FP) 1. 100µg b.d 2. 250µg b.d</td>
<td>Total study length of 6 months with many repeated ex tests &amp; methacholine challenges. Placebo group re-randomised to an active treatment after 6/52</td>
<td>Severity of EIB reduced at 3/52 similarly in both treatment arms &amp; sustained for 6 months. PD₂0 to methacholine increased at 6/52 in both FP arms &amp; steadily increased over 6 months with between arm difference approaching significance</td>
<td>In childhood asthma FP protects from methacholine in a time &amp; dose dependant manner</td>
</tr>
<tr>
<td>Jónasson 2000 (73)</td>
<td>57 children, 7-16yrs old with EIB</td>
<td>Randomised, double-blinded, placebo controlled, parallel group trial</td>
<td>Budesonide: 1. 100µg 1x daily 2. 200µg 1x daily 3. 100µg b.d</td>
<td>Treatment for 12/52 then repeat exercise test</td>
<td>Drop in post-exercise FEV₁ and daytime symptoms scores less in all budesonide groups compared to placebo with no difference between treatment groups</td>
<td>Low dose ICS improved exercise tolerance &amp; symptoms in children with EIB after 3 months treatment</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Design</td>
<td>Treatment</td>
<td>Method</td>
<td>Results</td>
<td>Conclusions</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------</td>
<td>---------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Vathenen 1991 (74)</td>
<td>40 asthmatics with PV&lt;sub&gt;20&lt;/sub&gt; EVH&lt;sub&gt;&lt;640L&lt;/sub&gt; &amp; PD&lt;sub&gt;20&lt;/sub&gt; Histamine&lt;sub&gt;&lt;4µmol&lt;/sub&gt;</td>
<td>Randomised, double-blinded, placebo controlled, parallel group trial</td>
<td>Budesonide 800µg b.d</td>
<td>Histamine challenge, EVH &amp; ex test on treatment day 1, 6/52 either active or placebo then repeat the 3 bronchial challenges</td>
<td>After budesonide there was an increase from 0.48 to 2.8µmol in PD&lt;sub&gt;20&lt;/sub&gt; histamine and in PV&lt;sub&gt;20&lt;/sub&gt; EVH from 364 to 639L. These were significantly correlated.</td>
<td>Inhaled corticosteroids provide similar protection from histamine challenge, EVH and EIB, suggesting a common mechanism of protection</td>
</tr>
<tr>
<td>Leukotriene Antagonists Rundell 2005 (86)</td>
<td>11 physically active EIB positive subjects</td>
<td>Randomised double-blinded, placebo controlled, crossover trial</td>
<td>Montelukast 10mg</td>
<td>Montelukast or placebo 6hrs before EVH &amp; exercise test</td>
<td>Montelukast provided protection against AHR in 44% for EVH &amp; 53% for exercise testing</td>
<td>Single dose of montelukast provides reasonable protection from AHR</td>
</tr>
<tr>
<td>Helenius 2004 (107)</td>
<td>16 male ice hockey players with exercise-induced bronchial symptoms</td>
<td>Randomised double-blinded, placebo controlled, crossover trial</td>
<td>Montelukast</td>
<td>4/52 each treatment arm with repeated Histamine challenge, symptom scores, F&lt;sub&gt;NO&lt;/sub&gt;, sputum, spirometry &amp; morning PEF. 1/52 washout between</td>
<td>Montelukast did not differ from placebo in treatment of asthma-like symptoms in these ice-hockey athletes</td>
<td>Possibility of different pathophysiology in elite athletes compared to asthmatics</td>
</tr>
<tr>
<td>Peroni 2002 (108)</td>
<td>19 asthmatics, 7-13yrs old with EIA</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>Montelukast</td>
<td>Montelukast or placebo then ex test at 2, 12 or 24hrs after dose</td>
<td>Montelukast had protective effect only at 12hrs after dosing</td>
<td>Timing of montelukast dose before exercise very important to obtain drug protective effects</td>
</tr>
<tr>
<td>Study</td>
<td>Population Description</td>
<td>Design Description</td>
<td>Treatment</td>
<td>Method</td>
<td>Results Description</td>
<td>Conclusions Description</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------</td>
<td>--------------------</td>
<td>-----------</td>
<td>--------</td>
<td>----------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Leff 1998 (88)</td>
<td>110 mild asthmatics with EIB</td>
<td>Randomised, double-blinded, placebo controlled, parallel group trial</td>
<td>Montelukast</td>
<td>Montelukast or placebo for 12/52 then ex tests 20-24hrs after dose at weeks 4, 8 &amp; 12 followed by 2/52 single-blind placebo washout &amp; ex test</td>
<td>Montelukast provided significant protection from EIB over the 12 week period and following withdrawal of treatment, montelukast group approached values seen in placebo group</td>
<td>Montelukast provided protection from EIB over 12 weeks with no evidence of tolerance or rebound worsening of symptoms after 2 weeks withdrawal</td>
</tr>
<tr>
<td>Reiss 1997 (85)</td>
<td>19 asthmatic men, 18-46yrs old with EIB</td>
<td>Randomised double-blinded, placebo controlled, 3 arm, crossover trial</td>
<td>Montelukast 100mg 1x daily, 2. Montelukast 50mg b.d</td>
<td>Each treatment arm for 48hrs with 4 days wash-out between arms Urinary leukotriene E_4 measured before and after ex tests at end of each treatment arm</td>
<td>Both dosing regimens on Montelukast provided protection against EIB. Twice daily dosing resulted in higher plasma concentrations but not correlated to protection. Urinary LTE4 increased after exercise in placebo group</td>
<td>Montelukast provided similar protection from EIB at plasma conc. 0.12-1.27µg/mL the increase in urinary LTE4 post EIB provides further evidence for the role of leukotrienes in EIB</td>
</tr>
<tr>
<td>Knöpfli 2005 (109)</td>
<td>8 children, 13yrs old, with asthma and EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>Ipratropium bromide 500µg nebulised and relationship to vagal nerve activity</td>
<td>Ipratropium bromide or 0.9% NaCl 45mins before 8min ex test + 4sec ex test for vagal activity</td>
<td>Ipratropium bromide increased pre-exercise FEV_1, similar drop in FEV_1 seen in both IB and placebo</td>
<td>The beneficial response to ipratropium bromide was positively correlated to vagal activity</td>
</tr>
<tr>
<td>Brannan 2000 (97)</td>
<td>24 asthmatic subjects with PD_{15} mannitol &lt;350mg</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>Nedocromil Sodium 8mg</td>
<td>Mannitol challenges on separate days each after either Nedocromil sodium or placebo pre-treatment</td>
<td>Nedocromil sodium greatly attenuated the AHR to mannitol with 12 subjects no longer reaching PD_{15} after 635mg of mannitol</td>
<td>Nedocromil sodium inhibits responsiveness to mannitol in asthmatic subjects</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Design</td>
<td>Treatment</td>
<td>Method</td>
<td>Results</td>
<td>Conclusions</td>
</tr>
<tr>
<td>-------</td>
<td>------------</td>
<td>--------</td>
<td>-----------</td>
<td>--------</td>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>de Benedictis 1995 (110)</td>
<td>13 asthmatic children, 7-15yrs old, with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>1. Cromolyn sodium (10 mg) 2. Nedocromil sodium (4 mg),</td>
<td>Medication inhalation followed by ex test 20min and 140mins afterwards. Treatment arms on separate days</td>
<td>Both active treatments significantly more protective than placebo at 20min ex test but not at 140min. No difference between active treatments</td>
<td>At the clinically recommended doses Cromolyn sodium &amp; Nedocromil sodium protect similarly against EIB but only for up to 2hrs</td>
</tr>
<tr>
<td>de Benedictis 1994 (111)</td>
<td>17 asthmatic children, 7-15yrs old, with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>1.. Cromolyn sodium (10 mg) 2. Nedocromil sodium (4 mg),</td>
<td>Medication inhalation followed by ex test 20mins later</td>
<td>Both active treatments significantly more protective than placebo against EIB. No difference between active treatments</td>
<td>Equal protection from Cromolyn sodium &amp; Nedocromil sodium against EIB in asthmatic children</td>
</tr>
<tr>
<td>Histamine Antagonist</td>
<td>Baki 2002 (112)</td>
<td>11 asthmatic children, 7-17yrs old, with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>Loratadine 10mg</td>
<td>Loratadine reduced post-exercise fall in FEV$_1$ compared to placebo (16.1% vs. 28% respectively)</td>
<td>Loratadine reduces EIB but does not offer clinical protection</td>
</tr>
<tr>
<td>Manning 1992 (113)</td>
<td>11 atopic asthmatics with EIB and refractoriness</td>
<td>2 studies: 8 people in each &amp; randomised, double-blinded, placebo controlled, crossover trials</td>
<td>1. Ranitidine 50mg 2. Cimetidine 400mg</td>
<td>Treatment twice daily for 3 days, last dose 1hr before ex test then 1hr gap before 2nd ex test</td>
<td>No difference in treatment groups compared to placebo in baseline FEV$_1$, EIB or degree of refractoriness.</td>
<td>EIB and refractoriness is not inhibited by H$_2$ receptor antagonists ranitidine &amp; cimetidine in these studies</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Design</td>
<td>Treatment</td>
<td>Method</td>
<td>Results</td>
<td>Conclusions</td>
</tr>
<tr>
<td>-------</td>
<td>------------</td>
<td>--------</td>
<td>-----------</td>
<td>--------</td>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>Ghosh 1991 (114)</td>
<td>20 mild asthmatics with EIB</td>
<td>3 studies: Randomised, double-blinded, placebo controlled, crossover trials</td>
<td>1. Cetrizine 10mg 1mL Cetrizine (5mg/mL nebulised) 2. 1mL Cetrizine (10mg/mL nebulised)</td>
<td>1. Treatment b.d for 1/52 then ex test (12 patients) 2. Single dose then ex test 30mins afterwards 3. Single dose then histamine challenge 30mins afterwards</td>
<td>Oral cetrizine did not affect baseline FEV\textsubscript{1} or offer protection from EIB Both doses of nebulised cetrizine offered significant protection from EIB with no difference between doses Nebulised cetrizine increased the PC\textsubscript{20} of histamine 13.1 fold compared to placebo</td>
<td>There is a difference between the protective effect of cetrizine when administered orally compared to nebulised &amp; this reason is not clear but may be due to differences in local airway concentrations</td>
</tr>
<tr>
<td>Storms 2004 (90)</td>
<td>122 asthmatics with uncontrolled symptoms on low dose fluticasone &amp; worsening with exercise</td>
<td>Randomised, double-blinded, placebo controlled trial parallel arm trial</td>
<td>Addition of montelukast (10mg) or salmeterol (50µg b.d) to open label fluticasone (100µg b.d)</td>
<td>Ex test and beta2-agonist rescue at baseline, week 1 &amp; week 4</td>
<td>Pre-exercise FEV\textsubscript{1} greatest with salmeterol but greater protection from ex test, increased rate and magnitude to rescue beta\textsubscript{2} agonist for those taking montelukast</td>
<td>Addition of montelukast to treatment regimen for asthmatics who remain uncontrolled on ICS provides protection from EIB and greater response to rescue beta\textsubscript{2}-agonists</td>
</tr>
<tr>
<td>Edelman 2000 (89)</td>
<td>191 asthmatics with EIB</td>
<td>Randomised, double-blinded, double dummy parallel arm trial</td>
<td>1. Montelukast 10mg 1x daily 2. Salmeterol 100µg b.d</td>
<td>Treatment for 8/52 with ex tests at day 3, week 4 &amp; week 8</td>
<td>Montelukast was superior to salmeterol in protection from EIB at weeks 4 &amp; 8 with comparable protection at day 3</td>
<td>Montelukast may be an appropriate controller medication for patients with EIB</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Design</td>
<td>Treatment</td>
<td>Method</td>
<td>Results</td>
<td>Conclusions</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------------</td>
<td>------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Villaran 1999</td>
<td>197 asthmatics with post exercise fall in FEV$_1$ $&gt;$18%</td>
<td>Randomised, double-blinded, double dummy parallel arm trial</td>
<td>1. Montelukast 10mg 1x daily 2. Salmeterol 50µg b.d</td>
<td>Treatment for 8/52 with ex tests at day 3, week 4 &amp; week 8 (comparable protection at day 3)</td>
<td>Montelukast was superior to salmeterol in protection from EIB at weeks 4 &amp; 8</td>
<td>Montelukast may be an appropriate controller medication for patients with EIB as there does not appear to be any tachyphylaxis</td>
</tr>
<tr>
<td>Woolley 1990 (99)</td>
<td>12 subjects with EIA</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>1. Terbutaline 0.5mg aerosol 2. Cromoglycate 2mg 3. Terbutaline 0.5mg + cromoglycate 2mg</td>
<td>Single dosing of treatment arm then four ex tests separated by 2hr intervals. Each treatment arm tested on separate days but within total space of 2/52</td>
<td>Both individual active treatments provided protection from EIB for up to 2hrs &amp; the combination of both provided protection for up to 4hrs</td>
<td>Combination of beta agonist and mast cell stabiliser provided prolonged protection from EIB</td>
</tr>
<tr>
<td>Stelmach 2008</td>
<td>100 atopic asthmatic children, 6-18yrs old with EIB</td>
<td>Randomised, double-blinded, placebo controlled, parallel group trial</td>
<td>1. Budesonide (200µg) + formoterol (9µg) 2. Budesonide (200µg) + montelukast (5 or 10µg) 3. Montelukast (5 or 10µg) 4. Budesonide (200µg)</td>
<td>Ex test then treatment for 4/52 with repeat ex test at end of treatment arm</td>
<td>EIB significantly diminished in all active groups compared to placebo. Greatest protection in group #2 (budesonide + montelukast) and group 3# (montelukast alone)</td>
<td>There are different protection effects between the therapeutic options available in the treatment of EIB</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Design</td>
<td>Treatment</td>
<td>Method</td>
<td>Results</td>
<td>Conclusions</td>
</tr>
<tr>
<td>-------</td>
<td>------------</td>
<td>--------</td>
<td>-----------</td>
<td>--------</td>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>Leukotriene Antagonist Vs. Beta Agonist Vs. Mast Cell Stabilisers</strong></td>
<td>Cowan 2010 (98)</td>
<td>39 patients with EIW &amp; AHR to mannitol &amp;/or EIB</td>
<td>Baseline F_E NO &lt;35ppb: Randomised, double-blinded, crossover trial</td>
<td>1. Montelukast 10mg mane 2. Formoterol 12µg b.d 3. Cromoglycate 5mg 4b.d</td>
<td>Stratification by baseline F_E NO then: Non-steroidal treatments for 2/52 with 1/52 washouts &amp; mannitol &amp; ex tests at end of each arm. Also pre-challenge dosing 15min before ex test and mannitol for formoterol and cromoglycate arms</td>
<td>Significant reduction in AHR to mannitol by montelukast, formoterol &amp; cromoglycate (47%, 61% &amp; 67% respectively) &amp; protection from EIB was 50%, 83% &amp; 67%</td>
</tr>
<tr>
<td><strong>Leukotriene Antagonists Vs. Beta Agonist Vs. 5-Lipoxygenase inhibitor</strong></td>
<td>Coreno 2000 (115)</td>
<td>10 asthmatic patients with EIB</td>
<td>Randomised, blinded, double dummy crossover trial</td>
<td>1. Salmeterol 42µg 2. Montelukast 10mg 3. Zafirlukast 20mg 4. Zileuton 600mg</td>
<td>Single dose of medication then exercise 1, 4, 8 &amp; 12hrs afterwards</td>
<td>All treatment arms blunted EIB within 1hr. Salmeterol &amp; the Leukotriene antagonists continued working at 12hrs whereas Zileuton did not differ from placebo at 8hr test</td>
</tr>
<tr>
<td><strong>Leukotriene Antagonist and Histamine Antagonist combination</strong></td>
<td>Peroni 2002 (116)</td>
<td>19 allergic asthmatic children with EIB</td>
<td>Randomised, double-blinded, placebo controlled, crossover trial</td>
<td>1. Montelukast 2. Loratadine 3. Montelukast + Loratadine</td>
<td>Treatment dose at 08:00h then exercise tests at 10:00h and 20:00h (separate days) 3-5 day washout between treatments</td>
<td>Protection seen at 12hrs after dosing with Montelukast and combination treatment</td>
</tr>
</tbody>
</table>
### Legend for Table 2.1:

**Abbreviations used:**

- **AHR** - Airway hyper-responsiveness
- **b.d** - Taken twice daily
- **conc.** - Concentration
- **EIB** - Exercise-induced bronchoconstriction
- **EVH** - Eucapnic voluntary hyperventilation
- **Ex test** - Exercise test
- **FEV<sub>1</sub>** - Forced Expiratory Volume in one second
- **hrs** - Hours
- **ICS** - Inhaled corticosteroid
- **mane** - Taken in the morning
- **MDI** - Metered dose inhaler
- **PC<sub>20</sub>** - Provocative concentration causing a 20% fall in FEV<sub>1</sub>
- **PD<sub>20</sub>** - Provocative dose causing a 20% fall in FEV<sub>1</sub>
- **PD<sub>15</sub>** - Provocative dose causing a 15% fall in FEV<sub>1</sub>
- **PV<sub>20</sub>** - Provocative volume causing a 20% fall in FEV<sub>1</sub>

### Table 2.1: Study Design and Results for Mast Cell Stabilisers Vs. NKCC2 inhibitor

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Design</th>
<th>Treatment</th>
<th>Method</th>
<th>Results</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mast Cell Stabilisers Vs. NKCC2 inhibitor</strong></td>
<td>Melo 1997 (117)</td>
<td>15 asthmatic children, 8-14 yrs old with EIB</td>
<td>Single-blinded, placebo controlled, crossover trial</td>
<td>Nebulised: 1. Disodium cromoglycate 20mg 2. Frusemide 20mg/m2 body area</td>
<td>Single dose then ex test 30mins after administration on 3 separate days</td>
<td>No difference in resting lung function between arms. Both treatments provided protection from EIB compared to placebo</td>
</tr>
<tr>
<td>Novembre 1994 (100)</td>
<td>24 children, 6-16yrs old with EIA</td>
<td>Randomised, placebo controlled, crossover trial</td>
<td>1. Nedocromil 4mg via MDI 2. Frusemide 30mg nebulised 3. Nedocromil + Frusemide</td>
<td>4 separate treatment days with ex test 20mins after dosing</td>
<td>Both active treatments significantly protected against EIB and the combination treatment arm provided further protection than either treatment alone</td>
<td>Nedocromil and frusemide provide comparable effects against EIB and together have an additive protective effect</td>
</tr>
</tbody>
</table>
Chapter Three: Exhaled Nitric Oxide

Airway inflammation is characteristic and in the airways of most patients with asthma. This has been identified through the study of bronchial biopsies (118-119), bronchoalveolar lavage fluid assays (120), induced sputum samples (121) and post-mortem airway histology (122). Inflammation is likely to be present in patients experiencing exercise-induced symptoms suggestive of asthma (123). Advances in research have allowed for non-invasive detection and measurement of eosinophilic airway inflammation, by utilisation of the surrogate biomarker exhaled nitric oxide ($F_{E}NO$) (124).

3.1 Measurement

Previous techniques for quantifying the degree of airway inflammation, such as induced sputum samples, bronchial biopsies and bronchoalveolar lavage fluid assays, are technically demanding and invasive. $F_{E}NO$ is a surrogate biomarker for the steroid responsive eosinophilic airway inflammation (125) and can easily be measured in the breath of adults (126-127) and children (128-129). The development of portable analysers enable the opportunity for this clinical tool to be utilised in the primary care setting for diagnosing and differentiating airway pathology, guiding therapeutic management and predicting treatment outcomes.

3.2 Clinical Applications

3.2.1 $F_{E}NO$ and Asthma

Asthma can be classified in many different ways based on clinical, physiological and pathological characteristics. The last of these allows for phenotyping based on the type of bronchial inflammation predominating in the airways. Induced sputum samples have identified two major inflammatory phenotypes; eosinophilic and non-eosinophilic inflammation (130). The ATS/ERS have recommended the cut-point for classification of eosinophilic inflammation as $>1.9\%$ of eosinophils present in a representative sputum sample (76). Importantly eosinophilic airway inflammation is steroid responsive (131-132), whereas non-eosinophilic inflammation, comprising paucigranulocytic and neutrophilic subtypes, is less likely to show clinical benefits with inhaled steroids (133-134).
Increased levels of \( \text{FE} \text{NO} \) present in patients with bronchial asthma were first reported by Alving et al. in 1993 (124). \( \text{FE} \text{NO} \) levels are significantly correlated to the degree of eosinophilic airway inflammation measured by more invasive methods (125, 135-137). Following treatment with ICS, \( \text{FE} \text{NO} \) levels decrease over time in a dose-dependent manner (138) and these changes in \( \text{FE} \text{NO} \) are correlated to the changes in eosinophils seen in induced sputum samples (139).

\( \text{FE} \text{NO} \) measurements have a role in the assessment of asthmatic patients. The utility of \( \text{FE} \text{NO} \) measurements for diagnosing the presence of asthma is significant, with studies demonstrating high test sensitivity and specificity (140-141). Further, \( \text{FE} \text{NO} \) measurements in combination with abnormal spirometry demonstrate even greater sensitivity and specificity (94% and 93% respectively) (141-142). Although, low \( \text{FE} \text{NO} \) levels do not necessarily exclude an asthma diagnosis, especially in non-atopic individuals with non-eosinophilic airway inflammation, the negative predictive value of a low \( \text{FE} \text{NO} \) for the absence of eosinophilic inflammation is high (143). However, conventional tests such as spirometry and bronchial challenges are still warranted in such patients (144).

### 3.2.2 \( \text{FE} \text{NO} \) and EIA

\( \text{FE} \text{NO} \) has a potential diagnostic role in asthmatics whose symptoms are predominately exercise-induced. The severity of EIB has been shown to be significantly correlated to sputum eosinophilia (145). Further, raised \( \text{FE} \text{NO} \) levels are highly predictive for the presence of EIB (129, 146) and correlate to the severity of EIB (147-148).

### 3.2.3 \( \text{FE} \text{NO} \) and Steroid responsiveness

As detailed, eosinophilic airway inflammation is steroid responsive and \( \text{FE} \text{NO} \) can be used to non-invasively quantify the degree of eosinophilic airway inflammation. Conversely, low \( \text{FE} \text{NO} \) levels are associated with the absence of eosinophilic airway inflammation, with high negative predictive values (143). Against this background, the ability of \( \text{FE} \text{NO} \) to predict steroid responsiveness was tested by Smith et al. (149). They demonstrated, that regardless of the diagnosis, raised \( \text{FE} \text{NO} \) measurements in patients with non-specific respiratory symptoms significantly correlated with a steroid responsive airway dysfunction. In contrast, patients with low \( \text{FE} \text{NO} \) did not respond. In other studies, asthmatic patients
with increased \( F_{E}NO \) levels demonstrate steroid responsiveness and greater clinical benefits (150-151).

The ability to detect underlying airway inflammation, likely to respond to ICS, easily in patients with exercise-induced symptoms of asthma is therefore potentially possible using \( F_{E}NO \). This would allow those patients unlikely to respond to ICS to be initiated on an alternative therapy, such as a leukotriene antagonist or mast cell stabiliser, and ICS would only be given to those likely to benefit. This could potentially eliminate the need for costly empiric trials.

Previously in the department, Cowan et al. demonstrated the effectiveness of ICS in patients with high \( F_{E}NO \) and the potential role of alternative therapies for those with low \( F_{E}NO \) (98). The study contained in this thesis is designed to extend these previous findings in the first placebo-controlled trial, to our knowledge, designed to assess the predictive utility of \( F_{E}NO \), for steroid responsiveness in patients with exercise-induced respiratory symptoms.

This thesis addresses the following hypothesis and aims:

**Hypothesis:**
Patients with exercise-induced respiratory symptoms and high \( F_{E}NO \) are more likely to respond to ICS treatment, compared to those with low \( F_{E}NO \)

**Aims:**
1. To calculate the performance characteristics of \( F_{E}NO \) measurements as a predictor of response to ICS in patients with EIA symptoms and AHR.
2. To compare the effectiveness of ICS in the management of EIA symptoms in patients with low versus high \( F_{E}NO \)
3. To confirm that pre-treatment measurement of \( F_{E}NO \) is an important way to approach the management of patients with EIA symptoms.
Chapter Four: Detailed Methods

This chapter contains detailed descriptions of the materials and methods used throughout the study contained in this thesis.

4.1 Laboratory Testing

4.1.1 Fraction of Exhaled Nitric Oxide (FE\textsubscript{NO})

Measurement of FE\textsubscript{NO} was undertaken before all other laboratory tests in accordance with ATS/ERS guidelines (126). FE\textsubscript{NO} was evaluated using the NiOX MiNO (Aerocrine, Solna, Sweden) at a flow rate of 50mL/sec and the mean of two acceptable manoeuvres recorded. Values were recorded in parts per billion (ppb).

4.1.2 Skin Prick Testing

Skin Prick Testing was undertaken after anti-histamines had been withheld for 72 hours. A single drop of each allergen, Cat pelt, Grass mix and House Dust Mite, plus positive and negative controls, (Hollister-Stier Laboratories, LLC, Spokane, WA99207, USA) were evenly spaced along the volar aspect of the patient’s forearm, avoiding surface veins. Skin pricks were administered at 90° to the skin using five sterile, single 1mm prick, microlancets (STALLERGENES, Ebos Ltd, New Zealand). Allergen and control drops were left for one minute on the skin before removal with absorptive tissues. Careful attention was given to ensure individual drops and reaction sites did not mix. Maximal wheal and flare diameters were measured fifteen minutes after initial skin puncture. Atopy was considered present if a wheal >2mm compared to the negative control was observed, with one or more allergens (134).

4.1.3 Spirometry and Assessment of Reversibility

Spirometry was undertaken on a rolling seal spirometer (SensorMedics Corporation, Yorba Linda, CA, USA) which was calibrated daily, before use, using a standard 3L syringe. Forced Expiratory Volume in one second (FEV\textsubscript{1}), Forced Vital Capacity (FVC), Forced
Expiratory Flow between 25 to 75% of Forced Vital Capacity (FEF\textsubscript{25-75}) and Peak Expiratory Flow Rate (PEFR) were measured following standardised ATS/ERS criteria (35). Short- and long-acting beta\textsubscript{2} agonists were withheld for six and sixteen hours respectively before all spirometry and bronchial challenges. Greater precision than outlined by the ATS/ERS criteria was achieved with the best of two reproducible, within 100mL as opposed to 150mL, FEV\textsubscript{1} manoeuvres being recorded. Predicted values were calculated using the reference set by Morris \textit{et al.} (152). Airway reversibility was assessed fifteen minutes after inhalation of 400µg salbutamol (Ventolin, GlaxoSmithKline, Greenford, UK) via a large volume spacer (Volumatic, GlaxoSmithKline, Greenford, UK). Reversible airway obstruction was deemed clinically significant if a FEV\textsubscript{1} improvement of at least 12% and 200mL was observed following bronchodilator, as defined by the latest ATS/ERS task force recommendations (36).

4.1.4 Peak Expiratory Flow Rate

Peak expiratory flow measurements were regularly recorded by patients who were previously taking inhaled corticosteroids upon entry into the study. This was used as the basis for their individualised action plan during subsequent withdrawal of their ICS, as later described. Patients were given detailed instructions on how to perform peak flow recordings and supplied with portable peak flow meters (Breath-Alert, Medical Developments International, Springvale, Victoria, Australia). In accordance with ATS/ERS criteria, patient technique was initially carefully observed in the laboratory to ensure accurate recordings and the best of three manoeuvres was used for assessment (35).

4.2 Bronchial Challenges

All bronchial challenges were undertaken at a location with sufficient medical cover and emergency equipment. For safety reasons bronchial challenges did not proceed if the pre-test FEV\textsubscript{1} was <60% predicted or <1.2L on the day of testing. If at any time the patient was distressed or particularly uncomfortable, the challenge was ceased and appropriate management commenced. Reversing of bronchoconstriction was achieved with either salbutamol (Ventolin, GlaxoSmithKline, Greenford, UK) via a large volume spacer (Volumatic, GlaxoSmithKline, Greenford, UK) or nebulised Duolin; salbutamol and ipratropium bromide.
4.2.1 Mannitol Challenge

All mannitol challenges were administered following a standardised protocol (58). Spirometry recorded throughout the challenge was performed as previously described. Abbreviated manoeuvres were employed during the challenge when only FEV$_1$ was necessary and not FVC. Mannitol was supplied by Pharmaxis, Sydney, Australia. The following doses were administered: 0 (empty capsule acting as the placebo for baseline reference), 5, 10, 20, 40, 80, 160, 160 and 160mg. Doses in individual capsules did not go higher than 40mg so the final four steps were administered in 2x 40mg capsules (80mg step) and 4x 40mg capsules (160mg steps). Mannitol was administered via the Osmohaler$^\text{TM}$ (Plastiape S.p.A., Osnago-Lecco, Italy) with subsequent FEV$_1$ recordings one minute after inhalation of each dose. The best reproducible FEV$_1$ following the placebo capsule, was used as the baseline value to calculate percent decrease in FEV$_1$ during the mannitol challenge. The challenge was ceased when a 15% reduction in FEV$_1$ was demonstrated (see calculation below) or until a total cumulative dose of 635mg of mannitol had been inhaled. The provocative dose of mannitol causing a 15% reduction in FEV$_1$ (PD$_{15}$) was calculated using linear interpolation of the dose response curve and the challenge was considered positive for airway hyper-responsiveness if the PD$_{15}$ was <635mg.

\[
\text{Target FEV}_1 = \text{Baseline FEV}_1 \times 0.85
\]

4.2.2 Exercise Challenge

Exercise challenges were conducted in the Department of Physiology, in accordance with ATS guidelines (47). Pre-exercise spirometry was undertaken using the same technique as previously described. All pre- and post-exercise spirometry was performed on a portable KoKo spirometer (nSpire Health Inc, Longmont, Germany), which was calibrated daily using a standard 3L syringe. The best reproducible FEV$_1$ prior to exercise was used as the baseline value to calculate the target ventilation range required during the exercise challenge (46):
**Target Ventilation Range**  

\[(\text{Baseline } \text{FEV}_1 \times 17) \text{ --- } (\text{Baseline } \text{FEV}_1 \times 21)\]

\[50\% \text{ Max Ventilation} \text{ --- } 60\% \text{ Max Ventilation}\]

Exercise was performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode BV, Groningen, The Netherlands). Patients wore a leak free respiratory mask (Hans-Rudolph 8980, Kansas City, MO, USA), breathing dry air (21% O\(_2\)) from a 200L Douglas bag, through one-way non-rebreathing valve (Hans-Rudolph 2700, Kansas City, MO, USA). Patients exercised for a total duration of eight minutes. The predicted FEV\(_1\) was used to calculate increasing target workloads expected to achieve the target ventilation range (46):

**Target Workload \text{Watts} = (\text{Predicted } \text{FEV}_1 \times 53.76) – 11.07**

Exercise began at 60% of the target workload and was increased at minute intervals to 70%, 90% and then sustained at the target workload for the final five minutes as tolerated (total exercise time of eight minutes). Ventilation rates were constantly monitored through-out the exercise test and workloads were adjusted as necessary to maintain the target ventilation range for the final four minutes.

Expiratory flow during the challenge was measured using a heated pneumotach (Hans-Rudolph HR800, Kansas City, MO, USA). Exhaled gas samples from the mask, end-tidal CO\(_2\) (PETCO\(_2\)) and O\(_2\) (PETO\(_2\)) were measured through-out the challenge by a gas analyser (model CD-3A, AEI Technologies, Pittsburgh, PA). A running 3-lead electrocardiogram (ECG) was constantly monitored during exercise for heart rate and potential arrhythmias. Real time recordings of ventilation, gas samples, ECG rhythm and heart rate were displayed during testing (Power Lab, ADI Instruments, Colorado Springs, CO, USA). A pulse oximeter was continually monitoring oxygen saturation and additionally provided secondary recordings of heart rate, during and after exercise until recovery.

Immediately on completion of exercise patients were asked to quantify their level of dyspnoea, “shortness of breath”, on the Borg Scale (153) and a Visual Analogue Scale. Post-exercise FEV\(_1\) was recorded at 1, 3, 5, 10, 15, 20 and 30 minutes, continuing until
either the FEV\textsubscript{1} had reduced by $\geq 10\%$ (warranting reversing of bronchoconstriction), two consecutive recordings were higher than the lowest at twenty minutes, or until thirty minutes had been reached. Exercise-induced bronchoconstriction was defined as a $\geq 10\%$ reduction in post-exercise FEV\textsubscript{1} compared to baseline.

**Positive exercise challenge: Post-exercise FEV\textsubscript{1} $\leq$ Pre-exercise FEV\textsubscript{1} x 0.9**

### 4.3 Qualitative methods

Throughout the study three different qualitative methods were used to quantify asthma symptoms and control; The Borg Scale (153) and Visual Analogue Scale after exercise challenges and the Asthma Control Questionnaire (154) completed at the beginning of each clinic visit.

#### 4.3.1 Borg Scale

This scale was used immediately following the exercise challenge. Patients were instructed to point to or say a number on the scale that best described their level of breathlessness at that moment. The scale began at 0=nothing at all, to 10=maximally breathless (see Figure 4.1). Post-treatment, a reduction of $\geq 1$ was considered a significant response (153).

#### 4.3.2 Visual Analogue Scale

This scale was used without delay following the Borg Scale after exercise challenges. The identical question of “How breathless do you feel at this moment” was asked and patients were instructed to mark a point along a 10cm line from 0=nothing at all, to 10=maximally breathless.

#### 4.3.3 Asthma Control Questionnaire

Before laboratory testing and bronchial challenges were undertaken in the clinic, patients were asked to complete the validated Asthma Control Questionnaire (ACQ) (154). This
comprises a total of seven questions; five of which relate to asthma symptoms, one regarding bronchodilator use and one concerning airway calibre (see Figure 4.2). Each question has seven possible answers assigned values from 0 to 6, with identical weightings on each question. The overall score is calculated as a mean of the seven answers and ranges from 0 to 6. A cut point of 1 can be used to differentiate well controlled asthma (score <1) from poorly controlled asthma (score >1) (155). However there is greater certainty if two cut points are used; defining well controlled asthma as scores <0.75 and poorly controlled asthma as scores >1.5 (155). Shortened versions of the ACQ can be used in clinical trials without loss of validity or change in measurement interpretations (156). These versions include the ACQ-5 (comprising only the first five symptom based questions) and the ACQ-6 (omitting the final question on FEV\(_1\) measurement). The study in this thesis used the ACQ-6. A reduction in ACQ score of ≥0.5 was defined as a positive response to treatment (156).

<table>
<thead>
<tr>
<th>How breathless do you feel at this moment?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

Figure 4.1: The Borg Scale
### Figure 4.2: Asthma Control Questionnaire

Taken from Juniper *et al.* (154)
4.4 Additional methods and considerations

4.4.1 Inhaled Corticosteroid treatment withdrawal

All patients currently on inhaled corticosteroids at the time of enrolment underwent withdrawal of their steroid before baseline testing. This aspect of my study has previously been well described by Gibson et al. (157) and allows steroid-free assessment of the airways to be undertaken.

Before any alteration in treatment regimen was undertaken, initial on-treatment spirometry, $F_{\text{E}}NO$, and PEFR were obtained. If these tests were satisfactory the patient was given a diary and peak flow meter. All patients enrolled in the study were provided with salbutamol (Ventolin, GlaxoSmithKline, Greenford, UK), an emergency supply of prednisone tablets and an emergency contact card for 24/7 access to the study investigators and Respiratory Consultants.

Diary data was collected for two weeks whilst the patient was taking all their usual medications. Data on morning and evening peak flows, reliever use, night waking due to asthma and symptoms were collected (see Figure 4.3). Additional pages at the back of the diary also allowed documentation of any changes in medication or descriptions of relevant information to be recorded by the patient. Frequency and severity of symptoms were quantified using the Asthma Symptom Score (see Table 4.1) which described respiratory symptoms in the previous 24 hours.

At the end of two weeks a review of the diary data was undertaken and in conjunction with one of the respiratory physicians, the safety of ICS withdrawal for each individual patient was assessed. If ICS withdrawal was deemed appropriate, an action plan was generated based on the run-in data. Individualised peak flow cut-offs comprising part of the “loss of control” criteria were calculated (see Table 4.2) and written in the front of the patient diaries. The importance of contacting the researchers should a patients peak flows reduce to these values was carefully explained and emphasised. At this stage, patients were then asked to stop their inhaled steroid and to keep recording all the aforementioned diary data. Patients were permitted to use their reliever as required and were closely followed with regular phone calls. They were followed until either a predetermined “loss of control”
occurred, as defined by their personalised action plan, or for 28 days, depending on which came first.

### Table 4.1: Asthma Symptom Score

<table>
<thead>
<tr>
<th>Asthma Symptom Score:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0</strong> = no symptoms at all</td>
</tr>
<tr>
<td><strong>1</strong> = symptoms for one short period during the day</td>
</tr>
<tr>
<td><strong>2</strong> = symptoms for two or more short periods of the day</td>
</tr>
<tr>
<td><strong>3</strong> = symptoms for most of the day which did not affect normal daily activities</td>
</tr>
<tr>
<td><strong>4</strong> = symptoms for most of the day which affected normal daily activities</td>
</tr>
<tr>
<td><strong>5</strong> = symptoms so severe that patient could not go to work or perform daily activities</td>
</tr>
</tbody>
</table>
# Week 1 of Phase 2/1 – record between Visits 3 and 4

<table>
<thead>
<tr>
<th></th>
<th>Mon</th>
<th>Tues</th>
<th>Wed</th>
<th>Thurs</th>
<th>Fri</th>
<th>Sat</th>
<th>Sun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Complete this section in the morning**

- Best of 3 peak flows
- Awoke because of asthma? Y/N
- No. of puffs of reliever “Ventolin” (overnight)

**Complete this section in the evening**

- Best of 3 peak flows
- Asthma symptom score 0-5
- No. of puffs of reliever “Ventolin” (daytime)

---

*Figure 4.3: Example page from patient diary*
### 4.4.2 “Loss of Control” Criteria for ICS withdrawal

The loss of control criteria used to determine the need for assessment in the Respiratory Research Unit for baseline testing, was modified from previous criteria described by Jones et al. (139). As shown in the criteria below, the term loss of control does not infer acute, severe asthma, but allows assessment of steroid-free airways. At any stage during the ICS withdrawal phase or treatment arms, participation in the study could be terminated by the patient through indicating the presence of distressing or intolerable symptoms, irrespective of their peak flow measurements.

#### Table 4.2: Loss of Control Criteria

<table>
<thead>
<tr>
<th>Any one or more of the following clinical features:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average morning PEFR &lt;90% over the last week (minimum of 5 days)</td>
</tr>
<tr>
<td>Morning PEFR &lt;80% any two consecutive days</td>
</tr>
<tr>
<td>Evening PEFR &lt;80% any two consecutive days</td>
</tr>
<tr>
<td>Morning PEFR &lt;60% on any day</td>
</tr>
<tr>
<td>Evening PEFR &lt;60% on any day</td>
</tr>
<tr>
<td>Night waking due to asthma – 2 more nights/week than run-in phase</td>
</tr>
<tr>
<td>Bronchodilator – 4 more puffs on average/day than run-in phase over previous week</td>
</tr>
<tr>
<td>Any distressing or intolerable symptoms</td>
</tr>
</tbody>
</table>
4.4.3 Randomisation technique

The treatment phase of this study was assigned in a double blinded, random order. Two respiratory technicians were responsible for generating the randomisation codes, maintaining allocation concealment and blinding the study medications. They generated the simple randomisation sequence by pulling two identically shaped pieces of card from an envelope, each labelled with either “active” or “placebo”. The order in which these pieces of card were drawn determined the order of treatment, repeated for each patient number. A list of study numbers, assigned to patients based on order of consent into the study, was thus generated with pre-determined treatment allocations.

Allocation concealment was achieved by keeping this list in a sealed, impermeable, brown envelope away from the author who was consenting and conducting laboratory tests. When each patient completed the study, the senior technician unblinded the treatment order at the end of the final visit.

4.4.4 Blinding of treatments

Blinding of medications was achieved by using identical Turbuhalers; one a standard inhaler containing Pulmicort 400µg and one with only the desiccant remaining. These two inhalers had all original labels removed and thus looked, tasted and were otherwise identical. Each inhaler was placed into a bag labelled with the patient study number and either arm one or arm two. This allowed the author to administer each of the treatment arms without needing to know the allocation sequences. A prior pilot study in the department had shown this method of inhaler blinding to be highly effective.
Chapter Five: Methods

5.1 Patients

Patients aged 16-70 years old with predominant symptoms of exercise-induced cough, wheeze and/or dyspnoea were eligible for this study. Recruitment was achieved using community flyers, global company emails (University of Otago and Dunedin City Council) and physician referrals to the pulmonary function laboratory of Dunedin Hospital. Exclusion criteria included co-existing cardiac disease, bronchiectasis, pregnancy, planning a pregnancy, breastfeeding, smoking history >10 pack years and/or smoking within the last three months, FEV$_1$ <60% predicted or <1.2L, BMI >35, severe hypertension (>180/100mmHg at rest), uncontrolled diabetes or history of unstable, severe asthma. All patients were free of illness for at least four weeks prior to all laboratory testing.

5.2 Study Design

A randomised, double blinded, placebo-controlled, cross-over, clinical trial was conducted at the Otago Respiratory Research Unit, Department of Medical and Surgical Sciences, University of Otago, based in Dunedin Public Hospital. The author was responsible for the day to day running of the study. Exercise testing for this study was undertaken in the Department of Physiology. Ethical approval was granted by the Lower South Regional Ethics Committee (LRS/09/10/042). Standing Committee on Therapeutic Trials (SCOTT) authority for the use of mannitol was not required by the New Zealand Ministry of Health.

At the first visit all patients gave full written, informed consent. Demographic data were collected including information on age, sex, height, weight, smoking history and ethnicity. A 12-lead ECG was also carried out to screen for any prior cardiac damage or current anomalies warranting exclusion. A full medical history and clinical examination of blood pressure, heart sounds and review of the ECG was undertaken by one of two Respiratory Consultants. Additionally if the patient was female they also underwent either a pregnancy test or signed a pregnancy test waiver.
Skin prick testing was undertaken to determine atopic status. Responses to cat, grass and house dust mite allergens were measured. Throughout the duration of the study all patients were equipped with a metered dose inhaler of salbutamol (Ventolin 100µg, GlaxoSmithKline, Greenford, UK), an emergency supply of prednisone tablets and an emergency contact card for 24/7 access to the study investigators and on call Respiratory Consultants. Short- and long-acting beta\textsubscript{2} agonists were withheld for 6/16 hours respectively before all laboratory testing.

All patients who were currently using inhaled corticosteroids (ICS) upon entry into the study underwent a period of ICS withdrawal. This has previously been described in Chapter Four and received full ethical approval. Patients entering the study who were steroid naïve or had not used ICS in the preceding twenty-eight days were permitted to undergo baseline testing at the first visit.

5.2.1 Phase One: Screening

Before any laboratory testing was undertaken all patients completed an Asthma Control Questionnaire. Baseline testing proceeded with measurement of exhaled nitric oxide (F\textsubscript{E}NO), spirometry, mannitol challenge and bronchodilator response. These were all conducted as previously described in Chapter Two according to standardised protocols. On a separate day, an exercise challenge was performed on an electromagnetically braked cycle ergometer. This was performed within forty eight hours of other baseline testing but at least eighteen hours apart from the other tests. At the immediate conclusion of the exercise challenge patients were asked to indicate their level of breathlessness on the Borg Scale and on a Visual Analogue Scale.

Only patients who demonstrated airway hyper-responsiveness (a provocative dose of mannitol causing a decrease in FEV\textsubscript{1} of \(\geq 15\%\)) and/or exercise-induced bronchoconstriction (\(\geq 10\%\) decrease in FEV\textsubscript{1} post-exercise) were eligible to continue to the treatment phase of the study. It was estimated, based on data from a previous study in the department (98), that a target enrolment of thirty patients would give the study enough power to detect clinically significant treatment-related changes between three F\textsubscript{E}NO tertile groups. The original study design aimed for ten patients with F\textsubscript{E}NO <25ppb, ten with F\textsubscript{E}NO 25ppb-45ppb and ten with F\textsubscript{E}NO >45ppb. These cut-points were selected based on the
results of previous research that had calculated 47 ppb as the optimum cut-point for steroid responsiveness in patients with non-specific respiratory symptoms (149). This study by Smith et al. also presented a very high negative predicted value for low \( F_{E\text{NO}} \) measurements and steroid responsiveness.

After screening a large number of symptomatic patients it became apparent that we would not find a sufficient number of patients with \( F_{E\text{NO}} < 25 \text{ ppb} \) and airway hyper-responsiveness (AHR). Of the forty-five people screened for eligibility only 2/45 (4%) had a \( F_{E\text{NO}} < 25 \text{ ppb} \) and AHR. On the basis of this we made the decision to undertake the analysis comparing a high versus a low \( F_{E\text{NO}} \) group, keeping 45 ppb as the cut-point. It was the intention to recruit fifteen patients in each group.

### 5.2.2 Phase Two: Trial of treatment

Eligible patients underwent a randomised order, placebo controlled, cross-over study of at least twenty eight days per treatment arm with budesonide (Pulmicort-Turbuhaler 400µg, Astra Zeneca, London, UK). Each treatment arm was separated by a two week washout period. Patients were instructed to use the study inhaler two doses in the morning and two doses at night with mouth rinsing after use. Thus, the total dose of active medication was 1,600µg of budesonide daily. During this time, patients were permitted to use their salbutamol inhaler as required for relief of asthma symptoms.

At the end of each treatment period patients underwent repeat testing of ACQ, \( F_{E\text{NO}} \), spirometry, mannitol challenge, exercise challenge, Borg Score and Visual Analogue Scale using the same methods as previously described.

### 5.3 Statistical Analyses

Baseline characteristics of the low and high \( F_{E\text{NO}} \) groups were compared using independent samples t-tests, chi-squared tests, Fishers exact tests and Mann Whitney U tests where appropriate. \( PD_{15} \) mannitol and \( F_{E\text{NO}} \) data were logarithmically transformed and expressed as geometric means (95% confidence interval). Comparisons between patients enrolled into the study with those who did not have proven AHR or EIB were also conducted using the same statistical analyses.
For each of the low and high FE\textsubscript{NO} groups, results after budesonide were compared to post-placebo values using paired t-tests and Wilcoxon signed ranked tests. PD\textsubscript{15} mannitol and FE\textsubscript{NO} were again analysed after logarithmic transformation and expressed as geometric means (95% confidence interval). The change in PD\textsubscript{15} mannitol following budesonide treatment in relation to baseline FE\textsubscript{NO} was analysed using simple linear regression.

Patients were classified as responders or non-responders based on the difference after budesonide compared to placebo. Responders were defined as: PD\textsubscript{15} mannitol increase ≥1 doubling dose (158); PD\textsubscript{15} mannitol increase ≥2 doubling doses; exercise FE\textsubscript{V}\textsubscript{1} \% fall\textsubscript{max} changed from ≥10% to <10% with treatment (complete protection) (104); ACQ reduction ≥0.5 (156); Borg Score reduction ≥1 (153).

Receiver Operating Characteristic (ROC) curves were constructed for FE\textsubscript{NO} as a predictor of steroid responsiveness (defined as at least one of: PD\textsubscript{15} mannitol increase ≥1 doubling dose, exercise FE\textsubscript{V}\textsubscript{1} \% fall\textsubscript{max} ≥10% to <10% or ACQ reduction ≥0.5). The optimum cut points for FE\textsubscript{NO} were selected from the measure with maximum combined sensitivity and specificity. Additionally, the corresponding positive predicted values, negative predictive values and percentage of correctly classified cases were reported.

Secondary analyses were done on the screening data to assess the correlation between baseline FE\textsubscript{NO} and the degree of AHR to mannitol (PD\textsubscript{15}) using linear regression. A ROC curve was also constructed to assess the predictive utility of FE\textsubscript{NO} in detecting those who would be hyper-responsive to either of the bronchial challenges.
Figure 5.1: CONSORT diagram of participant flow through study

Visit One
- Written informed consent
- Demographic Data collection
- Clinical Examination + Blood Pressure
- ECG
- Run-in diary for ICS patients
- FeNO and Spirometry for ICS patients
- Skin Prick Test

Visit One
- Asthma Control questionnaire
- FeNO measurement
- Spirometry
- Mannitol Challenge and bronchodilator response

Visit Three A
- Randomisation and Progression if either:
  - A provocative dose of mannitol causing a 15% fall in FEV₁ of less than 635mg
  - A maximum percentage fall in post-exercise FEV₁ of 10% or more from baseline

Visit Three B
- Exercise Challenge + Borg Score + Visual Analogue Score

Visit Two
- Steroid Withdrawal process if appropriate

Visit Four/Five A
- Asthma Control questionnaire
- FeNO measurement
- Spirometry
- Mannitol Challenge

Visit Four/Five B
- Exercise Challenge + Borg Score + Visual Analogue Score

FₑNO <45ppb
- Low Group
  - Inhaled Pulmicort 400µg 2b.d or matched placebo 2b.d for 28 days
  - 2 week wash out

FₑNO >45ppb
- High Group
  - Inhaled Pulmicort 400µg 2b.d or matched placebo 2b.d for 28 days
  - 2 week wash out

Tests repeated after each treatment arm

Phase One

Assessed for Eligibility (n=45)
- Excluded (n=26)
  - Inclusion/Exclusion (20)
  - Withdrew Consent (3)
  - Other (3)

Randomised (n=19)
- Excluded (n=2)
  - Withdrew Consent (1)
  - No longer met eligibility criteria (1)

Analysed (n=17)
- Low FₑNO (7)
  - Low FₑNO (7)
- High FₑNO (10)

n= 7
n= 10
Chapter Six: Results

6.1 Primary Analyses

6.1.1 Baseline Characteristics

(Tables 6.1 and 6.2)
Of the forty-five patients screened for this study, seventeen had AHR to mannitol and/or the exercise challenge and went on to complete the study (see Figure 5.1 for reasons for exclusion). Baseline characteristics of these patients and the twenty who did not meet the inclusion criteria, due to negative bronchial challenges, were compared. Those who were eligible had significantly higher baseline exhaled nitric oxide levels and asthma control questionnaire scores (56.6ppb vs. 22.7ppb; p=<0.001 and 1.0 vs. 0.5; p=0.022 respectively). There was also a greater prevalence of atopy in the eligible compared to the ineligible population (94% vs. 50%; p=0.008). The baseline FEV₁ as percent predicted was significantly lower in the eligible versus ineligible patients (99% vs. 109%; p=0.027).

After the seventeen eligible patients were divided into a low FₑNO group, (<45ppb; n=7), and a high FₑNO group, (>45ppb; n=10) baseline characteristics of these two populations were compared. As expected, there was a significant difference in the baseline levels of FₑNO between these two groups (31.6ppb vs. 85.0ppb; p=0.001). There were no other significant differences in any other baseline measures.

6.1.2 Comparison of results after placebo and budesonide for all patients

(Table 6.3)
Lung function measures, subjective asthma control, degree of airway hyper-responsiveness and presence of exercise-induced bronchoconstriction following treatment with inhaled budesonide were compared to placebo. Endpoints were firstly analysed for the seventeen eligible patients as one population. Following active treatment there were significant subjective improvements in asthma control as represented by a lower ACQ score (1.0 vs. 0.6; p=0.045). Following budesonide airway hyper-responsiveness to both the mannitol (PD₁₅) and exercise challenge (FEV₁% fall_max) was reduced (303mg vs. 545mg; p=0.018 and 4% vs. 0%; p=0.019 respectively).
Legend for Tables 6.1 and 6.2

Groups were compared using independent samples t-tests with the results expressed as mean (standard deviation) unless otherwise stated.

* Chi squared test with results expressed as n (%)  
† Fishers Exact test with results expressed as n (%)  
‡ Mann Whitney U test with results expressed as median (interquartile range)  
†† Analysed after logarithmic transformation with results expressed as geometric mean (95% confidence interval).
Table 6.1: Baseline Characteristics of Eligible vs. Ineligible Populations

<table>
<thead>
<tr>
<th></th>
<th>Eligible (n=17)</th>
<th>Ineligible – no AHR or EIB (n=20)</th>
<th>Mean Difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male*</td>
<td>6 (35%)</td>
<td>7 (35%)</td>
<td>-</td>
<td>0.985</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31 (11)</td>
<td>36 (16)</td>
<td>-5 (-14 to 4)</td>
<td>0.283</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170 (12)</td>
<td>170 (10)</td>
<td>0 (-7 to 7)</td>
<td>0.946</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 (4)</td>
<td>25 (4)</td>
<td>1 (-2 to 3)</td>
<td>0.608</td>
</tr>
<tr>
<td>Ex-Smokers†</td>
<td>2 (12%)</td>
<td>3 (15%)</td>
<td>-</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Previous Diagnosis of Asthma*</td>
<td>9 (53%)</td>
<td>10 (50%)</td>
<td>-</td>
<td>0.858</td>
</tr>
<tr>
<td>Steroid Naïve at entry+</td>
<td>16 (94%)</td>
<td>17 (85%)</td>
<td>-</td>
<td>0.609</td>
</tr>
<tr>
<td>ICS user at entry+</td>
<td>1 (6%)</td>
<td>3 (15%)</td>
<td>-</td>
<td>0.609</td>
</tr>
<tr>
<td>FeNO †</td>
<td>56.6 (41.1 to 77.8)</td>
<td>22.7 (18.1 to 28.6)</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atopic+ (n=34 had SPT)</td>
<td>15 (94%)</td>
<td>9 (50%)</td>
<td>-</td>
<td>0.008</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>3.41 (0.95)</td>
<td>3.64 (0.95)</td>
<td>-0.24 (-0.88 to 0.40)</td>
<td>0.453</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>99 (15)</td>
<td>109 (11)</td>
<td>-10 (-18 to -1)</td>
<td>0.027</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>77 (7)</td>
<td>80 (7)</td>
<td>-3 (-7 to 2)</td>
<td>0.269</td>
</tr>
<tr>
<td>FEV₁ % change post bronchodilator</td>
<td>3 (5)</td>
<td>3 (4)</td>
<td>0 (-3to 3)</td>
<td>0.853</td>
</tr>
<tr>
<td>ACQ</td>
<td>1.0 (0.7)</td>
<td>0.5 (0.4)</td>
<td>0.5 (0.1 to 0.9)</td>
<td>0.022</td>
</tr>
<tr>
<td>ACQ &gt;1.5+</td>
<td>6 (35%)</td>
<td>1 (5%)</td>
<td>-</td>
<td>0.033</td>
</tr>
<tr>
<td>PD₁₅ mannitol† (mg)</td>
<td>231 (142 to 374)</td>
<td>1230 (1176 to 1286)</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PD₁₅ mannitol &lt;635mg+</td>
<td>15 (88%)</td>
<td>0 (0%)</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exercise FEV₁ %fall_max ‡</td>
<td>7 (0 to 11)</td>
<td>0 (0 to 0)</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exercise FEV₁ %fall_max &gt;10% ‡</td>
<td>7 (41%)</td>
<td>0 (0%)</td>
<td>-</td>
<td>0.002</td>
</tr>
<tr>
<td>Borg</td>
<td>3 (2)</td>
<td>4 (1)</td>
<td>-1 (-2 to 0)</td>
<td>0.129</td>
</tr>
</tbody>
</table>
Table 6.2: Baseline Characteristics of low vs. high \(F_E\)NO groups

<table>
<thead>
<tr>
<th></th>
<th>Low (F_E)NO (n=7)</th>
<th>High (F_E)NO (n=10)</th>
<th>Mean Difference (95% CI)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male*</td>
<td>3 (43%)</td>
<td>3 (30%)</td>
<td>-</td>
<td>0.644</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33 (11)</td>
<td>30 (10)</td>
<td>3 (-8 to 14)</td>
<td>0.572</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170 (16)</td>
<td>170 (9)</td>
<td>0 (-15 to 15)</td>
<td>0.998</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25 (5)</td>
<td>25 (4)</td>
<td>0 (-5 to 5)</td>
<td>0.959</td>
</tr>
<tr>
<td>Ex-Smokers(\dagger)</td>
<td>1 (14%)</td>
<td>1 (10%)</td>
<td>-</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Previous Diagnosis of Asthma(\dagger)</td>
<td>4 (57%)</td>
<td>5 (50%)</td>
<td>-</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Steroid Naïve at entry(\dagger)</td>
<td>7 (100%)</td>
<td>9 (90%)</td>
<td>-</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>ICS user at entry(\dagger)</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
<td>-</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>(F_E)NO (\dagger)</td>
<td>31.6 (23.8 to 42.0)</td>
<td>85.0 (62.1 to 116.3)</td>
<td>-</td>
<td>0.001</td>
</tr>
<tr>
<td>Atopic(\dagger) (n=16 had SPT)</td>
<td>6 (86%)</td>
<td>9 (100%)</td>
<td>-</td>
<td>0.438</td>
</tr>
<tr>
<td>(FEV_1) (L)</td>
<td>3.37 (0.97)</td>
<td>3.43 (0.99)</td>
<td>-0.06 (-1.09 to 0.97)</td>
<td>0.900</td>
</tr>
<tr>
<td>(FEV_1) (% predicted)</td>
<td>97 (8)</td>
<td>100 (18)</td>
<td>-3 (-18 to 13)</td>
<td>0.719</td>
</tr>
<tr>
<td>(FEV_1/FVC)</td>
<td>75 (9)</td>
<td>79 (5)</td>
<td>-4 (-11 to 4)</td>
<td>0.343</td>
</tr>
<tr>
<td>(FEV_1) % change post bronchodilator</td>
<td>5 (4)</td>
<td>2 (5)</td>
<td>3 (-1 to 8)</td>
<td>0.135</td>
</tr>
<tr>
<td>ACQ</td>
<td>1.0 (0.8)</td>
<td>1.0 (0.7)</td>
<td>0 (-0.8 to 0.8)</td>
<td>0.957</td>
</tr>
<tr>
<td>ACQ &gt;1.5*</td>
<td>3 (43%)</td>
<td>3 (30%)</td>
<td>-</td>
<td>0.644</td>
</tr>
<tr>
<td>(PD_{15}) mannitol(\dagger) (mg)</td>
<td>324 (185 to 567)</td>
<td>182 (90 to 370)</td>
<td>-</td>
<td>0.264</td>
</tr>
<tr>
<td>(PD_{15}) mannitol &lt;635mg(\dagger)</td>
<td>6 (86%)</td>
<td>9 (90%)</td>
<td>-</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Exercise (FEV_1) %fall_{max}(\dagger)</td>
<td>3 (1 to 9)</td>
<td>11 (0 to 14)</td>
<td>-</td>
<td>0.767</td>
</tr>
<tr>
<td>Exercise (FEV_1) %fall_{max}&gt;10%(\dagger)</td>
<td>1 (14%)</td>
<td>6 (60%)</td>
<td>-</td>
<td>0.134</td>
</tr>
<tr>
<td>Borg</td>
<td>3 (1)</td>
<td>3 (2)</td>
<td>0 (-1 to 2)</td>
<td>0.683</td>
</tr>
</tbody>
</table>
Table 6.3: Comparison of post-placebo vs. budesonide results in all patients

<table>
<thead>
<tr>
<th></th>
<th>All eligible patients (n=17)</th>
<th>Mean Difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Budesonide</td>
<td></td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>3.37 (1.00)</td>
<td>3.41 (0.92)</td>
<td>0.04 (-0.04 to 0.12)</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>97 (15)</td>
<td>99 (14)</td>
<td>2 (-1 to 5)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>77 (8)</td>
<td>78 (8)</td>
<td>1 (0 to 2)</td>
</tr>
<tr>
<td>ACQ</td>
<td>1.0 (0.7)</td>
<td>0.6 (0.6)</td>
<td>-0.4 (-0.8 to 0.0)</td>
</tr>
<tr>
<td>PD₁₅ mannitol† (mg)</td>
<td>303 (172 to 534)</td>
<td>545 (364 to 816)</td>
<td>-</td>
</tr>
<tr>
<td>Exercise FEV₁ %fallₘ₉ᵃₓ‡</td>
<td>4 (0 to 11)</td>
<td>0 (0 to 3)</td>
<td>-</td>
</tr>
<tr>
<td>Borg</td>
<td>3 (2)</td>
<td>3 (1)</td>
<td>0 (0 to 1)</td>
</tr>
<tr>
<td>FₑNO†</td>
<td>54.3 (39.9 to 74.0)</td>
<td>26.8 (19.0 to 38.0)</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend for Table 6.5:
Groups were compared using paired t-tests with the results expressed as mean (standard deviation) unless otherwise stated.

‡ Wilcoxon signed ranks test with results expressed as median (interquartile range)

† Analysed after logarithmic transformation with results expressed as geometric mean (95% confidence interval)
6.1.3 Comparison of results after placebo and budesonide by $F_{\text{E}}$NO stratification

(Table 6.4 and 6.5)

Comparisons between study outcomes with placebo and budesonide were made for low and high $F_{\text{E}}$NO groups individually. There were no significant improvements in any of the measured endpoints following steroid treatment in the low $F_{\text{E}}$NO group, except for $F_{\text{E}}$NO itself which significantly reduced (33.4 ppb vs. 17.6 ppb; $p=0.006$).

In contrast, patients classified as having a high baseline $F_{\text{E}}$NO demonstrated a reduction in the degree of airway hyper-responsiveness, as measured by a significant increase in the PD$_{15}$ to mannitol (193 mg vs. 443 mg; $p=0.010$). Furthermore, in this high $F_{\text{E}}$NO group, budesonide reduced the degree of airway inflammation as measured by a significantly decreased $F_{\text{E}}$NO (76.5 ppb vs. 36.1 ppb; $p=0.007$). Improvements in asthma control (ACQ score) and AHR to exercise challenge approached, but did not reach, significance ($p=0.071$ and 0.063 respectively).

6.1.4 Proportion of responders in each $F_{\text{E}}$NO group

(Table 6.6)

The number of responders, characterised dichotomously for each of the study end-points was measured. In the low $F_{\text{E}}$NO group 43% had a PD$_{15}$ mannitol increase $\geq$1 doubling dose. In the high $F_{\text{E}}$NO group 60% had a PD$_{15}$ mannitol increase $\geq$1 doubling dose (of which one third of these had $\geq$2 doubling dose increases). Complete protection against EIB during the exercise challenge was seen in one person who had a low $F_{\text{E}}$NO and EIB, and in 4/5 of the subjects with high $F_{\text{E}}$NO and EIB. A significant reduction in ACQ score ($\geq0.5$) was seen in 43% of the low $F_{\text{E}}$NO group and 60% of the high $F_{\text{E}}$NO group.
Table 6.4: Comparison of post-placebo vs. budesonide results in low F\textsubscript{E}NO group

<table>
<thead>
<tr>
<th></th>
<th>Baseline F\textsubscript{E}NO &lt;45ppb (n=7)</th>
<th>Mean Difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Budesonide</td>
<td>Placebo</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (L)</td>
<td>3.31 (1.07)</td>
<td>3.37 (0.93)</td>
<td>0.05 (-0.13 to 2.39)</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (% predicted)</td>
<td>95 (9)</td>
<td>98 (9)</td>
<td>3 (-4 to 10)</td>
</tr>
<tr>
<td>FEV\textsubscript{1}/FVC</td>
<td>74 (10)</td>
<td>75 (10)</td>
<td>1 (-2 to 4)</td>
</tr>
<tr>
<td>ACQ</td>
<td>1.1 (0.9)</td>
<td>0.6 (0.7)</td>
<td>-0.4 (-1.4 to 0.5)</td>
</tr>
<tr>
<td>PD\textsubscript{15} mannitol\textsuperscript{†} (mg)</td>
<td>577 (235 to 1416)</td>
<td>733 (370 to 1451)</td>
<td>-</td>
</tr>
<tr>
<td>Exercise FEV\textsubscript{1} %fall\textsubscript{max} \textsuperscript{‡}</td>
<td>4 (0 to 6)</td>
<td>2 (0 to 3)</td>
<td>-</td>
</tr>
<tr>
<td>Borg</td>
<td>3 (1)</td>
<td>3 (2)</td>
<td>0 (-1 to 1)</td>
</tr>
<tr>
<td>F\textsubscript{E}NO (ppb)\textsuperscript{†}</td>
<td>33.4 (25.0 to 44.5)</td>
<td>17.6 (13.7 to 22.5)</td>
<td>-</td>
</tr>
</tbody>
</table>

**Legend for Table 6.4:**

Groups were compared using paired t-tests with the results expressed as mean (standard deviation) unless otherwise stated.

\textsuperscript{†}Wilcoxon signed ranks test with results expressed as median (interquartile range)

\textsuperscript{‡}Analysed after logarithmic transformation with results expressed as geometric mean (95% confidence interval)
### Table 6.5: Comparison of post-placebo vs. budesonide results in high FeNO group

<table>
<thead>
<tr>
<th></th>
<th>Baseline FeNO &gt;45ppb (n=10)</th>
<th>Mean Difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Budesonide</td>
<td></td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>3.30 (1.01)</td>
<td>3.44 (0.96)</td>
<td>0.14 (-0.11 to 0.38)</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>99 (19)</td>
<td>100 (17)</td>
<td>1 (-2 to 4)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>78 (6)</td>
<td>79 (5)</td>
<td>1 (-1 to 3)</td>
</tr>
<tr>
<td>ACQ</td>
<td>1.0 (0.6)</td>
<td>0.6 (0.5)</td>
<td>-0.5 (-0.9 to 0.0)</td>
</tr>
<tr>
<td>PD₁₅ mannitol† (mg)</td>
<td>193 (103 to 359)</td>
<td>443 (274 to 716)</td>
<td>-</td>
</tr>
<tr>
<td>Exercise FEV₁ %fallₘₐₓ‡</td>
<td>7 (0 to 11)</td>
<td>0 (0 to 1)</td>
<td>-</td>
</tr>
<tr>
<td>Borg</td>
<td>3 (2)</td>
<td>3 (1)</td>
<td>0 (-1 to 1)</td>
</tr>
<tr>
<td>FeNO†</td>
<td>76.5 (53.5 to 109.3)</td>
<td>36.1 (22.0 to 59.2)</td>
<td>-</td>
</tr>
</tbody>
</table>

**Legend for Table 6.5:**

Groups were compared using paired t-tests with the results expressed as mean (standard deviation) unless otherwise stated.

†Wilcoxon signed ranks test with results expressed as median (interquartile range)

‡Analysed after logarithmic transformation with results expressed as geometric mean (95% confidence interval)
Table 6.6: Proportion of responders to budesonide in each $F_E NO$ group

<table>
<thead>
<tr>
<th>Outcome Description</th>
<th>$F_E NO &lt;45$ppb (n=7) % responders</th>
<th>$F_E NO &gt;45$ppb (n=10) % responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD$_{15}$ mannitol increase $\geq$ 1 doubling dose</td>
<td>3/7 (43%)</td>
<td>6/10 (60%)</td>
</tr>
<tr>
<td>PD$_{15}$ mannitol increase $\geq$ 2 doubling doses</td>
<td>0/7 (0%)</td>
<td>2/10 (20%)</td>
</tr>
<tr>
<td>Exercise FEV$<em>1$ % fall$</em>{\text{max}}$ $\geq$ 10% to &lt;10% (complete protection)</td>
<td>1/1 (100%)</td>
<td>4/5 (80%)</td>
</tr>
<tr>
<td>ACQ reduction $\geq$ 0.5</td>
<td>3/7 (43%)</td>
<td>6/10 (60%)</td>
</tr>
<tr>
<td>Borg Score reduction $\geq$ 1</td>
<td>1/7 (14%)</td>
<td>2/10 (20%)</td>
</tr>
</tbody>
</table>
6.1.5 Relationship between baseline FeNO and the change in AHR following treatment with budesonide

(Figures 6.1 and 6.2)

Using linear regression, the correlation between baseline FeNO (logarithmically transformed) in the seventeen study patients and the change in log PD_{15} to mannitol (post-budesonide logPD_{15} – post-placebo logPD_{15}) was very weak with an r-value of 0.384 (p=0.129).

Over the duration of the study, concerns of inadequate steroid deposition in the airways and subsequent sub-therapeutic dosing in one particular patient were raised (clinical justifications discussed further in Chapter Seven). The linear regression analysis was repeated with the results from this patient excluded (n=16) and a newly calculated r-value of 0.647 was generated, with a highly significant relationship between these variables (p=0.007).
Figure 6.1: The correlation between the change in PD$_{15}$ to mannitol with ICS compared to placebo and baseline F$_{E}$NO (n=17)
Figure 6.2: The correlation between the change in PD$_{15}$ to mannitol with ICS compared to placebo and baseline F$_{E}$NO (n=16)
6.1.6 Utility of $F_{E}NO$ for predicting steroid responsiveness

(Figures 6.3 - 6.6 and Tables 6.7 – 6.10)
Receiver Operating Characteristic (ROC) curves were constructed for the utility of $F_{E}NO$ in predicting steroid responsiveness defined as one or more of the following: $PD_{15}$ mannitol increase of $\geq 1$ doubling dose, ACQ score reduction of $\geq 0.5$, or complete protection from exercise challenge in those with EIB. This yielded an area under the curve (AUC) of 0.833 and an optimum $F_{E}NO$ cut-point of 41.0ppb. At this cut-point the corresponding sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 78.6%, 66.7%, 91.7% and 40.0% respectively. Additionally at this cut-point just over three quarters (76.5%) of patients were correctly classified for steroid responsiveness.

ROC curves and corresponding $F_{E}NO$ cut points were also constructed for individual measures of steroid response ($PD_{15}$ mannitol increase of $\geq 1$ doubling dose, ACQ score reduction of $\geq 0.5$ and complete protection from exercise challenge in those with EIB). The calculated AUC values were 0.639, 0.569 and 0.000 respectively.
Figure 6.3: Receiver Operating Characteristic curve of different FeNO cut points for steroid responsiveness

Legend for Figure 6.3:
Steroid responsiveness defined by one or more of the following:
   PD_{15} mannitol increase of ≥1 doubling dose
   ACQ score reduction of ≥0.5
   Complete protection from exercise challenge in those with EIB
Table 6.7: Sensitivities and specificities of different FE\textsubscript{NO} cut points for steroid responsiveness

<table>
<thead>
<tr>
<th>FE\textsubscript{NO} (ppb)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Correctly Classified (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥14.0</td>
<td>100.0</td>
<td>0.0</td>
<td>82.4</td>
<td>-</td>
<td>82.4</td>
</tr>
<tr>
<td>≥27.5</td>
<td>100.0</td>
<td>33.3</td>
<td>87.5</td>
<td>100.0</td>
<td>88.2</td>
</tr>
<tr>
<td>≥35.5</td>
<td>92.9</td>
<td>33.3</td>
<td>86.7</td>
<td>50.1</td>
<td>82.4</td>
</tr>
<tr>
<td>≥41.0</td>
<td>78.6</td>
<td>66.7</td>
<td>91.7</td>
<td>40.0</td>
<td>76.5</td>
</tr>
<tr>
<td>≥52.0</td>
<td>64.3</td>
<td>100.0</td>
<td>100.0</td>
<td>37.5</td>
<td>70.6</td>
</tr>
<tr>
<td>≥58.0</td>
<td>57.1</td>
<td>100.0</td>
<td>100.0</td>
<td>33.3</td>
<td>64.7</td>
</tr>
<tr>
<td>≥75.5</td>
<td>42.9</td>
<td>100.0</td>
<td>100.0</td>
<td>27.3</td>
<td>52.9</td>
</tr>
<tr>
<td>≥80.5</td>
<td>35.7</td>
<td>100.0</td>
<td>100.0</td>
<td>25.0</td>
<td>47.1</td>
</tr>
<tr>
<td>≥164.0</td>
<td>14.3</td>
<td>100.0</td>
<td>100.0</td>
<td>20.0</td>
<td>29.4</td>
</tr>
<tr>
<td>&gt;237.0</td>
<td>0.0</td>
<td>100.0</td>
<td>-</td>
<td>17.6</td>
<td>17.7</td>
</tr>
</tbody>
</table>

Legend for Table 6.7:
Steroid responsiveness defined by one or more of the following:

- PD\textsubscript{15} mannitol increase of ≥1 doubling dose
- ACQ score reduction of ≥0.5
- Complete protection from exercise challenge in those with EIB

FE\textsubscript{NO} cut-points selected from data points on corresponding ROC curve (Figure 6.3)
Optimal cut-point (blue) represents value with the highest combined sensitivity and specificity

PPV = Positive Predicted Value
NPV = Negative Predicted Value
Figure 6.4: Receiver Operating Characteristic curve of different $F_{E\text{NO}}$ cut-points for $PD_{15}$ mannitol increase of $\geq 1$ doubling dose
Table 6.8: Sensitivities and specificities of different $F_{ENO}$ cut-points for PD$_{15}$ mannitol increase of $\geq 1$ doubling dose

<table>
<thead>
<tr>
<th>$F_{ENO}$ (ppb)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Correctly Classified (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 14.0$</td>
<td>100.0</td>
<td>0.0</td>
<td>52.9</td>
<td>-</td>
<td>52.9</td>
</tr>
<tr>
<td>$\geq 27.5$</td>
<td>100.0</td>
<td>12.5</td>
<td>53.6</td>
<td>100.0</td>
<td>58.8</td>
</tr>
<tr>
<td>$\geq 35.5$</td>
<td>100.0</td>
<td>25.0</td>
<td>60.0</td>
<td>100.0</td>
<td>64.7</td>
</tr>
<tr>
<td>$\geq 41.0$</td>
<td>77.8</td>
<td>37.5</td>
<td>58.3</td>
<td>60.0</td>
<td>58.8</td>
</tr>
<tr>
<td>$\geq 52.0$</td>
<td>66.7</td>
<td>62.5</td>
<td>66.7</td>
<td>62.5</td>
<td>64.7</td>
</tr>
<tr>
<td>$\geq 58.0$</td>
<td>66.7</td>
<td>75.0</td>
<td>75.0</td>
<td>66.7</td>
<td>70.6</td>
</tr>
<tr>
<td>$\geq 75.5$</td>
<td>44.4</td>
<td>75.0</td>
<td>66.6</td>
<td>54.5</td>
<td>58.8</td>
</tr>
<tr>
<td>$\geq 80.5$</td>
<td>33.3</td>
<td>75.0</td>
<td>60.0</td>
<td>50.0</td>
<td>52.9</td>
</tr>
<tr>
<td>$\geq 164.0$</td>
<td>11.1</td>
<td>87.5</td>
<td>50.0</td>
<td>46.7</td>
<td>47.1</td>
</tr>
<tr>
<td>$&gt; 237.0$</td>
<td>0.0</td>
<td>100.0</td>
<td>-</td>
<td>47.1</td>
<td>47.1</td>
</tr>
</tbody>
</table>

Legend for Table 6.10:
$F_{ENO}$ cut-points selected from data points on corresponding ROC curve (Figure 6.4)
Optimal cut-point (blue) represents value with the highest combined sensitivity and specificity
PPV= Positive Predicted Value
NPV= Negative Predicted Value
Figure 6.5: Receiver Operating Characteristic curve of different $F_{\text{E}}\text{NO}$ cut-points for Asthma Control Questionnaire reduction $\geq 0.5$
Table 6.9: Sensitivities and specificities of different $\text{FE}NO$ cut-points for Asthma Control Questionnaire reduction $\geq 0.5$

<table>
<thead>
<tr>
<th>$\text{FE}NO$ (ppb)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Correctly Classified (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 14.0$</td>
<td>100.0</td>
<td>0.0</td>
<td>52.9</td>
<td>-</td>
<td>52.9</td>
</tr>
<tr>
<td>$\geq 27.5$</td>
<td>100.0</td>
<td>12.5</td>
<td>56.3</td>
<td>100.0</td>
<td>58.8</td>
</tr>
<tr>
<td>$\geq 35.5$</td>
<td>88.9</td>
<td>12.5</td>
<td>53.3</td>
<td>50.0</td>
<td>52.9</td>
</tr>
<tr>
<td>$\geq 41.0$</td>
<td>77.9</td>
<td>37.5</td>
<td>58.4</td>
<td>60.1</td>
<td>58.8</td>
</tr>
<tr>
<td>$\geq 52.0$</td>
<td>66.7</td>
<td>62.5</td>
<td>66.7</td>
<td>62.5</td>
<td>64.7</td>
</tr>
<tr>
<td>$\geq 58.0$</td>
<td>55.6</td>
<td>62.5</td>
<td>62.5</td>
<td>55.6</td>
<td>58.8</td>
</tr>
<tr>
<td>$\geq 75.5$</td>
<td>33.3</td>
<td>62.5</td>
<td>50.0</td>
<td>45.4</td>
<td>47.1</td>
</tr>
<tr>
<td>$\geq 80.5$</td>
<td>22.2</td>
<td>62.5</td>
<td>40.0</td>
<td>41.7</td>
<td>41.2</td>
</tr>
<tr>
<td>$\geq 164.0$</td>
<td>22.2</td>
<td>100.0</td>
<td>100.0</td>
<td>53.3</td>
<td>58.8</td>
</tr>
<tr>
<td>$&gt;237.0$</td>
<td>0.0</td>
<td>100.0</td>
<td>-</td>
<td>47.1</td>
<td>47.1</td>
</tr>
</tbody>
</table>

Legend for Table 6.9:

$\text{FE}NO$ cut-points selected from data points on corresponding ROC curve (Figure 6.5)

Optimal cut-point (blue) represents value with the highest combined sensitivity and specificity

PPV= Positive Predicted Value

NPV= Negative Predicted Value
Figure 6.6: Receiver Operating Characteristic curve of different $F_{E\text{NO}}$ cut-points for complete protection from exercise-induced bronchoconstriction

Legend for Figure 6.6:
ROC curve constructed for those who had demonstrable EIB ($n=6$) and thus the calculated AUC is poor due to the small number of data points
(Further discussion of this in Chapter Seven)
Table 6.10: Sensitivities and specificities of different F\textsubscript{E}NO cut-points for complete protection from exercise-induced bronchoconstriction

<table>
<thead>
<tr>
<th>F\textsubscript{E}NO (ppb)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Correctly Classified (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥41.0</td>
<td>100.0</td>
<td>0.0</td>
<td>83.3</td>
<td>-</td>
<td>83.3</td>
</tr>
<tr>
<td>≥51.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.00</td>
</tr>
<tr>
<td>≥59.5</td>
<td>60.0</td>
<td>0.0</td>
<td>75.0</td>
<td>0.0</td>
<td>50.0</td>
</tr>
<tr>
<td>≥75.5</td>
<td>40.0</td>
<td>0.0</td>
<td>66.7</td>
<td>0.0</td>
<td>33.3</td>
</tr>
<tr>
<td>≥80.5</td>
<td>20.0</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
<td>16.7</td>
</tr>
<tr>
<td>≥237.0</td>
<td>0.0</td>
<td>100.0</td>
<td>-</td>
<td>16.7</td>
<td>16.7</td>
</tr>
<tr>
<td>&gt;237.0</td>
<td>0.0</td>
<td>100.0</td>
<td>-</td>
<td>16.7</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Legend for Table 6.10:
F\textsubscript{E}NO cut-points selected from data points on corresponding ROC curve (Figure 6.6)
An optimum cut-point was not selected due to the poor ROC curve with few data points (n=6)
PPV= Positive Predicted Value
NPV= Negative Predicted Value
6.2 Secondary Analyses

6.2.1 Relationship between $F_{E\text{NO}}$ and AHR in patients presenting with exercise symptoms suggestive of asthma

(Figures 6.7 and 6.8, Table 6.11)
Linear regression was used to assess the correlation between $F_{E\text{NO}}$ and degree of airway hyper-responsiveness to mannitol yielding an $r$-value of 0.577 ($p<0.001$). ROC curve of $F_{E\text{NO}}$ and presence of airway hyper-responsiveness ($PD_{15}$ mannitol $<635mg$ and/or EIB) produced an AUC of 0.858 and an optimum cut point for predicting airway hyper-responsiveness of 35.5ppb. At this cut-point the corresponding sensitivity, specificity, positive predictive value and negative predictive value of $F_{E\text{NO}}$ were 89.5%, 80.0%, 81.0% and 88.9% respectively. Using the calculated optimum $F_{E\text{NO}}$ cut-point (35.5ppb) 84.6% of this population were correctly classified for presence or absence of AHR.
Figure 6.7: Baseline $F_{E\text{NO}}$ and relationship to baseline $PD_{15}$ to mannitol
Figure 6.8: Receiver Operating Characteristic curve of different $F_{E\text{NO}}$ cut points for predicting airway hyper-responsiveness to mannitol and/or exercise-induced bronchoconstriction

Legend for Figure 6.8:
AHR to mannitol defined as $PD_{15} \leq 635\text{mg}$
EIB defined as a decrease of $\geq 10\%$ in $FEV_1$ after exercise challenge
Table 6.11: Sensitivities and specificities of different $F_{E}NO$ cut points for predicting hyper-responsiveness to mannitol and/or exercise-induced bronchoconstriction

<table>
<thead>
<tr>
<th>$F_{E}NO$ (ppb)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Correctly Classified (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 11.0$</td>
<td>100.0</td>
<td>0.0</td>
<td>48.7</td>
<td>-</td>
<td>48.7</td>
</tr>
<tr>
<td>$\geq 14.0$</td>
<td>100.0</td>
<td>10.0</td>
<td>51.4</td>
<td>100.0</td>
<td>53.9</td>
</tr>
<tr>
<td>$\geq 21.5$</td>
<td>94.7</td>
<td>60.0</td>
<td>69.2</td>
<td>92.3</td>
<td>77.0</td>
</tr>
<tr>
<td>$\geq 27.5$</td>
<td>94.7</td>
<td>70.0</td>
<td>75.0</td>
<td>93.3</td>
<td>82.1</td>
</tr>
<tr>
<td>$\geq 35.5$</td>
<td>89.5</td>
<td>80.0</td>
<td>81.0</td>
<td>88.9</td>
<td>84.6</td>
</tr>
<tr>
<td>$\geq 40.5$</td>
<td>73.7</td>
<td>80.0</td>
<td>77.8</td>
<td>76.2</td>
<td>76.9</td>
</tr>
<tr>
<td>$\geq 50.0$</td>
<td>63.2</td>
<td>90.0</td>
<td>85.7</td>
<td>72.0</td>
<td>76.9</td>
</tr>
<tr>
<td>$\geq 62.0$</td>
<td>31.6</td>
<td>90.0</td>
<td>75.0</td>
<td>58.1</td>
<td>61.5</td>
</tr>
<tr>
<td>$\geq 80.5$</td>
<td>26.3</td>
<td>100.0</td>
<td>100.0</td>
<td>58.8</td>
<td>64.1</td>
</tr>
<tr>
<td>$\geq 164.0$</td>
<td>10.5</td>
<td>100.0</td>
<td>100.0</td>
<td>54.0</td>
<td>56.4</td>
</tr>
<tr>
<td>$&gt;237.0$</td>
<td>0.0</td>
<td>100.0</td>
<td>-</td>
<td>51.2</td>
<td>51.3</td>
</tr>
</tbody>
</table>

Legend for Table 6.11:

$F_{E}NO$ cut-points selected from data points on corresponding ROC curve (Figure 6.8)

Optimal cut-point (blue) represents value with the highest combined sensitivity and specificity

PPV = Positive Predicted Value

NPV = Negative Predicted Value
Chapter Seven: Discussion

When taken regularly, inhaled corticosteroids can reduce bronchial inflammation (70), exercise-induced bronchoconstriction (70-74) and airway hyper-responsiveness (71, 74). For these reasons they are widely used in the treatment of asthma. However, there is variability in the individual treatment responses (75). It is now recognised that differing pathology in the airways of asthmatics may be responsible for the observed variation in steroid responsiveness. Patients with eosinophilic airway inflammation compared to non-eosinophilic inflammation are more likely to respond to inhaled steroid and demonstrate greater clinical benefits (70). The level of exhaled nitric oxide ($F_{E}NO$) in the breath can be used as a surrogate biomarker for eosinophilic inflammation (125) and is a simple, non-invasive test that can be successfully undertaken in children (128). Additionally, $F_{E}NO$ measurements have been shown to predict steroid responsiveness in patients with non-specific respiratory symptoms (149). The value of having a simple marker that could potentially provide evidence to identify treatment responders \textit{a priori}, and thus minimise the number of patients who are treated inappropriately, was explored by this thesis for this patient population.

The study contained in this thesis tested the hypothesis that non-smoking patients with exercise-induced respiratory symptoms and high $F_{E}NO$ are more likely to respond to inhaled corticosteroid treatment, compared to those with low $F_{E}NO$. The study which preceded this research (98) clearly demonstrated the effectiveness of inhaled corticosteroids in patients with high $F_{E}NO$ and the potential role of alternative therapies for those with low $F_{E}NO$. However, that study was not placebo controlled. To the best of our knowledge this latest study is the first placebo controlled trial used to assess the predictive utility of $F_{E}NO$ measurements for steroid responsiveness in patients with exercise-induced asthma symptoms and airway hyper-responsiveness to mannitol and/or exercise challenge.

It is acknowledged that at the time of writing this thesis, the study is underpowered. A target enrolment of fifteen patients per $F_{E}NO$ group was intended, however, due to the low inclusion rate this was not achieved in the allotted time to complete this BMedSc (Hons) thesis. It is intended that recruitment will continue for this study and the final analysis will
be undertaken when the target sample size has been reached. However there are still valuable insights to be made with the current results at hand.

The first aim of this study was to calculate the value of $F_E$NO measurements in patients with exercise-induced symptoms suggestive of asthma and AHR, as a predictor of their subsequent response to inhaled corticosteroids. With a calculated area under the ROC curve of 0.833, the predictive value was found to be adequate but not highly significant. The optimum cut-point for $F_E$NO to predict steroid responsiveness in this population was calculated to be 41.0ppb. The corresponding sensitivity and specificity at this cut-point was 78.6% and 66.7% respectively. The positive predicted value was very high at 91.7% suggesting that those patients with EIA and a pre-treatment $F_E$NO \(\geq\)41.0ppb are highly likely to respond to an inhaled corticosteroid. The negative predicted value at this optimum cut-point was 40.0%, somewhat lower than expected. Smith et al. reported high negative predicted values for $F_E$NO and measures of steroid response in undiagnosed symptomatic patients (149). In our population, the prevalence of steroid responsiveness was high and this may explain the observed differences. The value of a low $F_E$NO however still had a very high NPV which is in keeping with the literature. Further, 76.5% of our population were correctly classified for steroid responsiveness using 41.0ppb as a cut-point. Thus, $F_E$NO may be of value when clinicians are making decisions regarding which is the most appropriate treatment to prescribe for patients presenting with exercise-induced asthma symptoms.

The second aim of this study was to compare the effectiveness of inhaled corticosteroids in the management of patients with exercise-induced asthma symptoms who had low versus high $F_E$NO at presentation. After one month of treatment with budesonide, subjective asthma control and airway hyper-responsiveness, were compared to post-placebo results. There were no observed differences in the low $F_E$NO group. In contrast, the high $F_E$NO group demonstrated reduced AHR to the mannitol challenge, measured by increase in $PD_{15}$, following budesonide treatment. Furthermore, in this high $F_E$NO group, the mean difference for ACQ reduction and exercise challenge protection approached, but did not reach, statistical significance (p=0.071 and 0.063 respectively). It may be that with a larger sample size a significant difference will be detected.
It is important to note that for both FeNO groups, resting lung function measures, FEV1 and FEV1/FVC, did not change following treatment. This may be explained by the observation that the majority of the patients enrolled had near normal spirometry at rest. This is unsurprising as by definition all of the patients included in the study exclusively experienced exercise as their predominant provoking trigger and therefore at rest it is unlikely that abnormal lung function would have been detected. This is often the case in asthma: spirometry is very poorly sensitive except in patients with more severe disease.

The third aim of this study was to confirm that pre-treatment stratification using FeNO is an important way to approach the management of patients with exercise induced asthma symptoms. Current asthma guidelines advocate a stepwise approach to treatment (1). Short-acting beta2 agonists are recommended “as required” for relief of asthma symptoms in mild intermittent asthma. When symptoms become more frequent the addition of an inhaled corticosteroid is recommended. However, someone who only experiences these symptoms with exercise may utilise trigger avoidance, by not exercising, and consequently report a low frequency of asthma symptoms. Often such patients will still have active airway inflammation. The concern is that in some patients chronic airway inflammation may lead to permanent airway remodelling and fixed airway obstruction (159). In patients with severe asthma, the presence of sputum eosinophilia was strongly associated (OR=7.7) with persistent airflow limitation (160). Further, there is an increased rate of decline in lung function seen in asthmatic versus non-asthmatic patients; FEV1 decreased by 38mL and 22mL per year respectively (161). Although the precise mechanism has yet to be clarified, controlling airway inflammation in asthmatic airways with inhaled corticosteroids can potentially reduce this rate of decline in lung function (162). Additionally, Hahtela et al., showed that inhaled corticosteroids earlier rather than later in newly diagnosed asthmatics (by two years) resulted in a better response to treatment, in comparison to those who were treated later (163). Therefore, early detection and treatment of airway inflammation responsive to inhaled corticosteroids may reduce this rate of decline in airway calibre and limit the development of fixed airway obstruction. The ability to easily detect underlying airway inflammation likely to respond to inhaled corticosteroid is therefore clinically important.

Exhaled nitric oxide is a biomarker which can provide insight into the degree of airway inflammation (124, 164) and severity of hyper-responsiveness in asthmatic individuals (70,
Utilisation of this test by clinicians at time of presentation may allow detection of patients with underlying inflammation which is likely to respond to inhaled steroids. The study in this thesis demonstrated that $F_{E\text{NO}}$ had a high predictive utility for steroid responsiveness and provides further evidence that $F_{E\text{NO}}$ should be measured in the diagnostic work-up of patients with chronic respiratory symptoms. Additionally those classified in the high $F_{E\text{NO}}$ group showed clinically significant improvement in hyper-responsiveness compared to those with a low baseline $F_{E\text{NO}}$. Taken together, these findings confirm that pre-treatment stratification using $F_{E\text{NO}}$ is a helpful adjunct to approach the management of patients with exercise-induced asthma.

The results of our study do not mean that the amount of inhaled corticosteroids prescribed for exercise-induced asthma should be increased. Indeed the opposite is true. The identification of a steroid “responder” has the potential to facilitate more appropriate prescribing, by enabling pre-selection of those likely to have a favourable response. This would also allow those patients unlikely to respond to ICS to be promptly initiated on an alternative therapy such as a leukotriene antagonist or mast cell stabiliser (98).

As previously noted one of the limitations of this study is the relatively small number of patients, enrolled and subsequent study power. Despite screening forty five symptomatic patients only seventeen were eligible for the study and analysis. This low inclusion rate of 35% resulted in an inability to recruit the desired numbers. The study preceding this research in the department, by Cowan et al., shared a very similar inclusion rate of 38% (98). The major reason for exclusion for both of these studies was the negative responses to bronchial challenge testing. This study used the change in airway hyper-responsiveness to mannitol and exercise challenge following budesonide treatment as primary endpoints. Previous studies have used exercise challenge alone to assess treatment response (70, 72-73, 106), however the sensitivity of laboratory based exercise testing is low (31, 48) and therefore the results of these studies may not be generalisable to the wider population with exercise-induced asthma symptoms. Eucapnic voluntary hyperventilation (EVH) is recognised by the International Olympic Committee as the optimum test for detecting exercise-induced bronchoconstriction. However, there are currently no facilities with the required equipment in Dunedin and thus we were unable to utilise EVH for this study. The mannitol challenge has been shown to have a similar sensitivity and specificity to EVH and requires minimal equipment. For these reasons we elected to use the mannitol challenge, in
conjunction with standard exercise testing, to measure treatment outcomes in this study. At baseline only 41% of the enrolled patients had demonstrable exercise-induced bronchoconstriction on exercise testing in comparison to 88% who demonstrated airway hyper-responsiveness to the mannitol challenge.

Only 41% of the enrolled patients demonstrated EIB objectively and this reflects the previously reported low sensitivity of laboratory based exercise challenges (31, 48). A target ventilation range of 50-60% maximum voluntary ventilation for the final four minutes was used as the standardised stimulus for each patient. Workloads on the cycle ergometer were adjusted as necessary to maintain ventilation rates in this range. Subsequently, some patients, particularly those who were frequently active or competitive athletes, described the exercise challenge as significantly easier than what they would push themselves in training or competition where their symptoms are usually triggered. This may also account for the low number of patients having demonstrable bronchoconstriction following the exercise challenge.

When linear regression was used to assess the relationship between baseline \( F_{E}NO \) and change in \( PD_{15} \) to mannitol with budesonide a somewhat weak \( r \)-value of 0.383 (\( p=0.129 \)) was generated. As with all the treatment response analyses executed, every patient eligible (\( n=17 \)) was included. However, one patient with a particularly high \( F_{E}NO \) level remained unchanged in all endpoint outcomes throughout the duration of the study. Concerns of inadequate steroid deposition in the airways and subsequent sub-therapeutic treatment were raised. Additional clinically requested tests following completion of the study, demonstrated a marked improvement in baseline spirometry and reduction in \( F_{E}NO \) following a short oral steroid trial. This suggests that the results obtained using inhaled rather than systemic steroid treatment are possibly suboptimal, due to likely sub-therapeutic dosing. However, the clinical aim of the study required a pragmatic approach -it is not justified to use oral steroids to treat exercise induced asthma! Later, the linear regression analysis was repeated with the results from this patient excluded. A newly calculated \( r \)-value of 0.647 (\( p=0.007 \)) was generated, suggesting a highly significant relationship between these variables.

Increased levels of nitric oxide in the exhaled air of asthmatics were reported as early as 1993 (124). Patients who experience exercise-induced bronchoconstriction also have higher
levels of $F_E$NO compared to healthy controls (146). Additionally, the degree of exercise-induced bronchoconstriction has been shown to be significantly correlated with the level of exhaled nitric oxide (147-148). Secondary analyses in our study were consistent with these findings. Receiver Operating Characteristic curve of $F_E$NO as a predictor of the presence of airway hyper-responsiveness to mannitol and/or exercise challenge yielded an AUC of 0.858. The optimum diagnostic cut-point of 35.5ppb allowed 84.6% of this population to be correctly classified for presence or absence of AHR. These results support the use of $F_E$NO in providing evidence of likely airway hyper-responsiveness in symptomatic patients. It is easier and less expensive to measure $F_E$NO than to conduct a challenge test. Potentially, $F_E$NO measurements could reduce the need for time and resource consuming bronchial challenge tests.

At the study outset, we were aiming for three eligible $F_E$NO groups with cut-points of 25 and 45ppb. However after screening forty five people for the study only two patients (4%) with a $F_E$NO <25ppb demonstrated airway hyper-responsiveness. The decision was made to compare two $F_E$NO groups, high versus low, divided by the cut-point of 45ppb. This decision is supported by the very high negative predictive value of a low $F_E$NO for presence of AHR (~93% at a cut-point of 25ppb) detected by this study and similarly supported by other research (146). No alteration was made to the inclusion or exclusion criteria thus it is unlikely that this change would have introduced any bias.

The blinded, crossover design of this study should have eliminated the likelihood of selection bias. Reassuringly, the randomisation sequence split both the low and high $F_E$NO groups equally to order of treatments (4/7 of low $F_E$NO and 5/10 of high $F_E$NO group received budesonide first). There were additionally no differences in the baseline characteristics of these two study populations except for the classifying variable of $F_E$NO.
Chapter Eight: Summary and Conclusions

Exercise-induced symptoms are common, and the approach to diagnosis and treatment in primary care is usually empiric. This is because there is limited access to objective testing such as spirometry and bronchoprovocation tests. Even were they to be more readily available, the tests are insensitive. Further to this there are many treatment options for exercise-induced asthma, each with their own variable levels of efficacy. Inhaled corticosteroids remain the first line treatment in preventing asthma symptoms yet there is wide individual variability in their ability to reduce bronchial inflammation, exercise-induced bronchoconstriction and airway hyper-responsiveness. Therefore, selecting the most appropriate therapeutic intervention, if any, in patients presenting with exercise-induced cough, wheeze or dyspnoea can be somewhat of a challenge for physicians.

Based on our study results, the role of exhaled nitric oxide in the management of these patients appears promising. We know that there are a number of inflammatory phenotypes in asthma that respond differently to the first line treatment, inhaled corticosteroids. Pre-selecting those likely to respond to steroid would enable prompt initiation of treatment and potentially eliminate the need for empiric trials. Exhaled nitric oxide is a marker of the steroid responsive airway inflammation and the utility of exhaled nitric oxide in predicting steroid responsiveness is high for patients with non-specific respiratory symptoms.

To our knowledge this study is the first placebo-controlled trial to examine the role of exhaled nitric oxide measurements in managing patients with symptoms suggestive of exercise-induced asthma. Patients with a high pre-treatment $F_{E\text{NO}}$ demonstrated clinical improvements following treatment with budesonide, whereas those with a low $F_{E\text{NO}}$ showed no improvement. These data support the use of this simple test to aid clinical decisions in the management of patients with exercise-induced respiratory symptoms.
References


(ERS) and the European Academy of Allergy and Clinical Immunology (EAACI) in cooperation with GA2LEN. Allergy. 2008 Apr;63(4):387-403.


volume in one second (FEV1) during bronchoconstriction induced by different stimuli. J Asthma. 1997;34(2):105-11.


66. Simons FE, Gerstner TV, Cheang MS. Tolerance to the bronchoprotective effect of salmeterol in adolescents with exercise-


74. Vathenen AS, Knox AJ, Wisniewski A, Tattersfield AE. Effect of inhaled budesonide on bronchial reactivity to histamine, exercise, and


