SAFETY ASPECTS OF β_2-AGONISTS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Christopher Ian Whale
MBChB MRCP

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ABSTRACT

Chronic Obstructive Pulmonary Disease (COPD) presents an enormous public health challenge. Cigarette smoking remains the most important aetiological factor and although legislation to reduce smoking has been introduced in parts of the more developed world, consumption is increasing in many of the poorest parts of the world. With the predicted rise in disease prevalence, COPD is expected to become the world's third largest cause of death by 2020.

COPD is a disease state characterised by airflow limitation that is not fully reversible. Inhaled bronchodilators can only produce a small improvement in the airflow obstruction, but despite this, patients with COPD frequently use high doses of β_2-agonists as the disease progresses and they develop breathlessness and exercise limitation.

Short-acting β_2-agonists are generally used 'as required' to reduce breathlessness and reduce airflow obstruction whereas long-acting β_2-agonists are prescribed on a regular twice-daily basis to reduce symptoms and rescue medication use and because of a potential beneficial effect on quality of life and exacerbation rates.

Although generally well tolerated, the safety of inhaled β_2-agonists has been a source of some concern since the late 1960s, when an epidemic of asthma deaths was associated with the use of a high dose formulation of isoprenaline. Further controversy has followed and questions have extended to long-acting β_2-agonists, most notably after a recent large-scale post marketing surveillance study identified an association between the regular use of inhaled salmeterol and asthma-related deaths.

The safety of inhaled β_2-agonists is also an important consideration for patients with COPD. Being older and likely to have a history of cigarette consumption means that they are at risk of having symptomatic, or subclinical, ischaemic heart disease. β_2-agonists cause a number of systemic effects including an increase in heart rate, transient hypoxaemia and hypokalaemia. Since many patients with COPD are already hypoxaemic and may be taking other drugs that stimulate the myocardium and cause hypokalaemia, the additional systemic effects from β_2-agonists may be more likely to produce adverse cardiac events including dysrhythmia and ischaemia.

This thesis is concerned with the safety of inhaled β_2-agonists in the management of COPD. The introduction consists of an overview of the epidemiology, natural history and pathology of COPD (Chapter 1) and a review of human β_2-adrenoceptor function and inhaled β_2-agonist pharmacology (Chapter 2). This is followed by a systematic literature review of the results from long-term clinical studies of inhaled β_2-agonists in subjects with COPD (Chapter 3). The original work consists of three clinical studies that have examined aspects of the effect of high dose inhaled β_2-agonists in subjects with COPD and a discussion to place these findings in context.

Most published studies of inhaled β_2-agonists in subjects with COPD have focused on their efficacy, rather than safety. We were concerned that some individuals with COPD and limited bronchodilator reversibility may experience an increase in adverse systemic effects after inhaling high doses of β_2-agonists, which could lead to detrimental outcomes in certain clinical situations.

Apart from the cardiac effects mentioned above, β_2-agonists increase tremor, which causes CO_2 production, and cardiac output and tissue perfuson, which increases the transport of CO_2 to the lungs. The increase in CO_2 flux to the lungs will normally increase ventilation. We were concerned however that some subjects with severe COPD would not be able to increase ventilation appropriately in response to the β_2-agonist and this would lead to an increase in $PaCO_2$. Our hypothesis was that high dose inhaled β_2-agonists could worsen respiratory failure in some subjects with severe COPD.

The first two studies in the thesis examined the effect of high dose inhaled salbutamol on the partial pressure of arterial oxygen and carbon dioxide in subjects with severe COPD.

We initially conducted a double blind, randomised study on subjects within 48 hours of being admitted to hospital with an acute exacerbation of COPD (Chapter 4). The study was designed to determine whether high dose salbutamol caused an increase in the partial pressure of arterial carbon dioxide. We randomised subjects at a ratio of 3:1 to receive either salbutamol or ipratropium bromide and studied the pharmacodynamic effect on heart rate, PaO_2 and $PaCO_2$ over five hours. Over eighteen months and despite extensive efforts I was only able to recruit ten subjects, of whom five completed the study. I found that subjects who required hospital admission with an acute exacerbation of COPD were either too unwell for the study, had co-morbidities that precluded participation or the individuals were unwilling to participate. Although the study was terminated prematurely and we were unable to perform statistical analysis, I have presented the findings from the five subjects who completed the study, of whom four were randomised to receive salbutamol. We used ascending

doses of salbutamol (1.25mg, 1.25mg, 25mg, 5mg, 5mg) and found no consistent effect on $PaCO_2$ or PaO_2 and no dose response relationship. The subject with the highest baseline $PaCO_2$ did however have a rise in $PaCO_2$ with the highest 5mg doses of salbutamol.

To test the hypothesis further we conducted a randomised, double blind, crossover study and examined the effect of salbutamol on the arterial blood gas tensions of fourteen patients with stable severe COPD and a history of chronic or intermittent hypercapnia. The study was designed to determine whether high dose salbutamol causes a rise in $PaCO_2$ when inhaled by subjects with severe COPD and a history of alveolar hypoventilation. We compared the effect of two 5mg doses with two 200 µg doses of salbutamol on PaO_2 and $PaCO_2$ and heart rate. The subjects had severe COPD with a mean FEV_1 of 0.71 L (27% predicted) and a mean smoking history of 53 pack years. The mean baseline PaO_2 was 7.9 kPa and the mean baseline $PaCO_2$ was 7.0 kPa. The high dose of salbutamol caused a mean fall in both PaO_2 and $PaCO_2$ and a small increase in heart rate. There was some support for our hypothesis however as three subjects had a small rise in $PaCO_2$ after high dose nebulised salbutamol (Chapter 5).

The third study was a double blind, crossover, dose-response examination of the bronchodilator and systemic effects of inhaled formoterol in subjects with COPD (Chapter 6). The rapid onset and prolonged duration of action of formoterol offers potential for the drug to be used as rescue medication in addition to twice daily maintenance therapy, as is the case in the management of asthma. Our hypothesis was that high doses of formoterol would produce adverse systemic effects that would outweigh the beneficial bronchodilator effects in subjects with COPD and limited bronchodilator

response to salbutamol. We studied 20 subjects, with a mean FEV_1 of 1.32 L (47% predicted) and a mean smoking history of 42 pack years. Each subject was studied on five days and after receiving placebo, formoterol 6, 12, 24 and 48 μg in a random sequence, we examined the effect of each dose on FEV_1, tremor, dyspnoea, heart rate, blood pressure, SpO_2, walk distance, potassium and satisfaction. We found that all doses were well tolerated and although there was a small dose related increase in FEV_1 and the mean satisfaction scores were higher with each dose of formoterol than placebo, there was no dose related improvement in measures that are important to the patient, including breathlessness and walk distance. Apart from a dose related increase in tremor, other systemic effects were limited.

All three studies found that high dose inhaled β_2-agonists produced relatively modest systemic effects in subjects with COPD. This probably reflects the fact that almost all subjects were taking β_2-agonists on a regular basis and had developed tolerance to the systemic effects of an inhaled β_2-agonist. Although the results from the three studies were generally reassuring, questions still remain about the balance between beneficial and adverse effects with high dose inhaled β_2-agonists in subjects with COPD. The results may have been different if subjects had more severe disease, were exposed to higher doses of β_2-agonists, had certain β_2-adrenoceptor polymorphisms or were β_2-agonist naïve.

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I would like to thank Professor Anne Tattersfield and Dr Tim Harrison for their patience and for the support, advice and encouragement they have provided throughout my research and during the preparation of this thesis.

I am grateful to my family and friends who have encouraged me at every stage. Special thanks to my wife, Claudia, who has provided unwavering support throughout the research cycle. I recognise the sacrifices she has made.

DECLARATION

I declare that this thesis is the result of my own work. This thesis has not, in this or any other form, been presented to this or any other university in support of an application for another degree.

Much of chapter four is taken from the COPD section of the paper 'A benefit-risk assessment of inhaled long-acting β_2-agonists in the management of obstructive pulmonary disease'. As a co-author Professor Anne Tattersfield contributed to the final version of the paper although I did the initial review of all the papers.

The studies described in this thesis were designed, executed, analysed and written up in Nottingham. I contributed to the design of these studies and was responsible for patient recruitment, day to day running of the studies, data collection and analysis. Dr Milind Sovani and Dr Kevin Mortimer provided medical support for these studies and I received help with study administration from Janet Oborne and Sue Cooper. Dr Tim Harrison and Professor Anne Tattersfield contributed to study design and the papers resulting from the studies.

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Chapter One

Overview of Chronic Obstructive Pulmonary Disease

1.1 INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the accepted name for a condition known previously as chronic airflow limitation (CAL), chronic obstructive airways disease (COAD), chronic obstructive lung disease (COLD) and chronic bronchitis and emphysema. The term COPD was coined in the early 1960s and is used because it recognises that the disease is not confined to the airways, but also affects the lung parenchyma and pulmonary circulation. The Global Initiative for Obstructive Lung Disease (GOLD, 2001) defined COPD as 'a disease state characterised by progressive development of airflow limitation that is not fully reversible [1]. The airflow limitation is usually progressive and usually results from an abnormal response of the lungs to noxious particles or gases.' The clinical syndrome of COPD encompasses different disease processes from chronic obstructive bronchitis with small airway obstruction, to emphysema characterised by destruction of lung parenchyma, enlargement of air spaces, loss of elasticity and small airway closure. It is difficult to determine the precise prevalence of COPD, but it is expected to become the third largest cause of death worldwide by 2020 [2].

1.2 HISTORICAL BACKGROUND

Emphysema was first identified in the seventeenth century as enlarged respiratory air spaces on the lung surface. Ruysch (1691) noted that these distended airspaces could not be emptied by pressing on them and concluded that the bronchi were obstructed [3]. Matthew Baillie, a British physician, used a lung from the autopsy of the writer and philosopher Samuel Johnson to produce the first illustration of widespread emphysema

in 1793 [4] and in 1819, four years after inventing the stethoscope, Rene Laennec used air-dried inflated lung specimens to provide a basis for the description of emphysema that was accepted for over a hundred years [5]. Bronchitis had more humble origins and was described in 1808 as “chronic pectoral (chest) complaints, especially those of people advanced in life” [6].

The importance of respiratory illnesses in the UK was highlighted by ‘the great smog’ of December 1952. Death rates in London rose following the five-day smog, with many deaths among people suffering from chronic respiratory or cardiovascular disease. There was a more than sevenfold increase in mortality from bronchitis and pneumonia. The subsequent City of London Act of 1954 and the Clean Air Acts of 1956 and 1968 ensured the use of smokeless fuels in urban areas, reducing the likelihood that smogs of such severity would occur again. The increased profile of chronic respiratory diseases in this period and the need for internationally accepted terminology led to the CIBA symposium in 1959, which suggested definitions for chronic bronchitis, emphysema, and variable and fixed airflow obstruction [7]. Emphysema was defined in anatomical terms, whilst chronic bronchitis was defined clinically as “chronic or recurrent excessive mucus secretion in the bronchial tree”. The introduction of the physiological concept of airflow limitation as a diagnostic term was new, and the report emphasised that mucus hypersecretion, reversible airflow obstruction and irreversible airflow obstruction frequently coexisted in different combinations.

The British Medical Research Council report in 1965 classified chronic bronchitis as ‘simple’, i.e. mucus hypersecretion without airflow obstruction or ‘obstructive’ i.e. mucus hypersecretion associated with chronic airflow

obstruction related to airway pathology and/or coexistent emphysema [8]. ‘Chronic obstructive pulmonary disease’ was proposed as a diagnostic term in the 1975 report of the American College of Chest Physicians-American Thoracic Society Joint Committee on Pulmonary Nomenclature [9]. Definitions of the disease evolved in the latter half of the last century through further British, European and American guidelines, before the publication of consensus guidelines (GOLD) in 2001 [1].

In 1951, Doll and colleagues initiated a questionnaire study of over 40000 doctors in Britain, to examine the relationship between smoking habits and mortality. The follow-up over five decades has provided clear evidence of the increased mortality associated with cigarette smoking and an increased incidence of lung cancer and chronic obstructive lung disease [10-15]. Fletcher and Peto (1976) published a landmark epidemiological study with data collected from working men in West London between 1961 and 1969, showing that cigarette smoking causes an accelerated decline of FEV_1 and that smoking cessation slows this decline [16].

Attempts to explain the aetiology of chronic airflow obstruction led to opposing theories in the 1960s. The “British hypothesis” considered asthma and COPD to be different conditions, postulating that infections cause airway narrowing and the infections occurred because prior mucus hypersecretion impaired the defence of the airways against infection and led to bacterial colonization. Workers in the Netherlands proposed that asthma and chronic airflow obstruction were opposing ends of the same disease spectrum [17]. They postulated that an interaction of endogenous host factors (airway hyperresponsiveness and allergy) and exogenous environmental factors (cigarette smoking) caused airway obstruction termed

'chronic nonspecific lung disease', a theory later termed the "Dutch hypothesis".

In 1963, α_1-antitrypsin deficiency was identified and the high incidence of severe early onset emphysema among a small cohort of patients led to the serendipitous discovery of the only currently recognised genetic cause of COPD [18].

1.3 EPIDEMIOLOGY

1.3.1 Prevalence

Accurate determination of the worldwide prevalence of COPD is difficult, since most data has been obtained from the developed world. The condition is under-diagnosed, particularly in its milder forms and there is often diagnostic uncertainty and overlap with other obstructive lung diseases, notably asthma [19]. The definition of COPD has evolved with time and even now different international guidelines do not have complete agreement in the way COPD is defined using spirometry [20]. The use of mortality data to determine prevalence is also flawed, since COPD is not accurately reported as a contributing factor to mortality or the cause of death.

The prevalence of COPD is highest in countries where cigarette smoking is common. Smoking history has been used as a surrogate marker for the risk of developing COPD and estimated smoking rates suggest that there are approximately fifteen million cases in the USA and three million cases in the UK among adults over 40 years of age [21].

In the UK, prevalence of COPD among males reached a plateau in the 1990s, while annual rates among women continued to rise during the same time period. The Global Burden of Disease study calculated the worldwide

prevalence of COPD to be 9.34 per 1000 men and 7.33 per 1000 women in 1990 [22]. In this analysis of over thirty studies worldwide COPD prevalence ranged from 4 to 10%, generally with a higher prevalence among males.

Studies have also shown that the prevalence of COPD is greatest in areas of social deprivation [23] and since estimated prevalence rates include all age groups, they underestimate rates among the elderly.

Although age-standardised mortality rates from COPD have fallen progressively amongst men in most developed countries, there has been a small but progressive increase in mortality rates among women over the last 20 years, reflecting changing patterns of tobacco consumption. Between 1979 and 1993, there was a 17% rise in age-adjusted mortality rates among American males, while the same time period saw a 126% rise among females [24]. In Australia, a study published in 1994 reported a 2.6 fold increase in age-standardised mortality among females between 1964 and 1990 and predicted that female mortality rates would soon equal that of males [25].

In 1999, over 32,000-recorded deaths were attributed to COPD in the UK, representing 4.3% of male deaths and 5.9% of female deaths. Patients with COPD use many services in primary and secondary care and cost analysis for 1996-97 showed that the total NHS expenditure on COPD was more than £818 million. In the United Kingdom, around 35% of resources are used for care of emergency admissions, with an average admission costing £3000. In the 1990s there was a 50% rise in emergency hospital admissions for COPD and between 2000 and 2001 the 308,000 admissions cost the

NHS over £587 million. Much of the burden on health resources comes from the morbidity associated with COPD.

Work assessing the cost of COPD has mainly been carried out in the developed world, particularly the USA, where the National Heart, Lung and Blood Institute estimated the annual cost of COPD in 1993 to be $23.9 billion [26]. Although often considered a disease of the elderly, over 9 million people of working age in the USA have COPD, accounting for total lost productivity of approximately $9.9 billion [27]. Estimating the future burden of COPD is important and researchers in the Netherlands looked at anticipated changes in disease prevalence between 1994 and 2015 and predicted a rise in healthcare costs of 90% [28].

An ageing population and increasing cigarette consumption makes a worldwide increase in the prevalence and burden of COPD inevitable. In 2020, it is estimated that COPD will be the fifth leading cause of disability-adjusted life years (DALYs) worldwide, after ischaemic heart disease, unipolar major depression, road traffic accidents and cerebrovascular disease [2].

1.3.2 Aetiology

COPD is due to a combination of environmental and genetic factors.

1.3.2a Cigarette smoking

Cigarette smoking is the most important risk factor for COPD, with 10-15% of smokers developing COPD. In the industrialized world, smoking accounts for 85-90% of cases of COPD among males [29]. The prevalence of smoking has fallen slightly among men, but has risen among women,

who accounted for 19% of deaths from COPD in 1970 and 38.5% in 1993 [24].

In the 1800's most tobacco was smoked in pipes or cigars, but the 20th century saw a rapid rise in the consumption of manufactured cigarettes. The health hazards of cigarette smoking were not apparent until the second half of the century, when data collected between 1922 and 1947 showed a fifteenfold increase in deaths from lung carcinoma [30].

Initial results from the landmark questionnaire study of doctors showed higher mortality rates among smokers than non-smokers and an increased death rate from chronic bronchitis among smokers [11]. A decade later, the authors concluded that chronic bronchitis and emphysema 'chronic obstructive lung disease' was as important a fatal effect of smoking as lung cancer [12], although they acknowledged that other factors such as air pollution may contribute. Data published after 40 years of follow up showed the risks from persistent smoking were greater than previously suspected, concluding that around half of chronic smokers would die as a consequence of their habit [14]. The final paper in the series showed that lifelong non-smokers had an age standardized mortality rate from COPD of 0.11 per 1000 men/year. Among current smokers the equivalent mortality rate from COPD was 1.56, increasing to 2.61 per 1000 men/year among those smoking more than twenty-five cigarettes per day [15].

Lung function increases through childhood and reaches a plateau in early adult life, after which it declines gradually. An early study concluded that smoking was not associated with an additional fall in FEV_1 [31], but many subsequent cross-sectional and longitudinal studies have found that smokers have a more rapid decline in FEV_1 than non-smokers. Fletcher and Peto

(1976) found that smokers had a steeper decline in lung function than non-smokers, with the decline in FEV_1 returning towards the same level as non-smokers after stopping smoking [16].

Despite differences between study populations, all longitudinal studies carried out over at least five years have found smokers to have a greater decline in FEV_1 than non-smokers [16, 32-37]. Although large differences exist between individuals, the additional mean decline in FEV_1 due to cigarette smoking is around 15 ml/year among males and around 10 ml/year for females.

The detrimental effects of smoking are not limited to adult life, but can affect lung growth in utero, childhood and adolescence. Maternal smoking during pregnancy has been associated with a reduction in lung growth and the lung function of neonates [38], infants [39-41] and children of school age [42]. Exposure to maternal smoking during childhood and adolescence is also associated with impaired lung function [43-46]. Insults to the developing lung in utero and during childhood are likely to reduce the maximum lung function achieved and may put individuals at greater risk of developing COPD during adult life, depending on the individuals smoking habit and lifestyle. Smoking during adolescence is associated with mild airway obstruction and slowing of lung growth [47,48].

Most studies have examined the effect of cigarette smoking rather than the effects of pipe or cigar smoking. Cigar sales increased in the USA during the 1990s and although pipe smoking has declined since the 1960s, large numbers still use these methods of inhalation. The type of tobacco is probably less important than the amount smoked, the duration of smoking and the depth of inhalation [49], and both pipe and cigar smokers have also

been shown to have higher all cause mortality rates and higher rates of COPD than non-smokers [50-52].

The US Environmental Protection Agency has classified environmental tobacco smoke (ETS) ('passive smoking') as a human carcinogen. Although maternal smoking impairs lung growth during pregnancy and childhood and ETS has been linked with adult-onset asthma [53], little is known about the role of passive smoking in the development of COPD. A prospective study of Californian adults over 39 years recently found no association between ETS and mortality from COPD in men or women [54].

The Lung Health Study confirmed that smoking cessation reduces the rate of decline of FEV_1 among subjects with established COPD, with the greatest benefit occurring in the first year after stopping smoking and the subsequent decline among sustained quitters was half that of ongoing smokers and comparable to never smokers [55,56]. Smoking cessation among subjects with COPD also reduces the presence of respiratory symptoms [57].

1.3.2b Genetic factors

Many studies have investigated the potential genetic determinants of COPD, but the only confirmed predisposing genetic factor is alpha$_1$-antitrypsin (α_1-AT) deficiency. The first association between α_1-AT deficiency and emphysema was made in 1963, when Laurell, a Swedish protein research scientist, carried out serum electrophoresis on all the patients of a senior respiratory physician. He and Eriksson, a research physician identified five subjects with α_1-AT deficiency and noted that three had early onset severe emphysema [18]. Fourteen patients with α_1-AT deficiency were reported two years later, of whom nine had premature emphysema [58]. Later work

by the same group identified the importance of the interaction between smoking and α_1-AT deficiency and the genetic variations coding for α_1-antitrypsin.

The name α_1-antitrypsin originates from its ability to inhibit the pancreatic enzyme trypsin, although subsequent work found it also inhibited many other proteinases, including neutrophil elastase. Part of the serine proteinase inhibitor or 'serpin' superfamily, α_1-antitrypsin is a 394 amino acid acute phase glycoprotein encoded on the long arm of chromosome 14q32.1. It is mainly produced by the liver and synthesised in smaller amounts by macrophages, neutrophils and intestinal and bronchial epithelial cells [59,60]. In healthy subjects, α_1-AT prevents the neutrophil elastase released by triggered neutrophils from degrading elastic tissues in the lung [61,62]. The structure of α_1-AT is crucial to its role as a proteinase inhibitor; made up of three β sheets, it has an exposed reactive loop that binds to the target proteinase, stimulating a change in conformation, which deactivates the proteinase, allowing recognition by hepatic receptors and removal from the circulation.

Over 100 genetic variations of α_1-AT have been described and are characterised by their ability to migrate on isoelectric focusing gels, giving rise to the proteinase inhibitor or Pi system. The most common deficiency variants result from a single point mutation; PiS (slow) and PiZ (very slow) are named because the rate of migration is slower than the normal M (medium) α_1-AT. Occurring far less frequently are the so-called null alleles, which cause very low or even absent serum α_1-AT levels. The inheritance of

a Pi phenotype follows an autosomal co-dominant pattern, with a varied frequency of PiS and PiZ alleles throughout the world.

The highest prevalence of α_1-AT deficiency is seen in Europe where the original mutations are thought to have occurred, with much lower frequencies in parts of the Far East. Estimates suggest approximately 1.1 million subjects have severe α_1-AT deficiency worldwide and 116 million carriers of the deficiency allele [63]. Not all of those with severe α_1-AT deficiency have lung function impairment and it has been estimated that less than 6% of severely deficient individuals have been identified.

The PiS allele results from a single base alteration in the gene at position 264 (glutamic acid to valine) and the highest European frequency is on the Iberian peninsula [64]. Although homozygotes for PiS have α_1-AT levels 60% of normal, it is not usually associated with pulmonary complications. The PiS allele has a mean gene frequency in Southern Europe of 0.0564 (ie 1 in 17 are PI∗SS, PI∗SZ or PI∗MS) [63]. The PiZ allele results from an amino acid alteration at position 342 (glutamic acid to lysine) and has the highest gene frequency in southern Scandinavia, with the frequency falling as you move South and East across the continent. The PiZ gene frequency in Europe varies between 0.01 and 0.05, with two studies finding that approximately 1 in 1500 individuals are homozygotes [65,66].

Homozygotes for the Z variant have 10-15% of the plasma α_1-AT levels of the normal M allele. The Z mutation leads to α_1-AT accumulation in the inclusions of hepatic rough endoplasmic reticulum. This predisposes the homozygote to childhood liver cirrhosis and the lack of circulating anti-proteinase for neutrophil elastase renders individuals susceptible to

premature panlobular emphysema, particularly if they smoke. Debate exists around whether PiZ heterozygotes are at increased risk of developing COPD if they smoke. As well as reduced levels of α_1-AT in the lung, the available glycoprotein is less effective at clearing neutrophil elastase. Different processes are involved in the development of emphysema with PiZ α_1-AT, although the most important factor is cigarette smoking, which creates an environment where proteolytic enzymes are unopposed. The deficient and dysfunctional Z α_1-AT is deactivated by the direct oxidative effect of smoking, which may also predispose Z α_1-AT to polymerisation within the lung to an inactive form. The PiZ α_1-AT polymer may also be chemoattractant to inflammatory cells, which then contribute to tissue breakdown [67].

PiZ homozygotes have a varied response to cigarette smoking, with differences in the severity of disease and some individuals maintaining normal lung function. Individual modifier genes may mediate the differences in severity, although none has been identified to date. There is however, a dose-response relationship with cigarette smoking and decline in forced expiratory volume in one second (FEV_1) [68].

Genetic factors other than α_1-antitrypsin deficiency are probably involved in COPD aetiology, though none has been identified. A small study in 1970 found that first-degree relatives of patients with COPD have higher rates of airflow obstruction than relatives of spouses without COPD, although the study did not fully exclude subjects with α_1-antitrypsin deficiency and differences in smoking history existed between groups [69]. A subsequent well-matched case-control study that excluded subjects with α_1-antitrypsin

deficiency found a lower FEV_1 among siblings of subjects with COPD than among siblings of controls [70]. Current and ex-smoking relatives of patients with early onset severe COPD had an increased risk of having a low FEV_1 and FEV_1/FVC ratio compared with controls after adjusting for age and pack-years of smoking [71]. There were no differences between non-smoking relatives of cases and control, suggesting that genetic factors other than α_1-AT deficiency interact with smoking to increase the risk of developing COPD.

These findings and the variable clinical phenotype of individuals with α_1-AT deficiency suggest the development of COPD depends on a complex interaction of genetic and environmental factors.

1.3.2c Airway hyperresponsiveness

Airway hyperresponsiveness (AHR) describes an increased bronchoconstrictor response to pharmacological or physical stimuli. Non-specific AHR is present when the airways respond to a wide range of stimuli and is measured in the laboratory as a change in lung function after inhaling increasing concentrations (or doses) of bronchoconstrictor stimuli or after physical insults (eg cold air or exercise).

It was recognised in the 1960s that airway obstruction was often associated with airway hyperresponsiveness and subsequent work has examined the relationship between bronchial reactivity, smoking and FEV_1. Dutch investigators proposed that host factors including airway hyperresponsiveness predispose to the development of “chronic nonspecific lung disease” [17]. The “Dutch hypothesis” created debate about the role of airway responsiveness in the pathogenesis of COPD and, specifically,

whether AHR is a cause or consequence of accelerated decline in FEV_1. This issue is still being debated four decades later [72] as there is particular difficulty determining the exact impact of cigarette smoking and the geometric factors that occur with airway narrowing on airway hyperresponsiveness [73].

Smokers are more likely to have AHR than non-smokers. In a study of 227 men, smokers had increased bronchial reactivity, which was associated with low initial FEV_1 and the subsequent accelerated decline in FEV_1 [74]. Follow up of the male smokers confirmed an accelerated decline in FEV_1 and an increase in bronchial reactivity as FEV_1 fell [75].

Airway hyperresponsiveness was associated with an accelerated decline of FEV_1 in a four-year longitudinal study among subjects over 65 [76] and among middle-aged and older men in the Normative Aging Study [77]. The Lung Health study followed over 5000 patients with mild-moderate COPD for five years and measured AHR at intervals. The majority of subjects had increased bronchial reactivity at the start of the study and AHR predicted an accelerated decline in FEV_1 among continuing smokers [78].

A Dutch cohort study of risk factors for obstructive lung disease followed 2000 subjects over three decades and after accounting for cigarette smoking, found that AHR was associated with an accelerated decline in FEV_1, the development of respiratory symptoms (cough, sputum production, dyspnoea and wheeze) and increased mortality from COPD [79-81].

1.3.2d Atopy

Whether atopy predisposes or contributes to the pathophysiology of COPD also remains a matter for debate. Serum eosinophil count, total IgE and/or

skin prick testing have all been used to determine an allergic diathesis, but the interaction with cigarette smoking again complicates matters, since smoking has been associated with increased serum IgE [82] and eosinophil levels [83], but, a reduction in skin test reactivity [84].

Studies have examined the relationship between atopy and respiratory symptoms and, more importantly, atopy and rate of decline of FEV_1. The Vlagttwedde-Vlaardingen study found that serum eosinophilia was associated with increased respiratory symptoms and bronchial reactivity [85,86] and although an increased serum eosinophil count has been associated with a reduced FEV_1 in individual studies [87], longitudinal studies have not consistently found an accelerated decline in FEV_1 [88]. Similarly, elevated IgE levels have been associated with reduced FEV_1 [76], but not an accelerated decline in FEV_1 after adjusting for smoking [89].

Taylor and colleagues excluded asthma among 237 men and found no relationship between positive skin test results and decline in FEV_1 over seven years [83], a finding similar to that seen in working men in Paris [90]. However, skin test positivity predicted an additional decline in FEV_1 over three years in the Normative Aging Study [91] and a positive skin test result was associated with accelerated decline in FEV_1 over five years among smokers in an occupational setting [92].

1.3.2e Chronic mucus hypersecretion

The ‘British hypothesis’ that mucus hypersecretion is involved in the aetiology of COPD was undermined by the classic work of Fletcher and Peto [16]. They found that although both chronic mucus hypersecretion and progressive airflow obstruction occurred in cigarette smokers, their

influence on decline in FEV_1 and survival was different and concluded that they should be considered separately. No link was found between mucus hypersecretion and mortality from COPD after 20-25 years of follow-up; one section of the paper was entitled 'The irrelevance of mucus hypersecretion to COPD' [93].

A recent large longitudinal study reached different conclusions. The Copenhagen City Heart Study followed 9000 people for five years and found that chronic mucus hypersecretion was associated with an excess decline in FEV_1 of 13ml and 23ml per year in women and men respectively, after adjusting for smoking and age [94].

The study also found a relationship between mucus hypersecretion, all cause mortality and mortality from chronic obstructive lung disease [95]. The increased mortality may relate to systemic inflammation and chest infections. Subjects with chronic mucus hypersecretion had an increased risk of hospitalisation, even after adjusting for baseline FEV_1 and further analysis of the data found that subjects with COPD and chronic mucus hypersecretion were more likely to die from pulmonary infections than those without mucus hypersecretion [96].

1.3.2f Bronchopulmonary infections

Retrospective studies have shown that lower respiratory tract illnesses during childhood and adolescence are associated with a reduced level of lung function in later life [97-99]. It is however difficult to ascertain whether impaired lung function is the cause of infections or develops as a consequence.

The theory that recurrent bronchopulmonary infections during adult life are important in the development of chronic bronchitis and emphysema was originally rejected [16]. The cohort included subjects with mild or no airflow obstruction however and an infection was only diagnosed on individual recollection.

The Lung Health Study found that smokers and intermittent quitters had an increased decline in FEV_1 of 7ml/year for each lower respiratory tract illness [100]. Other recent evidence also supports the "British hypothesis". Patients with COPD classed as frequent exacerbators had a greater decline in FEV_1 over four years (by 8 ml/year) than infrequent exacerbators [101]. The same group found that a high or rising airway bacterial load was associated with an accelerated decline in FEV_1 [102] and bacterial colonisation of the airways was associated with an increase in exacerbation frequency [103].

1.3.2g Occupation

Although an association between occupational exposures and respiratory symptoms has been recognised since ancient times, the association between occupational exposures and COPD specifically has only been recognised recently. Teasing out the precise effect of occupation on lung function can be difficult in a workforce where smoking is prevelant and where the "healthy worker effect" (ie subjects with good lung function are more likely to enter the workforce) and the "survival effect" (ie affected workers are withdrawn early) may be operative. Although the effect of occupational exposure for a particular workforce may be small, a large population is at risk and interactions with smoking make occupational exposure an important factor in the incidence of COPD.

Many recent studies have examined the link between occupational exposures and COPD, including studies from Europe, China and the USA, and involving both urban and rural workforces. Despite geographical and population differences, the results from cross-sectional and longitudinal studies have generally found that those subjects exposed to dusty working environments are more likely to develop COPD than those working in non-dusty environment. Other specific factors include fume exposure though the effect is less marked than with dust exposure [104-107]. The risk of developing COPD from occupational exposure to dust or fumes is generally less than that associated with cigarette smoking.

A variety of specific occupational exposures (coal dust, silica, cadmium and cotton) have been associated with airflow obstruction and an accelerated decline of FEV_1.

COPD is now a compensatable occupational disease in coal miners in the United Kingdom, since it is now accepted that exposure to coal dust accelerates decline in FEV_1, independent of cigarette smoking and the presence of pneumoconiosis [108]. Centrilobular emphysema was more common at autopsy of miners than non-miners, with an association between dust exposure and degree of emphysema [109]. The effect of coal dust exposure and cigarette smoking appears to be additive on FEV_1 decline [110].

Many studies have demonstrated an increased risk of respiratory symptoms among workers exposed to silica, including foundry workers, quarrymen and goldminers. Longitudinal studies in South Africa found that decline in lung function was related to the silica content of gold mine dust [111] and the detrimental effects are particularly high among smokers [112]. The

higher risk of developing COPD among gold miners compared to coal miners is likely to relate to the higher silica content of gold mine dust [111].

Cadmium is an unusual occupational cause of COPD, since it causes emphysema. A long half-life and hepatic storage allows accurate measurement of cadmium exposure many years after exposure. In a case-control study of copper-cadmium alloy foundry workers, high occupational exposure to cadmium was associated with an accelerated decline in FEV_1 and the transfer factor for carbon monoxide [113].

Workers exposed to cotton dust have increased respiratory symptoms [114] and an accelerated decline in FEV_1. The effect on FEV_1 has been demonstrated in longitudinal occupational studies from Europe, India and China [115,116] and it has been proposed that adverse effects associated with cotton exposure relate to endotoxin content of cotton mill dust [117].

1.3.2h Gender

The prevalence of COPD has been generally greater among men than women, probably because men have smoked more cigarettes, inhaled more deeply and started smoking at an earlier age. Women may be more susceptible to developing COPD however. Compared with lifetime non-smokers, the estimated excess decline in FEV_1 for light, moderate, and heavy continuing smokers in the Vlagtwedde-Vlaardingen study was 4.4, 9.5 and 13.5 ml per year for men and 6.1, 10.8 and 18.8 ml per year for women, respectively [37]. Women had greater bronchial reactivity than men for all age groups in a study of 1700 Italian subjects, even after accounting for baseline lung function [118]. A large population study in Denmark found that women who smoked had a more rapid decline in FEV_1 and a higher risk

of hospitalisation from COPD than men [119]. A study of first-degree relatives of subjects with severe, early onset COPD found that female relatives were more likely to have a reduced FEV_1 [120].

1.3.2i Biomass fuels

Work in the 1960s in Papua New Guinea identified chronic bronchitis and obstructive arways disease in non-smokers and postulated the aetiolgical role of domestic wood smoke. It is now accepted that in the developing world, exposure to household smoke generated from the use of biomass fuels is an important cause of COPD, particularly among women [121]. Around 75% of households in the developing world use biomass fuels for cooking and indoor air pollution has been thought to account for half the cases of COPD in certain areas [122].

1.3.2j Air pollution

The nature of air pollution has changed over the last fifty years, particularly in the Western world. The great smogs of the 1950s in the UK were due to the accumulation of fog and air pollution and consisted mainly of black smoke and sulphur dioxide. The risk of death following air pollution episodes was dramatically demonstrated after the London smog of 1952 where there were over 4000 excess deaths [123]; many occurred in people with what would now be known as COPD. The subsequent ‘Clean Air Acts’ led to a move away from burning coal in urban areas, although emissions from other sources have increased. Air pollution in Westernised countries now consists mainly of nitrogen dioxide, ozone and particles generated by a

combination of motor vehicle emissions, burning of fossil fuels and heavy industry. Sulphur dioxide contributes to a lesser extent that in the 1950s.

Current levels of air pollution have detrimental effects on health, both chronic effects from long-term exposure and acute effects from air pollution episodes. Air pollutants can increase oxidative stress and produce a pro-inflammatory response in the lungs, increasing airway mucous production and impairing ciliary function.

Chronic exposure to elevated levels of air pollution contributes to all cause morbidity and mortality. To what extent chronic exposure influences subjects with COPD is less clear. Long-term exposure to high levels of air pollution has been associated with factors that may predispose to the development of COPD; impaired lung growth in childhood [124], mucus hypersecretion, bronchial hyperresponsiveness and low FEV_1 [125,126]. The 'Six Cities' study demonstrated increased mortality in urban areas with high levels of air pollution, without showing a specific effect in subjects with COPD [127,128].

Studies have examined the temporal relationship between air pollution episodes, rates of hospitalisation, overall mortality and mortality from COPD. Hospital admissions from COPD have risen following air pollution episodes [129,130] and fallen after periods of reduced air pollution [131]. Air pollution episodes have been associated with increased mortality from various causes, including COPD; particulate matter may be particularly harmful [132-135].

1.3.2k Social class and nutrition

The finding that the prevalence of respiratory symptoms increases with lower socio-economic status is likely to be multifactorial, with contributions from maternal smoking during pregnancy, increased childhood infections, passive smoke, urban air pollution, poor housing and diet.

In the Copenhagen City Heart Study, the incidence of COPD was greatest in subjects with low socio-economic status, with FEV_1 and exacerbation rates related to educational level and income after adjustment for smoking [136]. Unskilled workers had a greater decline in FEV_1 over twelve years than skilled workers in Paris after adjusting for occupation and smoking [137]. Education level has also been related to FEV_1, with adults who only had a primary school education being 2.9 times as likely to have obstructive lung disease than those with a university education after adjusting for age, sex, smoking and occupation [138].

Several epidemiological studies have examined the role of diet in the pathogenesis of COPD. Increased intake of certain vitamins (A, C and E) has been associated with improved lung function in some populations [139,140]. A diet rich in fresh fruit and vegetables appeared to reduce the risk of COPD in one study [141] and high fish and fruit intake was associated with reduced COPD mortality in the Seven Countries study [142]. Subjects with high dietary intake of fish (n-3 fatty acids) had a reduced risk of developing COPD in another study [143].

None of the dietary studies in COPD have been interventional however and it is not known whether poor diet simply reflects poor general health and socio-economic status and therefore exposure to other factors that would influence these outcomes.

1.4 NATURAL HISTORY

Healthy subjects have maximal lung function between the ages of 20 and 25. A relative plateau follows and the FEV_1 then gradually declines by around 30 ml per year. Smokers have a greater mean annual decline in lung function than non-smokers, with an average fall in FEV_1 of around 40 - 50 ml per year. Although most smokers have little additional loss of lung function, a minority of 'susceptible' smokers have an accelerated decline in FEV_1, with substantial damage occurring more frequently among heavy smokers.

In susceptible individuals, lung function deteriorates over time until symptoms of cough, sputum production and dyspnoea develop, often in the fifth and sixth decades when the FEV_1 has fallen to around 50% of the predicted value.

Symptoms will progress and gradually be accompanied by exercise limitation, disability and an impaired quality of life as the lung function declines. As COPD becomes more severe, gas exchange is impaired and patients develop hypoxaemia. This can lead to increased pulmonary artery pressures and the subsequent development of pulmonary hypertension [144-146]. The pulmonary artery pressure increases by 0.5 – 3.0 mmHg per year in severe COPD, causing right ventricular hypertrophy. In the late stages of COPD, individuals develop respiratory failure with associated cor pulmonale. Once present, the five-year survival is only 30-40%.

Some patients with severe COPD have significant alveolar hypoventilation and will develop chronic type 2 respiratory failure, with hypoxaemia and hypercapnia. Secondary polycythaemia occurs as a result of long-term hypoxaemia, with an associated increased risk of vascular events. Muscle

mass falls as COPD progresses and although this may be due to deconditioning, systemic inflammation may also contribute; weight loss often occurs and is associated with increased serum levels of tumour necrosis factor-alpha (TNF-α) [147]. Peripheral muscle weakness is associated with a transition from oxidative to the less energy-efficient glycolytic metabolism [148]. A body mass index (BMI) of less than 20 kg/m^2 in the presence of severe COPD is a strong predictor of mortality [149].

Exacerbations with worsening respiratory failure become more frequent as COPD deteriorates, with some exacerbations requiring hospitalisation. The development of acute type 2 respiratory failure has a poor prognosis, particulary when complicated by a respiratory acidosis. Other predictors of poor survival include smoking, ischaemic heart disease, chronic mucus hypersecretion and male gender.

Smoking cessation remains the only intervention known to alter the long-term rate of decline in FEV_1 in patients with COPD. Although oxygen can benefit some patients with COPD and severe hypoxaemia, it does not affect FEV_1. Bronchodilators do not appear to change the rate of decline in FEV_1, but controversy surrounds the relationship between inhaled steroids and rate of change in FEV_1. The difficulty in formulating a precise conclusion about the relationship is highlighted by the conflicting conclusions of two meta-analyses [150, 151]. Highland et al examined data from six randomised, placebo-controlled studies, encompassing 3571 patients with COPD and concluded that inhaled corticosteroid use was not associated with the rate of FEV_1 decline [150]. Sutherland et al used the same data and results from two other small studies, but used different methodology and statistical

analysis and concluded that inhaled steroids used for over two years slows the rate of FEV_1 decline in COPD by 7.7 ml per year [151].

1.5 PATHOLOGY

The main pathological changes seen in COPD include thickening of the airway wall, excessive mucus in the airway lumen and widespread emphysema. Inflammation leads to airway remodelling and narrowing, with neutrophils, macrophages and T lymphocytes identified as the most important inflammatory cells. Numerous techniques including lavage samples, induced sputum and bronchial biopsies have been used to identify the cells and inflammatory mediators involved in COPD pathogenesis, but the results have not always been consistent.

The central and peripheral airway walls are thickened, particularly the cartilaginous bronchi between two and four millimetres in diameter. Submucosal gland hypertrophy occurs and the volume and number of goblet cells is increased [152,153] and squamous metaplasia leads to fewer ciliated epithelial cells with shortened cilia [154,155]. Macrophages and T lymphocytes (predominantly CD8+ cells) are seen in the airway wall and smooth muscle and as airflow obstruction progresses, the inflammatory cell count increases.

There are increased numbers of macrophages and neutrophils in the airway lumen [156,157]. Neutrophils cause mucous metaplasia and luminal narrowing and are an important component of the protease/anti-protease imbalance implicated in pathogenesis of emphysema. Neutrophils can enhance connective tissue destruction through the release of elastase and

other inflammatory cytokines. These include matrix metalloproteases (MMPs), some of which degrade collagen and elastin *in vitro*.

Macrophages are the most prominent inflammatory cells seen in lavage and sputum studies and they also produce a range of inflammatory mediators (including MMPs). Studies examining the relationship between COPD and matrix metalloproteases have produced inconsistent results. Increased expression of gelatinase A and B (MMP2 and MMP9) and collagenase 1 and 2 (MMP1 and MMP8) have been found in BAL fluid from subjects with emphysema however [158].

CD8+ T cells play an important role in the defence against respiratory viruses, but whether they also contribute to COPD development is unclear. A positive viral culture can be detected in around 40% of acute exacerbations [159,160] and progression of emphysema has been associated with increased adenoviral E1A protein expression [161]. The defence against viruses may have some detrimental effects; the release of lytic agents from CD8+ cells and interactions with other agents could amplify the inflammatory response.

Pulmonary vessels are also infiltrated and thickened by inflammatory cells (particularly T lymphocytes) and the intimal thickening increases as airflow obstuction progresses [162,163].

There is limited information on the pathological changes that occur during an acute exacerbation of COPD. Bronchial biopsies taken during an exacerbation have found an increase in airway wall eosinophils, neutrophils and activated T lymphocytes, with increased chemokine activity [164,165].

The role of pro-inflammatory cytokines in an acute exacerbation remains unclear. Although studies have found increases in tumour necrosis factor

and interleukin-6 levels, the results for other cytokines (including interleukin-8) have been inconsistent [166,167].

1.6 PHYSIOLOGY

The aim of the respiratory system is to exchange oxygen and carbon dioxide in order to meet the metabolic needs of the body. The exchange of O_2 and CO_2 between air and blood is closely regulated and control of ventilation is a complex interaction between central controllers within the brain, peripheral receptors that sense changes around the body and respiratory muscles. The amount of oxygen in inspired air, cardiac output and the metabolic demands of the body are important contributors to gas exchange.

The central respiratory centres are collections of neurones found in the pons and medulla, with the dorsal medullary centre mainly involved in inspiration and the ventral medullary centre mainly involved in expiration. The inspiratory centre generates nervous impulses which are transmitted to the diaphragm and other respiratory muscles and a pontine 'pneumotaxic centre' can inhibit inspiration, regulating rate and inspiratory volume. Inspiratory centre output can be further modulated by the vagus and glossopharyngeal nerves. Expiratory centres are activated during more forceful breathing and are quiescent during passive breathing. Breathing is under voluntary control to a degree and the cortex can partially override the brainstem, with other parts of the brain influencing breathing during periods of high emotion.

Central chemoreceptors respond to a change in the chemical make up of the blood or surrounding fluid. The most important receptors are on the ventral surface of the medulla and respond to a change in pH of brain extracellular

fluid, with an increase in H+ concentration stimulating ventilation. The blood CO_2 level chiefly regulates breathing by its effect on the pH of CSF and the cerebral vasodilatation associated with hypercapnia enhances CO_2 transfer from blood to CSF.

Peripheral chemoreceptors in the carotid and aortic bodies, unlike central chemoreceptors, respond to a fall in PaO_2 as well as a rise in $PaCO_2$. The response to falls in partial pressure of arterial oxygen is very small with a PaO_2 of over 100 mmHg, but below this point the activity rises sharply. All human responses to hypoxaemia are regulated by peripheral chemoreceptors, whereas they contribute less to the response to hypercapnia than central chemoreceptors. The carotid, but not the aortic chemoreceptors also respond to a fall in pH.

Respiratory gas disturbances are the consequence of an interaction between ventilation-perfusion abnormalities, alveolar ventilation, cardiac output, oxygen consumption and carbon dioxide production.

Hypoxaemia occurs if one of four processes occurs: ventilation–perfusion mismatch, alveolar hypoventilation, shunt or impaired diffusion, whereas the presence of hypercapnia generally reflects alveolar hypoventilation.

The degree of ventilation-perfusion inequality is the main cause of hypoxaemia in disease and can be assessed from an arterial blood gas sample by calculating the alveolar-arterial PO_2 difference. In a perfect lung, ventilation and perfusion are matched. In reality, there are differences in the distribution of ventilation and perfusion in the healthy lung, which range from some areas of ventilated lungs having no perfusion and some perfused areas of lung having no ventilation. Alveoli with no ventilation will have the same PaO_2 and $PaCO_2$ as mixed venous blood and alveoli with no perfusion

have the same PaO_2 and $PaCO_2$ as inspired gas. Most alveoli lie between these extremes and have a PaO_2 and $PaCO_2$ which lie in the range between that of inspired gas and mixed venous blood.

In the healthy lung, most alveoli have a V/Q ratio of 0.5 to 2.0, a range that increases with age and disease. While unventilated alveolar units are classed as 'intrapulmonary shunt', unperfused units are classed as dead space. Increased dead space is overcome by an increase in minute volume and shunts up to 30% can be overcome by increasing the inspired oxygen concentration.

Cardiac output influences respiratory gas exchange. An increase in cardiac output increases pulmonary arterial and venous pressures and leads to recruitment and distension of pulmonary capillaries. If perfusion increases more than ventilation, V/Q mismatch can increase. Enhanced tissue perfusion will follow a rise in cardiac output and the subsequent increase in venous return will lead to a flux of CO_2 to the lungs which stimulates ventilation, a mechanism named 'cardiodynamic hyperpnoea'.

It is important to consider the influence of an increased carbon dioxide production on $PaCO_2$. The amount of CO_2 produced is a function of the metabolic rate and the substrate used for fuel. In healthy subjects, the absorption and metabolism of carbohydrate loads causes an increase in CO_2 output. Interventions which increase the metabolic rate increase CO_2 production and stimulate ventilation.

The work of breathing is produced by respiratory muscles stretching the elastic tissues of the chest wall and lungs and moving air through the respiratory passages. Work is proportional to the pressure change (change in transpulmonary pressure needed to overcome the elastic work of breathing

and the resistive work of breathing) times the volume change (tidal volume). In healthy lungs and during passive breathing, the work of breathing is performed by the inspiratory muscles alone. Half of the work is dissipated as heat overcoming the frictional forces opposing inspiration and half is stored as potential energy in the elastic tissues within the lung and chest wall. Stored energy becomes the energy source for expiration and is dissipated as heat to overcome the friction resisting expiration. The work of expiration in healthy lungs is therefore transferred to inspiratory muscles.

The work performed by the respiratory muscles is very small in the healthy resting subject, with oxygen consumption in the respiratory muscles contributing less than 3% of the metabolic rate. In this situation, the respiratory muscles are only 10% efficient. The oxygen cost of breathing can increase to as much as 30% in a healthy individual during exercise. During exercise larger tidal volumes result in increased work of breathing to overcome the elastic recoil of the lungs and chest wall during inspiration. The lungs are less compliant at high volumes and the elastic recoil of the lungs is inward at high thoracic volumes. High airflow rates result in greater airway resistance, with turbulence and dynamic airway collapse. Healthy adults can increase minute ventilation from 6 L/min to 150 L/min, but cardiac output can only increase 4-6 fold. In healthy adults the increase in oxygen transport with exercise can be 15-20 times the resting rate. P_aO_2 stays constant during exercise, as does P_aCO_2 until anaerobic threshold is reached and lactic acid is produced.

The work of breathing is increased during disease and efficiency falls as minute volume increases. In disease, individuals select a respiratory frequency close to the level that minimises respiratory work.

Subjects with severe COPD working at maximum exercise capacity can only increase oxygen consumption by a factor of 4. The limitation occurs because the lungs are unable to match pulmonary O_2 uptake and CO_2 elimination to the increased whole body level of O_2 consumption and CO_2 production that accompanies exercise.

In subjects with COPD, more work is required to overcome inspiratory frictional resistance as airway obstruction progresses and the oxygen cost of breathing is not only much higher at resting minute volumes, but rises steeply as ventilation is increased. As maximum ventilation is approached, a further increase in ventilation could mean that respiratory muscles are consuming more oxygen than is available to the rest of the body. When respiratory muscles have been severely stretched, they contract with less strength and muscle fatigue is likely to contribute to ventilatory failure. The high rate of activity of respiratory muscles makes them more susceptible to weakness in the context of reduced oxygen supply.

In COPD there is increased dead space as a result of alveoli being replaced by emphysematous air sacs and the presence of uneven alveolar ventilation and perfusion leads to the development of hypoxia. The mismatch between ventilation and perfusion is partially ameliorated by a compensatory mechanism of hypoxic vasoconstriction and collateral ventilation. The blood supply is thus limited in those areas of the lung with low alveolar PaO_2, minimising the degree of hypoxaemia. Some subjects with severe COPD develop a persistent high $PaCO_2$, which may reflect a reduction in the sensitivity of peripheral chemoreceptors.

Chapter Two

The human β_2-adrenoceptor and inhaled β_2-agonists

(Literature review)

A series of scientific breakthroughs in the 20th century led from the concept that cells have a "receptive substance" [168] to the identification and subdivision of adrenergic receptors and the evolution of pharmacological agents specifically directed towards these receptors. This chapter examines the nature of the most important pulmonary adrenoceptor; the β_2-adrenoceptor and looks at the pharmacology of β_2-agonists.

2.1 THE HUMAN β_2-ADRENOCEPTOR

2.1.1 Background

Bates, an American ophthalmologist, first reported the discovery of a substance produced by the adrenal gland in 1886, which was later isolated and identified as epinephrine by a Polish physiologist, Cybulski, and a research team at the John Hopkins University, Baltimore, led by Professor Abel. In 1901 a Japanese chemist, Takamine, isolated the same hormone from the adrenal medulla, and called it adrenaline. The German chemist, Stolz, synthesized adrenaline in 1904 and over forty years later, the Swedish scientist, von Euler, identified the sympathetic neurotransmitter noradrenaline.

The concept that the adrenergic receptor existed in two forms was first suggested following experimental work conducted in the 1940s to find a drug that relaxed the human uterus. Ahlquist compared the effects of catecholamines on a range of target tissues and after finding different responses, determined the rank order of potency of adrenaline, noradrenaline and isoprenaline (a synthetic derivative of noradrenaline) on each tissue. These results led him to propose that distinct adrenergic receptor types existed, which he classified as α- and β-receptors [169].

Lands and colleagues subdivided the β-receptor into β_1- and β_2- subtypes after correlating the effects of catecholamines on lipolysis and cardiac stimulation (β_1-effects) and bronchodilatation and vasodepression (β_2-effects). The order of potency was Isoprenaline > Adrenaline = Noradrenaline at the β_1-receptor and Isoprenaline > Adrenaline > Noradrenaline at the β_2-receptor [170].

β_1-receptors predominate in the heart, with β_2-receptors found in the lungs, heart, skeletal muscle, blood vessels, uterus, bladder and brain. β_3-receptors have since been identified and although not present in the human lung [171], they are found in the heart, adipose tissue, gastrointestinal tract, brain, skeletal muscle and urogenital tract. Their main functions appear to be lipolysis and thermogenesis.

2.1.2 Structure/Function

The β-adrenoceptor is a 413 amino acid protein (Figure 2.1), the product of a 1242 base pair gene located on the long arm of chromosome five (5q31-32). Like other members of the G protein-coupled receptor family, the β-adrenoceptor has an extracellular amino-terminus, seven hydrophobic transmembrane-spanning domains and an intracellular carboxy-terminus, with three extracellular and three intracellular hydrophilic loops. A fourth cytoplasmic loop forms when cysteine at position 341 in the carboxy-terminus forms a covalent bond with fatty acids in the membrane protein (palmitoylation) thus anchoring part of the cytoplasmic tail to the plasma membrane. The three β-adrenoceptor subtypes have structural similarities; the β_2-receptor shares around 54% amino acid sequence homology with the β_1-receptor and 50% with the β_3-receptor.

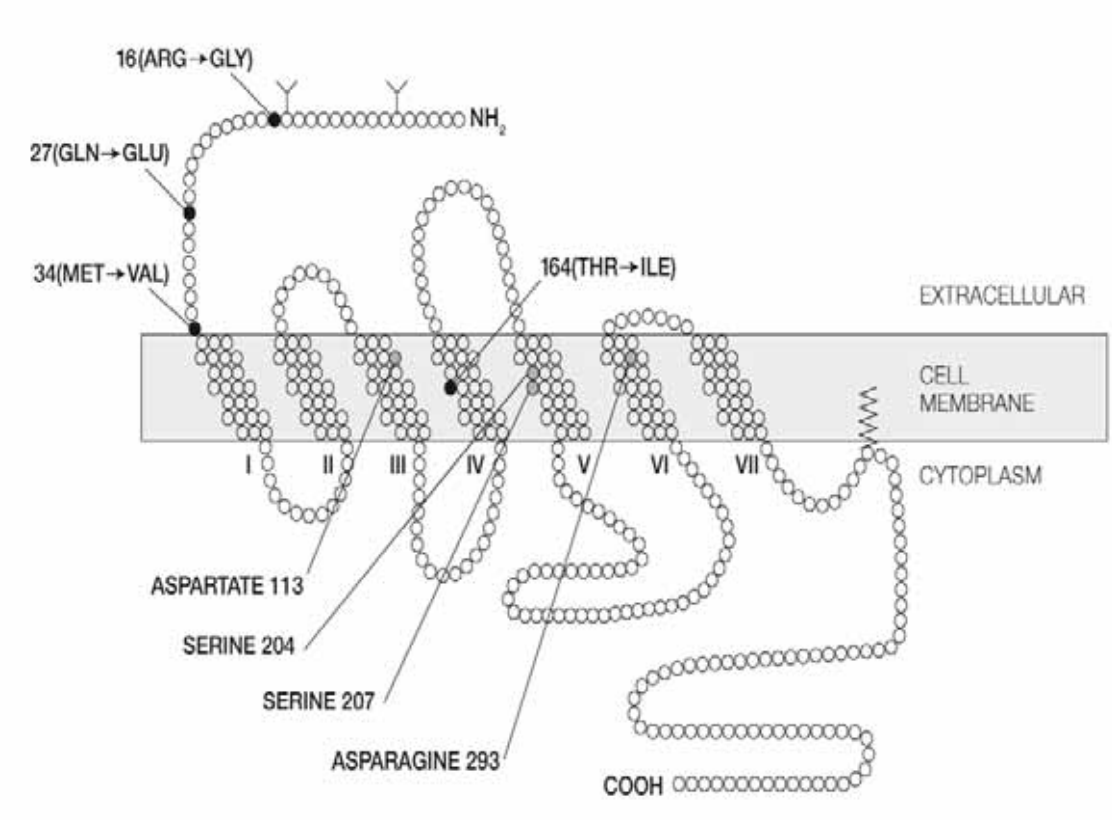

Figure 2.1: The human β_2-adrenoceptor. The sites of receptor polymorphisms are demonstrated (●), together with the amino acid residues involved in agonist binding (●).

Agonist binding to the β_2-adrenoceptor alters the receptor structure, causing activation of the guanine nucleotide-binding protein (G protein). Site-directed mutagenesis studies have determined that the critical sites of agonist binding are in a "pocket" between the third and sixth transmembrane domains [172]. β_2-agonists bind to amino acids on the third (Aspartate 113), fifth (Serine 204, 207) and sixth (Asparagine 293) transmembrane domains [173,174] (Figure 3.1). The part of the receptor that activates the G protein has been localised to part of the third and fourth intracellular loops [175](Figure 2.2).

The β_2-adrenoceptor oscillates between active and inactive states, achieving equilibrium in resting conditions, with the inactive state in predominance. In the active state the receptor is associated with the α subunit of the G protein and the inactive state when the α subunit is dissociated.

2.1.3 Location

Autoradiographic localization studies in ferrets and subsequently on human lungs have shown numerous β–receptors in all airways. The receptor density increases as airways became smaller, with the highest density on the smallest bronchioles, with β_2-receptors outnumbering β_1-receptors by approximately 3:1. β_2-receptors are present in higher density on airway epithelium and submucosal glands than airway smooth muscle, with greatest numbers in alveolar walls (90%). 10% of submucosal and 30% of alveolar receptors are of the β_1–subtype [176,177].

In situ hybridisation of human lung tissue showed that the distribution of β–receptor subtype mRNA was similar to that of binding sites [178]. Large

amounts of β_2-receptor mRNA are found in airway smooth muscle relative to the number of binding sites, which may reflect high receptor turnover and protect against the development of bronchodilator tolerance.

2.1.4 Intracellular mechanisms of action

Binding of the adrenoceptor by the β_2-agonist activates a G protein, changing the G protein conformation, allowing guanosine diphosphate (GDP) bound to the α subunit to be replaced by guanosine triphosphate (GTP). The activated α subunit then dissociates from the other two subunits ($\beta\gamma$), diffuses within the plasma membrane to bind and activate adenylyl cyclase. This catalyses the conversion of adenosine triphosphate (ATP) into cyclic 3'5' adenosine monphosphate (cyclic AMP). This intracellular second messenger causes airway smooth muscle relaxation through a number of protein kinase A (PKA)-mediated mechanisms which include:

1. Inhibition of inositol phospholipid hydrolysis [179]
2. Inhibition of myosin light chain phosphorylation [180]
3. Reduced release of intracellular calcium ions [181]
4. Increased Na+/K+ adenosine triphosphatase [181]
5. Stimulation of calcium-activated potassium channels (maxi-K channels) [182]

Not all β_2-agonist related smooth muscle relaxation is cAMP dependent since the G protein α-subunit can open large conductance potassium channels (maxi-K channels) in smooth muscle, an effect seen in vitro with concentrations of β-agonist that do not cause a rise in cAMP [183].

Protein kinase A can also increase β_2-adrenoceptor gene transcription by phosphorylating cAMP response element binding protein (CREB) in the nucleus, and thus activating cAMP response element (CRE) in the upstream promoter region of the β_2-adrenoceptor gene.

2.1.5 Desensitisation

Prolonged exposure to a given dose of β_2-agonist *in vitro* and *in vivo* leads to a reduced response over time ('desensitisation'). 'Tachyphylaxis' refers to short-term desensitisation and 'tolerance' to desensitisation after prolonged exposure. Several mechanisms contribute to these effects (Figure 2.2), including;

1) β-arrestin binding. Within seconds of agonist-receptor binding, β-adrenergic receptor kinase (βARK), a G protein kinase (GRK), anchors to the two dissociated G protein subunits (βγ) to phosphorylate sites on the carboxy tail [184,185]. Phosphorylation of the receptor by βARK does not directly cause desensitisation, but enhances binding of β-arrestin, a protein that uncouples the receptor from $G_{\alpha s}$ protein and limits receptor function. cAMP-dependant protein kinases also contribute to desensitisation by phosphorylating other parts of the third intracellular loop and carboxy tail.

2) Receptor internalisation / Sequestration. The β_2-adrenoceptor becomes internalised to a subcellular compartment within minutes of agonist exposure and cannot activate the G protein. The process is maximal around 30 minutes and is mediated by βARK/β-arrestin and a tyrosine residue in the seventh transmembrane domain [186]. Removal of the β_2-agonist allows the receptor to return to the cell surface 'resensitised'.

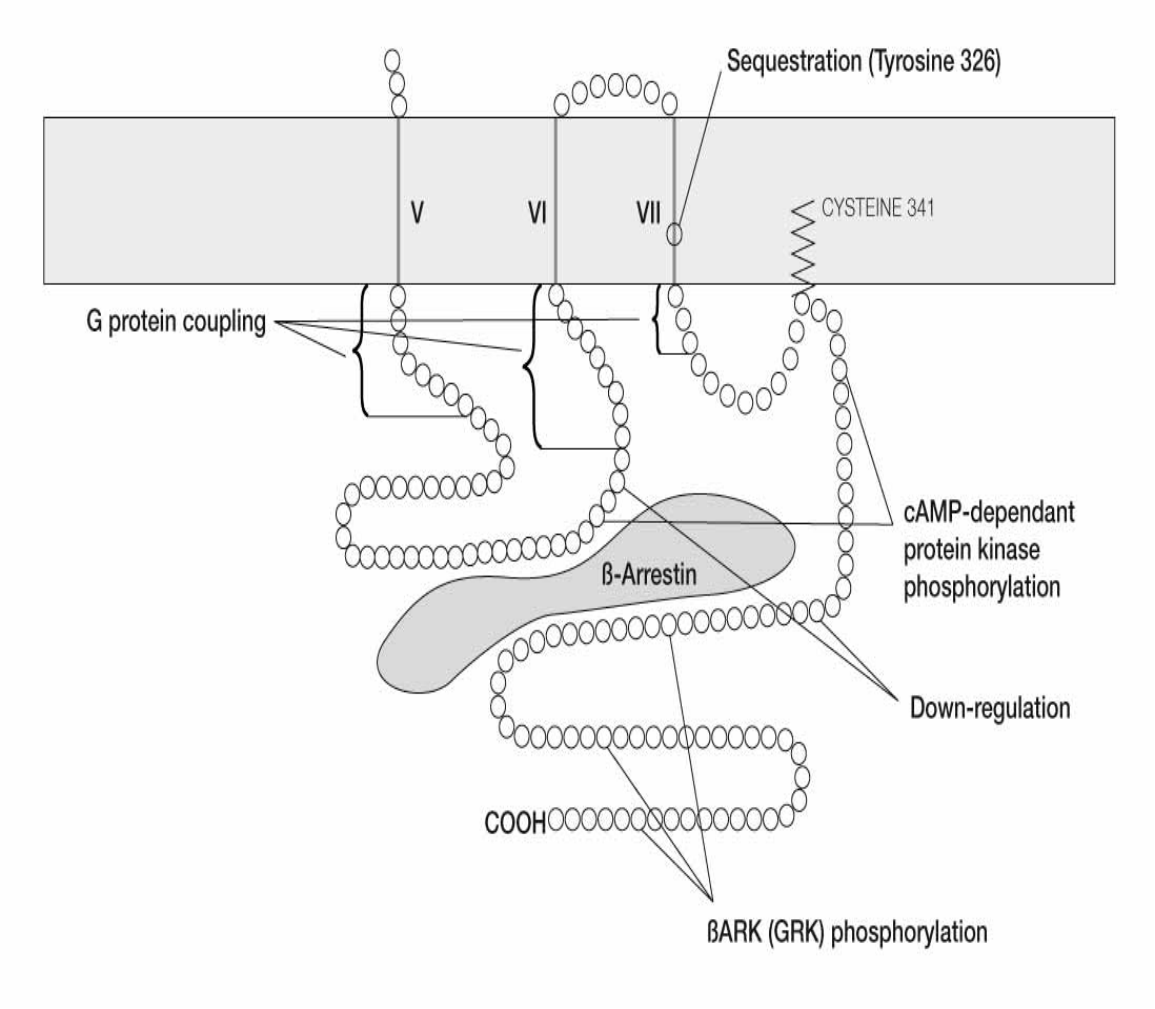

Figure 2.2: The third intracellular loop and carboxy terminal of the human β_2-adrenoceptor. The sites of G-protein coupling and areas involved in receptor desensitisation are identified.

3) Down-regulation. Longer-term exposure to β_2-agonists causes a reduction in the levels and stability of receptor mRNA and a decrease in the rate of receptor gene transcription. Once receptor degradation has occurred, new receptors must be synthesized to restore responsiveness.

'Homologous desensitisation' describes the reduced responsiveness to β-agonists. Desensitisation of the β_2-adrenoceptor to other agonists also occurs and is termed 'heterologous desensitisation'.

2.1.6 Receptor cross-talk

Stimulation of the β_2-adrenoceptor can alter the activity of other receptors and similarly, activity at other receptors can influence β_2-receptor function. The interactions with corticosteroid and muscarinic receptors are of particular importance.

Phosphorylation of the glucocorticoid receptor by β-arrestin-induced mitogen-activated protein kinase (MAPK) makes the β_2-receptor more sensitive to steroid-induced activation. Glucocorticoids can increase β_2-receptor expression in human lung tissue and restore downregulated receptors to normal levels, although high β_2-agonist concentrations may inhibit some of the anti-inflammory effect of glucocorticoids [187].

Furthermore, muscarinic M2 receptors have the opposite effect to β_2-agonists on maxi-K channels and, inflammatory mediators such as acetylcholine can activate smooth muscle receptors that uncouple and downregulate β_2-receptors via phospholipase C and protein kinase C.

2.1.7 Genetic polymorphism

Nine single base polymorphisms in the coding region of the β_2-adrenoceptor gene have been identified. Five are clinically silent because the encoded amino acid is unchanged, but four result in a single amino acid change in receptor structure. Studies using site-directed mutagenesis and recombitant expression suggest that the polymorphisms at positions 16, 27 and 164 may alter receptor function, whilst that at position 34 does not.

The two most common receptor variants are in the extracellular amino terminus, characterised by substitution of glycine (Gly) for arginine (Arg) at position 16 and glutamic acid (Glu) for glutamine (Gln) at position 27, occuring with an allelic frequency of around 60-70% and 40-50% respectively.

The other polymorphisms that alter the β_2-receptor amino acid sequence occur infrequently. Substitution of threonine (Thr) by isoleucine (Ile) at position 164 has an allelic frequency of less than 5%, whilst substitution of valine (Val) by methionine (Met) at position 34 occurs in less than 1% of subjects [188].

Cells with the Ile 164 polymorphism have lower affinity for adrenaline and noradrenaline [189] and lower adenylyl cyclase levels after agonist stimulation [190]. This may relate to the proximity of the polymorphism to serine at position 165, which has a role in agonist binding.

Linkage disequilibrium (when a combination of alleles occur at a higher or lower than expected frequency) occurs between the polymorphisms at positions 16 and 27. The combination of Arg 16-Glu 27 is rare and when individuals have Arg 16 they are likely to have Gln at position 27 [190]. Since most individuals are heterozygotes, large populations are required to

study the relationship between individual β_2-receptor polymorphisms and disease phenotypes.

Three combinations of polymorphisms (haplotypes) at positions 16 and 27 account for most of the population; Arg-Gly/Gln-Glu (26-33%), Gly-Gly/Glu-Glu (18-29%) and Gly-Gly/Gln-Glu (15-22%) [191-193].

Although early studies suggested that the Gly 16 genotype was associated with more severe asthma, a recent large study showed no difference in distribution of β_2-adrenoceptor polymorphisms between the normal population and those with airways disease and no relationship with asthma prevalence [194].

The question of whether β_2-adrenoceptor polymorphisms affect the respose to β_2-agonists has also been studied. In early studies *in vitro* using transfected cell lines or primary airway smooth muscle cells, the Gly 16 variant cells showed increased downregulation in response to β_2-agoinsts and the Glu 27 variant demonstrated attenuated downregulation [195,196].

Numerous studies have examined the association between β_2-receptor polymorphisms and clinical outcomes among subjects with asthma, and to a much lesser extent, chronic obstructive pulmonary disease.

Early clinical studies found that Gly 16 was associated with increased nocturnal symptoms and increased bronchial responsiveness among asthmatics [197-201].

Studies also showed an association between Gly 16 and desensitisation of the bronchodilator response [202,203].

Results have not been consistent however and retrospective analysis of three of the larger studies of subjects with asthma treated with regular salbutamol found that those homozygous for Arg 16 had worse outcomes than those

homozygous for Gly 16 [204-206]. This was corroborated in a recent prospective study, which found that asthmatics with the Gly-Gly 16 genotype using regular salbutamol had better outcomes than those homozygous for Arg 16 [207]. Studies in subjects with asthma found that the Gly-Gly/Glu-Glu combination was associated with a larger bronchodilator response and smaller systemic effects after β_2-agonists than those with the Arg-Arg/Gln-Gln combination [208,209].

Fewer studies have examined β_2-receptor polymorphisms among subjects with COPD. Recent post hoc analysis of patients in the Lung Health Study suggested that heterozygosity at position 27 might confer some protection against rapid FEV_1 decline [210].

2.2 β_2-AGONISTS

Ephedrine has been used in the treatment of respiratory diseases for over 5000 years. Derived from the plant *Ephedra* and called Ma Huang in Chinese medicine, ephedrine is the earliest known bronchodilator. Ephedrine was shown to be effective by inhalation in 1910 [211], producing bronchodilatation.

Solis-Cohen, a physician from Philadelphia, first showed that orally administered adrenal extract (adrenal substance pills) was beneficial in asthma [212]. The direct bronchodilator effect of adrenaline was subsequently demonstrated by Kahn in 1907 using precontracted tracheal strips *in vitro* [213]. Subcutaneous adrenaline injection became a widely used treatment, particularly for acute exacerbations of asthma. Isoprenaline was modified from early adrenergic drugs by German chemists in the 1940s

and became the most widely used treatment for asthma.

The first description of delivering adrenergic agonists by inhalation was by a general practitioner who described the efficacy of nebulising an adrenaline solution with oxygen in patients with acute exacerbations of asthma [214]. Maschberg, an American engineer, designed a special valve in 1956, which allowed patients with asthma to self-deliver bronchodilator medications by aerosol.

The discovery of adrenoceptor subtypes led to the development of β_2-agonists, drugs that were highly selective for the β_2-receptor and had a prolonged duration of action. The short-acting β_2-agonist, salbutamol was introduced as an inhaled treatment for asthma in 1969 and inhaled β_2-agonists with a long duration of action were introduced in the 1990s.

2.2.1 Chemistry

All adrenergic agonists have the same basic structure; a benzene ring with an attached chain of two carbon atoms linked to an amine group or a substituted amine head. Catecholamines are characterised by the presence of hydroxyl groups at positions three and four of the benzene ring. Modification to the basic catecholamine structure has produced non-catecholamines that are more selective to the β_2-receptor and are metabolised more slowly.

β_2-agonist non-catecholamines have a substitution or repositioning of the hydroxyl groups that reduces potency, but confers greater metabolic stability and resistance to breakdown. Increasing the size of the alkyl substitution on

the amine group also reduces potency, reduces drug metabolism and increases β_2-receptor selectivity.

All β_2-agonists contain at least one asymmetrical carbon which results in the molecule existing as a pair of optical isomers (mirror images), the R or S enantiomers, in a racemic mixture. Some β_2-agonists have two asymmetrical centres and four enantiomers, known as RR, SS, RS and SR. Experimental data has shown that the R-configuration mediates the beneficial adrenergic effects [215], whereas the S-configuration has weaker and often negligible effects. These differences may occur because the β-hydroxy group on the R-enantiomer is orientated downwards and could have a better interaction with key amino acids in the adrenoceptor [216].

2.2.2 Metabolism and elimination

Endogenous catecholamines are metabolised following active removal processes known as uptake-1 and uptake-2. Noradrenaline and adrenaline are taken into synaptic storage vesicles in sympathetic nerve terminals (uptake-1) where they are metabolised by monoamine oxidase (MAO), which inactivates them by cleaving or deaminating the side chain. All catecholamines are metabolised by uptake-2 in non-neuronal tissues with sympathetic innervation, where the enzyme catechol-O-methyl transferase (COMT) terminates their action by methylation of the catechol nucleus. Catecholamines have short half-lives due to rapid metabolism by COMT and MAO; the breakdown products are then excreted in urine.

Non-catecholamine β_2-agonists do not undergo metabolism via uptake-1 or uptake-2. Altering the hydroxyl groups on the catecholamine benzene ring creates resistance to COMT breakdown and substitution of the amine head

imparts resistance to MAO allowing a longer duration of action. Non-catecholamine β_2-agonists have longer half lives than catecholamines and are eliminated by metabolism or renal excretion of unchanged drug. Elimination depends on the route of administration; parenteral administration leads to most drug being excreted unchanged in urine, avoiding the first pass elimination in liver and small intestine seen after inhalation and oral administration.

2.2.3 Lipophilicity

Short acting β_2-agonists are generally hydrophilic and access the receptor from the extracellular aqueous compartment. Long-acting β_2-agonists have much greater lipophilicity and are rapidly taken up into the cell membrane, before interacting with the receptor. The lipophilicity of long-acting β_2-agonists plays an important role in determining the onset and duration of their action.

Gastrointestinal absorption is also affected by lipophilicity, since absorption of lipophilic β_2-agonists is almost complete, whereas absorption of hydrophilic β_2-agonists is generally much lower.

2.2.4 Systemic absorption and drug distribution

The inhaled route delivers drug directly to the airways, allowing lower doses to be given and reducing systemic blood levels. Although β_2-agonist reaching the lungs is rapidly absorbed, most inhaler devices only deliver around 20% of the drug to the lung (Section 2.6.2).

Following oral administration of β_2-agonists, the bioavailability will be reduced by pre-systemic first-pass metabolism, with conjugation to sulphates or glucoronides following uptake in the gut wall and liver.

Lipophilic β_2-agonists have high plasma protein binding and hydrophilic agents have low protein binding. Although β_2-agonists have a large volume of distribution, animal experiments have found that only modest amount of β_2-agonist cross the blood brain barrier [217].

2.2.5 Efficancy, potency and selectivity

β_2-agonists have variable efficacy (ability to produce the desired effect), potency (amount of drug needed to produce an effect) and selectivity for β_2- versus β_1-adrenoceptors (ability to relax airway smooth muscle (β_2-effects) versus the effect on cardiac β-receptors).

While a full agonist has high efficacy and an antagonist would have low or zero efficacy, most β_2-agonists have intermediate efficacy. Partial agonists can antagonise full agonists by occupying receptors and reducing the access of drugs that have fuller agonist activity. Although β_2-agonists have variable efficacy in studies *in vitro*, the bronchodilator efficacy of β_2-agonists in human studies shows little difference between drugs.

A highly selective β_2-agonist drug produces more beneficial airway effects and fewer positive inotropic and chronotropic effects than a non-selective drug. Although the short- and long-acting inhaled β_2-agonists in current use have similar selectivity and are more selective than isoprenaline, some inhaled β_2-agonists, including fenoterol, are less β_2-selective and are no longer widely used.

2.3 PHARMACOLOGY OF SPECIFIC β_2-AGONISTS

Differences in β_2-agonists relate to a combination of characteristics and of the many examples, only four are frequently prescribed to patients with COPD. Here I have summarised the pharmacology of the short-acting β_2-agonists, salbutamol and terbutaline, and the long-acting β_2-agonists, salmeterol and formoterol (Figure 2.3).

2.3.1 Salbutamol

Salbutamol is a saligenin, with a $–CH_2OH$ replacing the 3-hydroxy group and protecting the molecule from COMT. It has a N-tertiary butyl substituent side chain preventing MAO breakdown and conferring β_2-selectivity and exists as a mixture of R- and S- enantiomers.

Salbutamol is hydrophilic, so penetration of the lipid bilayer is poor and the drug accesses the β_2-adrenoceptor from the extracellular aqueous compartment. Inhaled salbutamol has a rapid onset of action, with bronchodilation occuring within five minutes, peak bronchodilation occurring around 60 minutes and the effect maintained for up to six hours.

A proportion of inhaled salbutamol is delivered to the oropharynx and subsequently to the gut after swallowing, with only negligible buccal absorption and a variable amount reaching the lungs. Salbutamol delivered to the lungs is rapidly absorbed and excreted unchanged by the kidneys. Salbutamol is almost completely absorbed from the gastrointestinal tract, with around half undergoing first-pass metabolism. Sulphate conjugation in the gastrointestinal mucosa and liver produces a virtually inactive metabolite, with renal excretion accounting for 60% of the total clearance of salbutamol and its 4,*O*-sulphate conjugate.

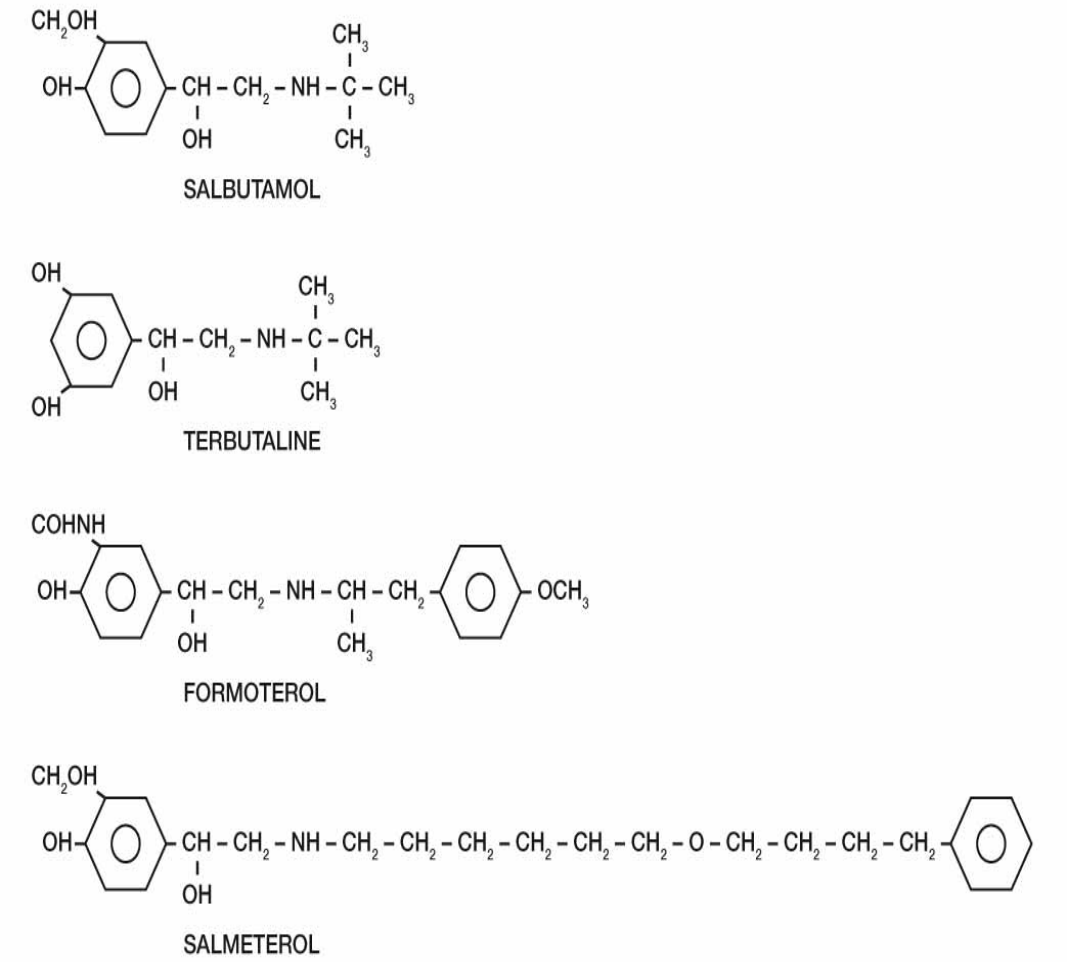

Figure 2.3: The chemical structure of the main β_2-agonists used in COPD.

Blood levels are low after inhaled salbutamol, reaching a peak between five and fifteen minutes post inhalation, with a half-life of four to six hours [218-220].

Differences have been found in the bioavailability of R- and S-enantiomers after inhaling racemic salbutamol and although results have been inconsistent [221,222], there is some evidence of preferential gut metabolism of the more pharmacologically active R-enantiomer [223].

2.3.2 Terbutaline

Terbutaline has the same amine side chain substitution as salbutamol, with hydroxyl groups at positions three and five of the benzene ring, and exists as a pair of optical isomers.

Terbutaline is highly hydrophilic with poor lipid bilayer penetration, acting directly on the β_2-adrenoceptor. The rapid onset and duration of action is similar to that of salbutamol. Of the terbutaline that is swallowed following inhalation, around half is absorbed from the gastrointestinal tract and two-thirds of the absorbed drug undergoes first pass metabolism in the gut wall and liver. It is conjugated with sulphuric acid and excreted as sulphate conjugate, with no active metabolites formed.

Peak serum concentrations of terbutaline are recorded 30-60 minutes after inhalation. Terbutaline and its metabolites are mainly excreted in urine and it has a plasma half-life of almost six hours.

2.3.3 Salmeterol

Salmeterol is a saligenin derivative of phenylethanolamine with a large, eleven-atom, aliphatic side chain.

It is a highly potent relaxant of human bronchial airway smooth muscle *in vitro*, with β_2:β_1 selectivity of 2800. However, *in vivo* salmeterol is a partial agonist and has a lower efficacy on airway smooth muscle than salbutamol.

Salmeterol is highly lipophilic, with a slower onset of action than salbutamol, but a long duration of action. Neutron diffraction studies show that salmeterol diffuses rapidly and completely into the lipid bilayer and then slowly binds to an exosite found on β_2- but not the β_1-adrenoceptor, in addition to the normal binding sites. The exosite, located at a relatively hydrophobic section of the fourth transmembrane regulator domain by sequence mutagenesis [224], binds to the long hydrophobic side chain of salmeterol preventing dissociation from the receptor and allowing the saligenin head of the molecule to engage repeatedly with the active receptor site and prolonging the drugs duration of action [225].

Salmeterol is well absorbed from the lung and gut and is metabolised by hydroxylation in the liver. The majority of the drug is eliminated within 72 hours, 23% in urine and 52% in faeces [226]. 28-36% of the systemic effects produced by inhaled salmeterol are caused by drug absorption from the gastrointestinal tract [227].

2.3.4 Formoterol

Formoterol, a formanilide-substituted phenoethanolamide, is a highly potent relaxant of human bronchial airway smooth muscle in vitro, with β_2:β_1 selectivity of 200-400. Formoterol is a fuller agonist than salbutamol, terbutaline and salmeterol [228].

Following inhalation, formoterol has a rapid onset and long duration of action. It is moderately lipophilic, which enables the drug to dissolve into

the cell membrane lipid bilayer, to diffuse and bind to traditional β_2-receptor binding sites. Some drug remains in the aqueous phase and reacts directly with the receptor, accounting for the rapid duration of action [228]. The onset of bronchodilatation after inhaled formoterol is similar to that seen with salbutamol [229]. Despite being lipophilic, absorption is poor after oral ingestion of formoterol. Oral formoterol does not produce high concentrations of drug in the lipid bilayer and it does not produce the long-acting effects seen with inhaled formoterol.

Formoterol is well absorbed from the lung and exhibits a biphasic serum concentration after inhalation with an initial peak after fifteen minutes and a smaller peak after ninety minutes. Formoterol is largely metabolised by hepatic conjugation with glucuronic acid and the metabolites are mainly excreted in urine. The mean plasma half-life of inhaled formoterol is around two hours and twelve hours after inhalation, 24% of the drug has been excreted in urine [230].

The clinical efficacy of these four inhaled β-agonists in subjects with chronic obstructive pulmonary disease is reviewed in chapter 3.

2.4 AIRWAY EFFECTS OF β_2-AGONISTS

β_2-agonists have a range of effects that can contribute to airway obstruction.

2.4.1 Airway smooth muscle

β_2-agonists relax airway smooth muscle and are functional antagonists in that they inhibit or reverse the airway response to bronchoconstrictor stimuli [231]. β_2-agonists have also been shown to inhibit smooth muscle proliferation after exposure to different agents, including epidermal growth factor [232,233].

2.4.2 Blood vessels

β_2-agonists cause vasodilatation and increase blood flow in the pulmonary circulation [234]. They reduce airway microvascular leakage induced by inflammatory mediators in animal airways [235,236] and formoterol reduced histamine-induced plasma exudation in the induced sputum of healthy subjects [237].

2.4.3 Mucus secretion and mucociliary clearance

β_2-agonists increase ciliary beat frequency in human and mammalian airway epithelial cells, enhancing mucus clearance [238-240]. Although β_2-agonists stimulate mucus secretion in animal models, consistent effects have not been demonstrated in human airways and their effect on human epithelial goblet cells is unclear [241-243]. β_2-agonists may prevent airway closure by stimulating the release of surfactant lipids from Clara cells [244].

2.4.4 Epithelial protection

β_2-agonists may counteract some of the detrimental effects of bacterial infection and may help to protect the airway epithelial barrier. Salmeterol has been associated with reduced pseudomonas aeruginosa and haemophilus influenzae induced epithelial damage [245,246]. The beneficial effect is likely to relate to improved structural integrity of the epithelium rather than a direct antibacterial effect. Evidence in this area is limited, but salmeterol also increased the expression of tight junction proteins in human airway epithelial cells; proteins which are important in preserving the epithelial barrier [247].

2.4.5 Inflammation and mediator release

Some studies *in vitro* have found that β_2-agonists have anti-inflammatory effects. Not all studies have demonstrated an effect however and some effects are only seen in high doses [248]. β_2-agonists have been associated with inhibited release of thromboxane, leukotrienes and eosinophil-activating cytokines from airway smooth muscle cells [249,250].

Although β_2-agonists have demonstrated *in vitro* anti-inflammatory properties, data from bronchial biopsy studies does not suggest that long-term treatment has a beneficial effect on chronic inflammation [251].

2.5 SYSTEMIC EFFECTS OF β_2-AGONISTS

Inhaled β_2-agonists are absorbed into the systemic circulation and cause adverse effects directly relating to the stimulation of β_2-adrenoceptors. These effects are summarised below and are opposed by β-receptor antagonists.

2.5.1 Tremor

β_2-agonists produce tremor by stimulating receptors on extrafusal fibres and spindles of skeletal muscle [252]. The tremor increases in a dose-dependant manner in healthy subjects and patients with asthma and COPD [253-256]. It is more apparent after oral administration of β_2-agonists and with high doses delivered via a nebuliser [257]. Tremor is reported by around 2 – 4% of patients with asthma taking a regular β_2-agonist [258]. Mann et al (1996) found that 1 in 296 asthmatics discontinued inhaled salmeterol because of

tremor. Blunting of the tremor response has been demonstrated among subjects taking regular inhaled β_2-agonists [259].

2.5.2 Hypokalaemia

Stimulation of β_2-receptors on skeletal muscle activate the Na+/K+ ATPase 'pump', which increases sodium efflux, hyperpolarazing the cell and causing flux of potassium ions into cells and reducing extracellular and plasma potassium concentrations [260,261]. Plasma potassium falls in a dose related manner after inhaled short- and long-acting β_2-agonists among both healthy volunteers and subjects with asthma and COPD [262-266]. The degree of hypokalaemia is reduced after prolonged use of inhaled β_2-agonists [267,268]. Studies involving healthy subjects have found that inhaled β_2-agonists reduce the plasma potassium by between 0.3 and 1.12 mmol/L, even when doses well above recommended levels are used [262-264]. Inhaled β_2-agonists appear to produce a similar or lesser degree of hypokalaemia when equivalent doses are administered to subjects with asthma and COPD.

2.5.3 Cardiac effects

β_2-agonists relax vascular smooth muscle and cause peripheral vasodilatation, with reflex cardiac stimulation leading to tachycardia; direct stimulation of cardiac β_1- and β_2- adrenoceptors also contributes to an increase in heart rate and contractility. Radioligand studies have demonstrated that β_2-receptors constitute around 35% of atrial and 25% of ventricular β-adrenoceptors [269,270].

β_2-agonists can cause T wave changes and a prolonged QT_c interval on the electrocardiograph. In a recent study nebulised salbutamol enhanced AV node conduction and reduced the AV nodal, atrial and ventricular refractory period, changes that were independent of heart rate and could contribute to the generation of arrhythmias [271].

Inhaled β_2-agonists cause a dose related increase in heart rate and systolic blood pressure, a fall in diastolic blood pressure and prolongation of the QT_c interval in healthy volunteers and patients with asthma and COPD [253,254,272-274]. Like other systemic effects, tolerance has been demonstrated for the acute cardiovascular effects, with a reduction in the change in heart rate, QTc interval and T wave response after long-term inhaled β_2-agonist use [275,276].

2.5.4 Hypoxaemia

Inhaled β_2-agonists transiently reduce the partial pressure of arterial oxygen (P_aO_2) in patients with asthma and COPD. A fall in P_aO_2 was originally seen in patients receiving intravenous aminophylline, but similar changes were noted in asthmatic patients with inhaled isoprenaline and salbutamol [277-279]. The fall in P_aO_2 is mainly attributed to a β_2-agonist mediated increase in ventilation-perfusion mismatch. The effect is generally small and returns to normal within thirty minutes [280].

The distribution of ventilation throughout the lung is more variable in COPD than in normal lungs and the variability increases as COPD becomes more severe. The mismatch between ventilation and perfusion in the lungs of a subject with COPD is partially ameliorated by an adaptive mechanism of hypoxic vasoconstriction in poorly ventilated areas of the lung.

β_2-agonists cause vasodilatation in the hypoxic areas of lung without equivalent brochodilatation, worsening ventilation-perfusion mismatch and reducing the partial pressure of arterial oxygen.

β_2-agonists can increase minute ventilation and although the exact mechanism of this action is unclear, potential mechanisms include stimulation of central or peripheral receptors. β_2-agonists increase oxygen consumption and carbon dioxide production and the subsequent flux of carbon dioxide to the lungs may directly stimulate ventilation (cardiodynamic hyperpnoea)[281,282].

2.5.5 Other effects

β_2-agonists can cause agitation, irritability and headaches. This has been attributed to stimulation of receptors in the central nervous system, with β_2-receptors most abundant in the cerebellum and hippocampus [283].

Other metabolic changes after administration of β_2-agonists include an increase in serum insulin, plasma glucose and lactate. The rise in insulin levels may result from direct stimulation of pancreatic islet cell β_2-receptors and would also contribute to the fall in plasma potassium [268,284]. Blood glucose levels increase with inhaled short- and long-acting β_2-agonists as a result of hepatic glycogenolysis and gluconeogensis [227,285]. β_2-agonists also increase muscle glycogenolysis and produce lactate [286]. It is unclear whether long-term β_2-agonist use attenuates the metabolic effects [268].

β_2-agonists are used in obstetrics as a tocolytic agent, when relaxation of the uterine smooth muscle can suppress contractions and delay the onset, or halt the progress, of labour.

2.5.6 Safety

Questions surrounding the safety of β_2-agonists date back to the 1960s, when it was suggested that a sharp increase in asthma deaths, particularly among young people, may be due to overuse of inhaled sympathomimetics [287,288]. Adrenergic agents can cause areas of myocardial necrosis when infused into animals, and similar lesions have been demonstated in humans who died from asthma [289,290].

A number of theories have been suggested to explain the association between asthma deaths and sympathomimetic use, including one that the introduction of isoprenaline 'forte' was responsible for the asthma epidemic. Isoprenaline is a poorly selective β_2-agonist and the dose of 'forte' was five times the standard dose. Drug sales showed a temporal relationship with asthma deaths and mortality increased in countries where the 'forte' preparation was used [288, 291]. As a result of the concerns, bronchodilator aerosols became available by presciption only in the United Kingdom.

It was also proposed that the freons found in fluorocarbon propellants might sensitise the heart to asphyxia-induced arrhythmias, but studies with placebo inhalers could not replicate the high blood levels of freon required to cause cardiotoxic effects in animals [292].

The safety of β_2-agonists was brought into question again a decade later after an increase in asthma deaths occurred in New Zealand and three case-control studies showed an association between use of inhaled fenoterol and an increased risk of death in young patients with severe asthma [293-295]. A Canadian case-control study noted that the odds ratio of death or near death from asthma was high with the regular use of inhaled β_2-agonists, particularly fenoterol [296].

Concerns remain about the safety of regular inhaled β_2-agonists in asthma and have extended to long-acting β_2-agonists. A recent large-scale surveillance study looking at the safety of regular inhaled salmeterol was halted prematurely after an increase in asthma-related deaths was identified in the active limb [297].

Debate about the safety of inhaled β_2-agonists in patients with COPD has followed on from concerns raised in patients with asthma. Cardiovascular safety is particularly relevant in patients with COPD, since a significant proportion of patients have co-existing, or subclinical, ischaemic heart disease [298]. The risk of developing a cardiac event (ischaemia or arrhythmia) may be particularly high in the presence of hypoxaemia, hypercapnia and acidosis and if concomitant drugs that also stimulate the myocardium (eg theophyllines) and cause hypokalaemia are used. The potential for risk may be increased further if β_2-agonists cause tachycardia, hypokalaemia and worsening of hypoxaemia. The effect of inhaled β_2-agonists on cardiovascular outcomes (heart rate, QTc, peripheral vascular resistance) are greater during hypoxaemic conditions [299,300]. The presence of hypoxia and hypercapnia can be particularly pro-arrythmogenic for subjects with COPD; in this situation, correction of hypoxia can shorten an already prolonged QTc interval [301].

Few studies have concentrated on the cardiovascular safety of inhaled β_2-agonists in COPD, with most safety data extracted from meta-analyses and prospective studies designed to assess effectiveness, many of which exclude patients with ischaemic heart disease.

Conradson et al concluded that patients with COPD and no evidence of ischaemic heart disease were not at increased risk of arrhythmia after

inhaled β_2-agonists [302]. Suissa et al (1996) used subjects from the Saskatchewan health insurance database to examine the relationship between bronchodilator use and acute cardiac death, but found no associated increased risk with the use of inhaled β_2-agonists in patients with cardiac disease [303]. A meta-analysis of seven long-term studies found no difference in cardiovascular events or death rates between patients with COPD treated with salmeterol 50 μg bd and those treated with placebo, even after stratifying by age or known cardiovascular disease [304]. A community-based study with a cohort of over 12000 subjects found that the use of short-acting inhaled β_2-agonists by subjects with COPD was not a risk factor for myocardial infarction [305].

Some studies have found an association between β_2-agonist use and adverse outcomes in COPD. Au et al (2000) conducted two case-control studies in North America and found an increased risk of myocardial infarction among subjects recently commenced on an inhaled β_2-agonist [306] and an increased risk of acute coronary syndrome among subjects with COPD filing a prescription for an inhaled β_2-agonist [307]. Although one meta-analysis concluded that β_2-agonist use in obstructive airways disease increased the risk of adverse cardiovascular events [308], major flaws in the methodology and analysis were identified [309]. Cazzola et al compared the effect of a single dose of salmterol 50 μg, formoterol 12 μg and 24 μg on electrocardiography in twelve subjects with COPD, hypoxaemia and previous cardiac dysrhythmia. Each dose increased the heart rate and caused a fall in serum potassium levels, but the effect was greatest with the higher

dose of formoterol and one third of subjects developed paired or multiform ventricular beats.

In the recent TORCH (Towards a Revolution in COPD Health) study [310], over 1500 subjects with COPD were randomised to receive salmeterol 50 μg twice daily for three years. The study found no difference between placebo and salmeterol for all-cause mortality rates or COPD-related deaths, with fewer subjects discontinuing salmeterol than placebo. Subjects randomised to salmeterol had fewer moderate or severe exacerbations, required fewer systemic corticosteroids and had fewer hospitalizations. Adverse events were similar in the placebo and salmeterol groups and there was no evidence of an increase in cardiac events in the salmeterol limb.

2.6 FACTORS AFFECTING DRUG DEPOSITION

Inhaler devices have the advantage of delivering high drug concentrations directly to the disease site, achieving a similar or superior therapeutic effect at a lower dose than is required with systemic administration.

2.6.1 Particle size

The deposition and distribution of inhaled drugs within the lung depends on several factors, including particle size. Fine particles are distributed in peripheral airways at low levels of drug per unit surface area, while large particle aerosols are deposited at higher density on central airways [311].

Inhaled drugs usually consist of particles of varying size, with the size calculated using mass median diameter (When 50% of particle mass appears above and below this point) and aerodynamic diameter (The diameter of a sphere of unit density that has the same settling velocity as the particle

regardless of shape or density). The mean mass aerodynamic diameter (MMAD) is calculated from a cumulative distribution curve of the different particle sizes and volumes and geometric standard deviation (GSD) measures the variability of particle sizes within the aerosol [312].

Lung deposition occurs by inertial impaction (>5μm), gravitational sedimentation (0.4-5μm) or diffusion (<0.4μm), with the majority of particles with a diameter of 5-10 μm deposited in the large conducting airways. In the alveoli there is negligible air velocity and deposition occurs by sedimentation and diffusion [313]. Particles > 10 μm are generally deposited in the oropharyngeal region and subsequently swallowed, while particles <3 μm have an 80% chance of reaching the lower airways with 50-60% being deposited in the alveoli [314].

2.6.2 Delivery device.

Various devices are available to deliver inhaled drugs including nebulisers, metered-dose inhalers (MDI) and dry-powder inhalers (DPI). Each has advantages and disadvantages.

There are two main types of nebuliser device. The jet nebuliser uses the Bernoulli principle, with compressed air passing through a narrow orifice to produce an area of low pressure, drawing drug solution from a fluid reservoir and producing shattered droplets. Ultrasonic nebulisers use a high frequency vibrating crystal to generate a fountain of small droplets.

Nebulisers do not require patient co-ordination or a specific inhalation technique, but the majority of nebulised drug deposits within the apparatus or is released into the environment. Small amounts are deposited in the oropharynx and often only around 10% of the drug reaches the lungs [315].

Nebuliser solutions with a low pH and/or hypo-/hyper-osmolality can cause bronchoconstriction and hence alter drug deposition [316,317].

Metered-dose inhalers are compact and portable, with the drug aerosol driven by chlorofluorocarbons (CFC) or hydrofluoroalkanes (HFAs) through a nozzle at a velocity of over 30 metres per second. Even with effective delivery (good hand-mouth coordination and an appropriate inspiratory flow rate), only around 10-20% of emitted drug reaches the lung with many inhalers, with approximately 50-80% delivered to the oropharynx due to the large particle size and velocity of delivery [318]. Although effective use of the MDI requires good hand-mouth co-ordination, over 50% of subjects were unable to manage this in one study [319]. The inspiratory flow rate is an important variable for drugs with a MMAD of 1-5 μm, since very high flow rates reduce total lung deposition and peripheral penetration, since more drug is deposited by inertial impaction in the conducting airways and oropharynx [320]. With slow inhalation and an increase in tidal volume, deposition in the peripheral regions of the lung is enhanced [321-323], while a ten-second-breath hold reduces the immediate exhalation of drug deposited in the peripheries.

The addition of a holding chamber or spacer device to a MDI can decrease the proportion of drug deposited in the oropharynx (reducing the mean particle size and slowing aerosol velocity) and can increase peripheral drug deposition [324].

Dry powder inhalers were designed to eliminate the need for hand-mouth co-ordination and have the environmental advantage of not using chlorofluorocarbon propellants. Various devices are available, producing variable lung deposition (12-40%), with around a quarter of the drug being

retained in the device [325-327]. Lung delivery is enhanced by a rapid inspiratory rate, since airflow through the inhaler provides the required turbulence to separate drug from carrier particles and produce particles small enough to be carried into the lower airways [328]. Poor deposition can relate to slow inspiration rates or factors (high humidity, changes in temperature) that impair the deaggregation of drug particles from larger carrier particles. Modern dry powder inhlers are breath actuated and require an inspiratory flow rate of 30-130 L/min to achieve an aerosol in the appropriate range [329].

2.6.3 Pulmonary factors.

2.6.3a Drug deposition

Pulmonary factors including airway narrowing and inflammation can affect the deposition of an inhaled drug. Airway obstruction increases turbulence and disturbs the normal laminar flow, with inhaled aerosols directed towards unobstructed airways and less drug delivered to obstructed areas. Airway obstruction is associated with central deposition of the inhaled drug, with deposition depth correlating with FEV_1 [322,330]. Severe obstruction causes a heterogeneous central distribution, unlike the uniform and peripheral distribution seen in normal lungs.

2.6.3b Drug clearance

Various factors protect the lung against inhaled particles, including airway geometry, humidity and clearance mechanisms. The complex airway branching system with progressively smaller airways encourages particles to deposit by impaction [331]. The high relative humidity ensures that

hygroscopic particles increase in size (upto five fold) as they move from an area of low temperature and humidity into the airways, encouraging central distribution within the lung [332-334].

Following deposition, inhaled drugs are degraded, absorbed into the systemic circulation or cleared from the lungs. Within conducting airways, most drug is cleared by ciliated epithelia which stretch from the trachea to terminal bronchioles. Particulate matter is trapped in the mucus layer and beating of the cilia generates upward movement of the particles towards the pharynx and subsequently, the gastrointestinal tract. Mucociliary function is usually impaired with airflow obstruction, with secretions and particulate matter removed by coughing. Despite this, there is an inverse relationship between FEV_1 and drug clearance [335].

Some soluble particles are absorbed, with lipophilic molecules passively transported through airway epithelium and hydrophilic molecules crossing extracellular pathways or being actively transported into the circulation or lymphatics [336]. Alveolar drug particles are either phagocytosed by macrophages and slowly cleared from the lungs or absorbed into the pulmonary circulation [337]. Drug metabolism within the lung plays a minor role in drug clearance; although metabolising agents are found throughout the airways and alveoli, more than 95% of inhaled proteins are absorbed intact [338].

Chapter Three

A risk-benefit assessment of inhaled β_2-agonists in the management of Chronic Obstructive Pulmonary Disease

(Literature review)

Patients with COPD have limited reversibility of airflow obstruction and therefore limited capacity to respond to pharmacological interventions. Inhaled β_2-agonists are frequently prescribed to patients with COPD and this chapter examines the balance between benefits and risks with long-term use.

3.1 EFFICACY OF REGULAR SHORT-ACTING β_2-AGONISTS IN PATIENTS WITH COPD

Inhaled short acting bronchodilators have a rapid onset of action and are the first line treatment for patients with symptomatic COPD. Inhaled β_2-agonists are used by millions of patients with COPD throughout the world but very few studies have examined the long-term effects of regular treatment.

The limited data from studies that have compared regular inhaled salbutamol or terbutaline taken at recommended doses with placebo, inhaled muscarinic receptor antagonists and oral theophyllines has been reviewed. Only studies that have been conducted over at least two weeks' duration have been included (Table 3.1).

3.1.1 Studies comparing short-acting β_2-agonists with placebo

3.1.1a Lung function

Regular inhaled short-acting β_2-agonists have been shown to cause bronchodilatation in subjects with COPD, improving morning FEV_1 [339-342], FVC [339,341,343] and PEFR [339,341].

3.1.1b Symptoms and quality of life

Salbutamol 200 μg, inhaled four times a day, has reduced breathlessness compared to placebo [340], although other authors have concluded that

regular salbutamol is no more effective than placebo at reducing symptoms among subjects with COPD [341,342].

Only two studies have examined the effect of regular inhaled short-acting β_2-agonists on quality of life. One study found that salbutamol 200 μg taken four times daily for two weeks had a beneficial effect on quality of life compared to placebo, improving the physical and emotional function domains of the Chronic Respiratory Disease Questionnaire [339]. The other study found that salbutamol 200 μg four times daily and placebo had comparable effects on health related quality of life (HRQOL) [342].

3.1.1c Effect on exacerbations

No published data exists to determine whether regular long-term inhaled short-acting β_2-agonists have a beneficial or detrimental effect on exacerbation rates in subjects with COPD.

3.1.1d Exercise tolerance

Two studies have compared the effect of two weeks of treatment with salbutamol or placebo on walk distance and obtained conflicting results. Regular salbutamol 200 μg four times daily increased six-minute walk distance in one study, with a corresponding reduction in exertional dyspnoea scores [339]. The second study found that the same dose of inhaled salbutamol and placebo had the same effect on twelve-minute walk distance [342].

Study	No of subjects	Duration SAβA (weeks)	SAβA qid dose (μg)	Mean FEV_1 % predicted	Control
Versus placebo					
Guyatt et al 1987	19 XO	2	Sb 200	1.02 L[a]	Placebo T bid Sb 200 + T bid
Guyatt et al 1989	24 XO	2	Sb 200	34	Placebo T bid Sb 200 + T bid
Jaeschke et al 1991	24 XO	2	Sb 200	34	Placebo T bid Sb 200 + T bid
Thomas et al 1992	12 XO	2	Sb 200	1.09 L[a]	Placebo T bid Sb 200 + T bid
Sansores et al 2003	20 XO	2	Sb 200	38	Placebo IB 80 μg qid Sb 200 + IB 80 μg qid
Versus other bronchodilators					
COMBIVENT study group 1994	534 PG	12	Sb 200	37	IB 42 μg qid Sb 200 + IB 42 μg qid
Man et al 1996	20 XO	2	Sb 200	1.18 L[a]	T bid
Campbell 1999	357 PG	4	Sb 200	36	Sb 200 + IB 40 μg qid
D'Urzo et al 2001	172 XO	3	Sb 200	51	F 12 bid
Cook et al 2001	53 XO	13	Sb 200	34	Sb 200 'as required'

FEV_1 = forced expiratory volume in one second; [a] Mean FEV_1 (% predicted not given); SAβA = short-acting β_2-agonist; XO = crossover; PG = parallel group; Sb = salbutamol; IB = ipratropium bromide; T = theophylline; F = formoterol; bid = twice daily; qid = four times daily.

Table 3.1: Controlled studies of >2 weeks' duration in adults with chronic obstructive pulmonary disease (COPD) in which the clinical response to regular short-acting β_2-agonist was compared with placebo or other bronchodilators.

3.1.2 Comparison with other bronchodilators

3.1.2a Long-acting β_2-agonists

The only comparison of short- and long-acting β_2-agonists in patients with COPD is a three-week study in which all patients were taking ipratropium bromide 40 μg four times daily [344]. The addition of formoterol 12 μg twice daily caused a greater improvement in FEV_1 over six hours, pre-treatment morning FEV_1, symptom scores and the symptoms component of the St George's Respiratory Questionnaire compared with the addition of salbutamol 200 μg four times daily.

3.1.2b Muscarinic receptor antagonists

Short-acting muscarinic receptor antagonists

Only two trials of reasonable duration have compared regular inhaled β_2-agonists with regular inhaled ipratropium bromide in subjects with COPD. Both had the primary objective of comparing the combination of salbutamol and ipratropium bromide with each individual agent and found no appreciable difference between regular inhaled salbutamol and ipratropium bromide for spirometric measurements [342,345], dyspnoea scores [342,345] or walk distance [342]. A shorter study conducted over one week had similar findings, with no difference between salbutamol and ipratropium for spirometry, dyspnoea scores or walk distance [346].

Long-acting muscarinic receptor antagonists

Although clinical studies have compared tiotropium bromide with placebo, ipratropium bromide and long-acting β_2-agonists in patients with COPD, no published studies have compared tiotropium bromide and regular short-acting inhaled β_2-agonists.

3.1.2c Theophylline

After two weeks of treatment with inhaled salbutamol 200 μg four times daily or an appropriately titrated dose of twice-daily oral theophylline, there was no difference between treatments for FEV_1, FVC or PEFR, symptom scores or side effects [341]. In a comparison of once-daily sustained release theophylline and inhaled salbutamol 200 μg four times daily, the main outcome was the effect of the two drugs on sleep quality. Results favoured theophylline for nocturnal hypoxaemia, symptoms, early morning FEV_1 and FEV_1/FVC ratio, but there were no differences between treatments for sleep quality [347].

3.1.3 Effect of adding short acting β_2-agonists to other bronchodilators

Regular inhaled salbutamol has been compared with 'as required' use in subjects with COPD already taking regular inhaled ipratropium bromide and corticosteroids. There were no clinically important differences between the two groups and the authors concluded that regular inhalation doubled the amount of salbutamol use without providing any significant physiological benefit [348].

The combination of a short-acting inhaled β_2-agonist and inhaled ipratropium bromide has been compared with inhaled salbutamol alone in three studies. The combination improved FEV_1 in two studies [345,349], symptom scores in one study [349] and exertional dyspnoea but not walk distance in the third study [342]. All the studies concluded that there were no additional adverse effects with the combination compared to salbutamol alone. A retrospective analysis, concluded that the combination might have

a beneficial effect on exacerbation rates compared to inhaled salbutamol alone [350].

Regular inhaled salbutamol taken with oral theophylline has produced additional improvements in spirometry and symptom scores than that seen with either individual drug [339,341]. The combination of the two drugs also produced a small improvement in six-minute walk distance, but this did not translate to a beneficial effect on quality of life measures [339]. Neither study reported differences in the adverse effects produced by salbutamol alone and the combination of inhaled salbutamol and oral theophylline.

3.1.4 Effect of different doses

All of the studies have examined a single dose of inhaled β_2-agonist, so no published data has looked at the effect of different doses over a period of at least two weeks.

3.2 EFFICACY OF REGULAR LONG-ACTING β_2-AGONISTS IN PATIENTS WITH COPD

Few studies have looked at the longer-term effects of long-acting β_2-agonists in patients with COPD and the results have been inconsistent.

Studies comparing long-acting β_2-agonists with placebo, muscarinic receptor antagonists and oral theophyllines, and of more than four weeks' duration, are detailed in table 3.2. The studies have involved between 29 and 6184 subjects and have lasted up to three years.

Study	No of subjects (No on LAβA)	Duration LAβA (weeks)	LAβA bid dose (μg)	Mean FEV_1 % predicted	% on ICS	Control
Versus placebo						
Ulrik 1995	66 XO	4	S 50	45	0	Placebo
Grove et al 1996	29 XO	4	S 50	42	83	Placebo
Boyd et al 1997	674 (447)	16	S 50 & 100	46	60 (5)	Placebo
Jones & Bosh1997	283 (188)	16	S 50 & 100	46	71 (16)	Placebo
Weiner et al 2000	30 (24)	6	S 50	33	NR	Placebo
Chapman et al 2002	408 (201)	24	S 50	45	61	Placebo
Aalbers et al 2002	692 (430)	12	F 4.5, 9 & 18	54	NR	Placebo
Stockley et al 2006	634 (316)	52	S 50	46	54	Placebo
Mahler et al 2002	691 (160)	24	S 50	41	25	Placebo FP 500 μg bid FP 500 μg bid plus S 50 bid
Szafranski et al 2003	812 (201)	52	F 9	36	26	Placebo BUD 400 μg bid BUD 320 μg bid plus F 9 bid
Calverley et al 2003a	1465 (372)	52	S 50	45	51	Placebo FP 500 μg bid FP 500 μg bid plus S 50 bid
Calverley et al 2003b	1022 (255)	52	F 9	36	48	Placebo BUD 400 μg bid BUD 320 μg bid plus F 9 bid
Hanania et al 2003	723 (177)	24	S 50	42	20	Placebo FP 500 μg bid FP 500 μg bid plus S 50 bid
O'Donnell et al 2006	185 (59)	8	S 50	1.21 L[a]	0	Placebo FP 250 μg bid plus S 50 bid
Calverley et al 2007	6184 (1521)	156	S 50	44	9	Placebo FP 500 μg bid FP 500 μg bid plus S 50 bid

Study	No of subjects (No on LAβA)	Duration LAβA (weeks)	LAβA bid dose (μg)	Mean FEV_1 % predicted	% on ICS	Control
Versus ipratropium bromide or tiotropium bromide and placebo						
Mahler et al 1999	411 (135)	12	S 42	40	NR	Placebo IB 36 μg qid
Rutten-van Molken et al 1999	144 (47 S) (47 S+IB)	12	S 50	44	77	Placebo S 50 + IB 40 μg qid
Van Noord et al 2000	144 (47 S) (47 S+IB)	12	S 50	44	77	Placebo S 50 + IB 40 μg qid
Dahl et al 2001	780 (386)	12	F 12 & 24	45	52	Placebo IB 40 μg qid
Rennard et al 2001	405 (132)	12	S 42	1.27 L[a]	NR	Placebo IB 36 μg qid
Wadbo et al 2002	183 (61)	12	F 18	33	71	Placebo IB 80 μg tid
Donohue et al 2002	623 (213)	26	S 50	40	66	Placebo TB 18 μg od
Versus theophylline						
Di Lorenzo et al 1998	178 open label (91)	52	S 50	2.0 L[a]	17	T bid
ZuWallack et al 2001	943 open label (310)	12	S 42	41	37	S 42 + T bid T bid
Rossi et al 2002	854 (425)	52	F 12 & 24	47	47	Placebo T bid

FEV_1 = forced expiratory volume in one second; [a] Mean FEV_1 (% predicted not given); LAβA = long-acting β_2-agonist; NR = not reported; XO = crossover; PG = parallel group; S = salbutamol; F = formoterol; IB = ipratropium bromide; T = theophylline; BUD = budesonide; FP = fluticasone propionate; ICS = inhaled corticosteroid ; TB = tiotropium bromide; OD = once daily; bid = twice daily; tid = three times daily; qid = four times daily.

Table 3.2: Controlled studies of >4 weeks' duration in adults with chronic obstructive pulmonary disease (COPD) in which the clinical response to salmeterol or formoterol was compared with placebo or other bronchodilators.

All seventeen studies of salmeterol (presented in nineteen papers) have looked at the 50 μg twice daily dose and one of the studies (two papers [351,352]) also looked at the 100 μg twice daily dose. Of the six studies of formoterol, five have looked at 12 μg twice daily, four at 24 μg twice daily and one at 6 μg twice daily. The main findings in these studies can be summarised as follows.

3.2.1 Studies comparing long-acting β_2-agonists with placebo

3.2.1a Lung function

Salmeterol and formoterol when taken twice daily have been shown to cause bronchodilatation in patients with COPD when compared to placebo. There is usually an increase in pre-treatment morning peak flow [353-364], pre-treatment morning FEV_1 [351,354,355,358-361,363-368] and FEV_1 twelve hours after drug administration [356,360]. Four studies found salmeterol or formoterol to be more effective than placebo at increasing evening peak flow [354,358,359,362] whilst two found no difference [353,357]. The increase in morning PEFR has been marginally greater than the increase in evening PEFR.

In the study in which salmeterol 50 μg and 100 μg were included, the bronchodilator response to both doses was greater than the response to placebo but there was no difference between the two doses [351]. In the three studies comparing formoterol 12 μg and 24 μg there was also no difference in change in FEV_1 or PEFR between the two doses, although again both doses differed from placebo [356,361,365]. The response to the

formoterol 6 µg dose produced a similar response to the other two doses in the study by Aalbers et al [365].

4.2.1b Symptoms and quality of life

Twice daily treatment with salmeterol and formoterol has reduced rescue medication use [351,353-356,358-362,366,368,369] and most studies have shown a beneficial effect on daytime or nocturnal symptoms compared with placebo [351-354,356,358,365,366,368,369]. In the three studies [356,361,365] comparing formoterol 12 µg and 24 µg with placebo, symptom scores were lower with both doses of formoterol compared to placebo, although this was not significant in one study [361] and was only significant for the 24µg dose in another [365]. The reduction in symptom scores for the 12 µg and 24 µg doses were almost identical in two of the studies, whereas in the third, which included the 6 µg dose, there was a dose-related reduction in symptom-free days [365].

Salmeterol and formoterol have generally shown a trend towards a beneficial effect on quality of life compared with placebo, irrespective of the tool used to measure quality of life. The beneficial effects have not always reached the level considered to be clinically important, however, and have not always been statistically significant. Of the twelve studies comparing quality of life after administration of salmeterol 50µg twice daily or placebo, three showed a significant improvement with salmeterol [352,368,369], seven showed an improvement that was not statistically significant [310,355,357,359,360,363] and two studies showed no difference [364,370]. Five studies have looked at the effect of formoterol on quality of life. In the two that included the 12 µg and 24 µg twice-daily doses, both showed an

improved quality of life compared to placebo, although the greater benefit was seen with the lower 12 µg dose in one study [356], and with the higher dose in the other [361]. Quality of life did not differ between the 24 µg twice daily dose and placebo in a third smaller study [358], while in the final two studies [362,366] the 12 µg twice-daily dose was superior to placebo, although the significance was only stated for one [362].

3.2.1c Effect on exacerbations

The effect of salmeterol on exacerbations of COPD has been assessed in some of the larger studies. Salmeterol 50 µg twice daily increased the time to first exacerbation in one study [369] and reduced the exacerbation rate in another [363] (1.3 versus 1.04 exacerbations per patient per year for placebo and salmeterol, respectively). The largest study found that salmeterol 50 µg twice daily reduced moderate and severe exacerbation rates and the number of exacerbations requiring systemic corticosteroids [310]. It did not cause a significant reduction in exacerbation rates in the other five studies, however, and although the trend was in favour of salmeterol in all the studies, the differences were small [351,354,355,357,359,368].

The effect of formoterol on exacerbations has also been rather variable. Formoterol 12 µg and 24 µg reduced mild exacerbations and the 24 µg dose reduced more severe exacerbations in one study [361]. However, neither the frequency of exacerbations nor use of additional therapy for exacerbations was reduced by twice daily treatment with either dose in a second study [356] or by the 12 µg dose in a further two studies [362,366].

3.2.1d Exercise tolerance and respiratory muscle strength

When compared with placebo, twice-daily salmeterol 50 μg reduced Borg scores for perceived exertion in a four-week study [371] and breathlessness scores in a sixteen week study [351], although the six-minute walk distance was unchanged in both studies.

When respiratory muscle strength and endurance were studied there was no change after six weeks of treatment with salmeterol 50 μg twice daily compared with placebo [372]. There was no change in exercise time after eight weeks treatment between placebo and salmeterol 50 μg twice daily, although the long-acting β_2-agonist produced a beneficial effect on some physiologic responses to exercise [367].

3.2.2 Comparison with other bronchodilators

3.2.2a Muscarinic receptor antagonists

Three studies have compared ipratropium bromide 40 μg four times daily with recommended doses of formoterol or salmeterol [355,356,369] and, although differences were not always statistically significant, the benefit has generally been in favour of the long-acting β_2-agonist. In a twelve-week study in which formoterol 12 μg and 24 μg twice daily were compared with ipratropium bromide 40 μg administered every six hours, both doses of formoterol were more effective at increasing pre-treatment morning PEFR, increasing the FEV_1 over 12 hours and reducing symptoms, rescue medication use and improving quality of life [356]. Two studies comparing salmeterol 50 μg twice daily and ipratropium 40 μg four times daily did not show any difference in morning FEV_1 or FEV_1 over twelve hours at the end

of the studies [355,369], but salmeterol was associated with a reduction in nocturnal breathlessness and increased the time to first exacerbation in one study [369].

When formoterol 12 μg twice daily was compared with a higher dose of ipratropium bromide (80 μg three times daily) there were no differences in spirometric outcomes, symptoms or overall quality of life [358].

A large study has compared salmeterol with once daily tiotropium bromide in 623 patients. Tiotropium bromide 18 μg daily was more effective than salmeterol 50 μg twice daily at increasing pre-treatment morning FEV_1, mean FEV_1 over twelve hours (measured as area under the curve) and evening PEFR. This was in part due to a greater initial response to tiotropium bromide and in part because the bronchodilator response to salmeterol fell over the course of the study, whereas the response to tiotropium bromide was maintained [359,373]. Tiotropium bromide also caused a greater improvement in the St George's Respiratory Questionnaire total score and in the number of patients achieving a four-point change in score, although only the latter differed significantly from the finding with salmeterol [359].

3.2.2b Theophylline

Three studies have compared a long-acting β_2-agonist with individually titrated oral theophylline, one with formoterol 12 μg and 24 μg twice daily [361] and two with salmeterol 50 μg twice daily [374,375]. For most endpoints the long acting β_2-agonists were superior to theophylline, although not all differences were statistically significant. Significant

differences in favour of the long acting β_2-agonist in at least one of the studies included morning PEFR and the twelve-hour change in FEV_1 [361], pre-treatment morning FEV_1, symptoms and rescue medication use [374], number of 'bad days' experienced [361] and satisfaction with treatment [375]. No consistent differences in quality of life measures were seen after long-term treatment with salmeterol or formoterol compared with theophylline despite the increased adverse events seen with theophylline [370,374,375]. Whether this is due to increased withdrawals with theophylline is unclear.

3.2.3 Effect of adding long-acting β_2-agonists to other bronchodilators

Combining salmeterol 50 μg twice daily with ipratropium 40 μg four times daily improved lung function (FEV_1, airway conductance and evening PEFR) more than salmeterol alone, although the combination was no more effective than salmeterol alone at improving symptoms, rescue medication use, morning PEFR or exacerbation rate [354]. The combination of ipratropium bromide and salmeterol caused a greater improvement in certain quality of life measures, namely the total score, emotional domain and proportion of patients improving by the four points considered to be clinically useful on the Chronic Respiratory Questionnaire, and the symptoms component but not total score of the St George's Respiratory Questionnaire [370].

When combined with orally titrated doses of theophylline, salmeterol caused greater bronchodilatation than was seen with either drug alone, with improved dyspnoea scores and salbutamol-free days. Other outcomes

favoured the combinations, although there was no difference in the rate of exacerbation between salmeterol and combined treatment [375].

3.2.4 Effect of different doses

No consistent bronchodilator dose response effects have been seen with formoterol doses ranging from 6 to 24 μg twice daily [356,361,365], although the lower dose was more effective among patients with more reversible airflow obstruction in the study by Rossi et al [361]. The effect on symptom scores has also been broadly similar when formoterol 12 μg and 24 μg doses have been compared. There was, however, a dose-response effect for reduction in symptom-free days for the three doses of formoterol [365] but no differences between doses for the six-minute walk distance, rescue medication use or exacerbation rates [356,361,365]. Formoterol 12 μg and 24 μg twice daily produced similar changes in quality of life in one study [361], while the lower dose caused greater improvements in the total score and all three domains of the St George's Respiratory Questionnaire in another [356].

For salmeterol, no difference was seen between the 50 μg and 100 μg twice daily doses in terms of effect on FEV_1, symptom scores, rescue medication use or exacerbation rates [351]. For several end points the lower 50 μg dose had the greater effect, including reduction in breathlessness score at the end of a six-minute walk, total score and impacts component of the St George's Respiratory Questionnaire. In the short form health survey (SF-36), the lower dose of salmeterol improved health score in four of the eight components while the higher dose caused deterioration in these components [351,352].

Thus, neither drug has shown a bronchodilator dose-response effect in patients with COPD. The finding that lower doses caused a greater improvement in quality of life than higher doses in some studies is interesting and presumably relates to the balance between the relatively small bronchodilator effect in these patients and increased adverse effects with the higher doses. A similar phenomenon was observed in a study of increasing doses of salbutamol ranging from 400 μg to 4 mg in patients with COPD [253]. In this study there was a dose related increase in PEFR and FEV_1, but the incremental effects were small and higher doses were associated with a progressive increase in heart rate and tremor and a fall in oxygen saturation. Patients preferred the middle dose (1mg) where presumably the benefits from bronchodilatation were not outweighed by excessive adverse effects. The fact that the quality of life measures favoured the lower dose of the long-acting β_2-agonist suggests that this dose achieves the optimum balance between beneficial and adverse effects.

3.3 SAFETY OF REGULAR LONG-ACTING β_2-AGONISTS IN COPD

The safety of long-acting β_2-agonists is an important consideration. Concern about the safety of regular short-acting β_2-agonists in the management of asthma has recently extended to long-acting β_2-agonists. Mann et al reviewed three studies (one in children) and found an increased number of severe asthma exacerbations among patients receiving formoterol 24 μg twice daily compared with formoterol 12 μg twice daily [376]. A randomised 28-week study was set up at the request of the FDA to determine the safety of salmeterol in a post-marketing context. The study

was halted prematurely after an interim analysis showed that salmeterol was associated with an increased risk of life-threatening asthma episodes or asthma-related deaths [297].

The adverse effect profile of β_2-agonists is well recognised, particularly at higher doses and although the systemic response to β_2-agonists may show tolerance with regular use, both salmeterol and formoterol have the potential to cause systemic adverse effects when used in the management of COPD.

To assess the safety of long-acting β_2-agonists in the management of COPD i have reviewed the adverse effects reported in long-term prospective studies of effectiveness. I have also discussed a small prospective study designed specifically to look at cardiovascular safety of long-acting β_2-agonists in COPD.

Most of the prospective studies that have assessed the effects of salmeterol and formoterol in patients with COPD were designed primarily to look at efficacy but have also reported the adverse effects relating to β_2-agonists. It is important to consider that most long-term studies conducted in patients with COPD excluded those individuals with a history of cardiac problems. The predictable adverse effects were reported in most studies and although there was not usually a difference between the treatment groups and placebo, the incidence of adverse effects was greater in some studies when the higher doses of salmeterol and formoterol were used [351,365].

None of the studies identified a difference between placebo and either salmeterol or formoterol in terms of effect on cardiovascular endpoints, with no increase in the rate of dysrhythmias. Occasional patients in the studies have developed a dysrhythmia with long-acting β_2-agonists, but it is not clear whether these were due to the drug itself or simply occurred by

chance. Two individuals developed atrial fibrillation with formoterol 12 μg twice daily [356,365] and one study found an increase in the rate of non-sustained ventricular tachycardia with salmeterol 50 μg twice daily [355].

A published meta-analysis of seven studies of salmeterol 50 μg twice daily covering 1443 patients with COPD found no difference in cardiovascular events or deaths compared with placebo, even when patients were stratified by age >65 years or known cardiovascular disease [304]. In this study 40% of patients had a cardiac problem and a serious cardiovascular adverse event occurred in 38 patients (2.6%) taking salmeterol and in 27 patients (1.9%) taking placebo.

A small single-dose study looked specifically at the effect of a long-acting β_2-agonist in patients with COPD who had experienced a previous cardiac dysthythmia and hypoxaemia. Formoterol 12 μg and 24 μg and salmeterol 50 μg were compared in 12 patients with continuous electrocardiography. All three doses caused an increase in heart rate and a fall in serum potassium levels. The effects were most marked with the higher dose of formoterol and four of these patients developed paired or multiform ventricular beats [377].

The TORCH (Towards a Revolution in COPD Health) study was established to assess the effect of inhaled corticosteroids and inhaled long acting β_2-agonists on mortality in the management of COPD [310]. The combination of inhaled fluticasone propionate and salmeterol was compared with inhaled salmeterol alone, inhaled fluticasone alone and placebo, with each subject randomised to a three-year study. Over 1500 subjects with COPD (mean FEV_1 44% predicted) were randomised to receive salmeterol 50 μg twice daily and 960 of these subjects completed the study.

This study covered a greater period of time than previous published studies of long-acting β_2-agonists in subjects with COPD and randomised the largest number of patients, so important safety data relating to the long-term use of salmeterol was obtained. The study found no difference between placebo and salmeterol for all-cause mortality rates or COPD-related deaths after three years. There was a large dropout rate in the study, but fewer subjects discontined salmeterol than placebo over the three years. Drug-related adverse events were similar in the placebo and salmeterol groups and there was no evidence of an increase in cardiac events in the salmeterol limb.

Chapter Four

Determining the optimum dose of nebulised salbutamol in acute exacerbations of Chronic Obstructive Pulmonary Disease

4.1 AIMS

This study was designed to examine the effect of nebulised salbutamol on arterial blood gas tensions during an acute exacerbation of COPD. We wished to test the hypothesis that increased carbon dioxide production and flux of carbon dioxide to the lungs following high doses of β_2-agonists could cause an increase in arterial carbon dioxide tension and thus worsen respiratory failure in some subjects with COPD.

Unfortunately the study had to be abandoned since we were only able to recruit five patients in eighteen months. Because this is an important clinical question and we have detailed information on the five patients, I am presenting the data in this chapter without a formal analysis.

4.2 INTRODUCTION

Acute exacerbations of chronic obstructive pulmonary disease (COPD) are a major cause of hospital admissions and patients with hypercapnia have particularly high mortality rates [378]. The response to treatment during acute admissions has been little studied and guidelines are unable to provide clear dose recommendations for bronchodilator treatment. Patients with severe COPD have a limited ability to bronchodilate, particularly during an acute exacerbation. In this situation high doses of β_2-agonists are often given although the bronchodilatation they produce is often minimal. The adverse effects following the high doses could then outweigh the limited beneficial effects as was seen in the study by Vathenen et al [253], where subjects with severe airflow obstruction and limited reversibility were given a range of doses of salbutamol and preferred a modest dose.

One potential problem with high dose β_2-agonists in patients with severe COPD could arise as a result of an increase in carbon dioxide output (VCO_2). Carbon dioxide output rose by 20% after healthy subjects inhaled salbutamol, with a direct relationship between dose and VCO_2 [277]. The increase has been attributed to a combination of factors, including an increase in CO_2 production as tremor increases and an increase in flux of CO_2 to the lungs as cardiac output increases [281,282,379]. The normal response to the increase in CO_2 flux to the lungs would be to increase ventilation (cardiodynamic hyperpnoea), but during an acute exacerbation patients with severe COPD and limited reversibility may not have the capacity to do this, in which case the P_aCO_2 would rise. In addition, inhaled β_2-agonists have been shown to cause transient hypoxaemia in patients with asthma and COPD, an effect that has been attributed to an increase in ventilation/perfusion mismatching.

We wished to examine the effect of a range of doses of nebulised salbutamol in patients during an acute exacerbation of COPD. We gave ipratropium bromide to the control subjects to try to avoid the need for additional bronchodilators during the study period and to avoid the bronchoconstriction that can occur with nebulised normal saline [380].

We planned a double blind study in which patients admitted to Nottingham City Hospital with an acute exacerbation of COPD received five doses of nebulised salbutamol or ipratropium, with each dose given an hour apart. The main endpoint was the change in partial pressure of carbon dioxide (P_aCO_2) from baseline.

4.3 SUBJECTS AND METHODS

4.3.1 Subjects

Patients aged 40 to 85 admitted to Nottingham City Hospital with an acute exacerbation of COPD were eligible to participate in the study. Subjects were required to have a smoking history of more than 20 pack years, no history of asthma or atopy, a previous diagnosis of COPD and a documented forced expiratory volume in one second (FEV_1) of less than 70% predicted. Exclusion criteria included the presence of another significant acute medical problem, radiographic evidence of pneumonia, anticoagulation, hypotension (<100/60 mmHg), hypokalaemia (<3.5 mmol/l) or a history of glaucoma. Nottingham City Hospital Ethics Committee approved the study and written informed consent was obtained on the day prior to the study day.

4.3.2 Measurements

P_aCO_2, P_aO_2 and pH were measured using standard electrodes on a blood gas analyser (Rapidlab 840, Bayer, Leverkusen, Germany), which calibrates automatically. One-point calibration with a reagent of known concentration occurs every 30 minutes and two-point calibration occurs every 120 minutes by measuring the response to two reagents of known concentration.

The electrocardiograph was recorded on a central monitor (Dynascope DS-5100E, Fukuda Denshi, Japan) and heart rate was calculated as mean of six consecutive beats.

4.3.3 Protocol

This was a double blind, single centre randomised study performed on patients within 48 hours of admission to Nottingham City Hospital with an acute exacerbation of COPD. Subjects were required to withhold all short-acting bronchodilators from 2200 on the night before the study and all long-

acting bronchodilators for at least 24 hours. Patients abstained from caffeine containing foods and beverages from midnight on the day before the study and following a light breakfast at 0700, abstained from eating throughout the study period.

Prior to randomization and after hand circulation has been assessed by 'Alan's test', an arterial cannula (FloSwitch, Becton Dickinson, Swindon, UK) was inserted into the radial artery to allow blood sampling. Three chest leads were attached to the subject, who was then placed on bed rest for 30 minutes. The subject received continuous oxygen, delivered at 28% via Venturi mask.

Subjects were randomised to receive one of two treatment options at a ratio of 3:1 with the treatment determined by computer-generated random sequence; the majority received five doses of salbutamol sulphate (Steri-Neb, Baker Norton, UK), delivered via a nebuliser, at hourly intervals from 0800 to 1200. A quarter of the patients acted as the control group and received five doses of nebulised ipratropium bromide (Steri-Neb, Baker Norton, UK) at the same time points. Resting heart rate and arterial blood gas tensions were recorded at ten-minute intervals for 30 minutes after insertion of the arterial cannula. The first dose of medication was given around 0800 when the heart rate, P_aCO_2 and P_aO_2 had reached a stable state. This was defined as two consecutive resting heart rates within ten beats per minute and consecutive mean P_aCO_2 and P_aO_2 readings within 0.5 kPa, with the baseline calculated as the mean of these two values. Two one ml blood samples were taken sequentially from the arterial cannula to measure P_aCO_2 and P_aO_2 (mean value recorded).

The subject was given either salbutamol sulphate, two doses of 1.25mg in 2.5ml, one dose of 2.5mg in 2.5ml and two doses of 5mg in 2.5ml or five doses of ipratropium bromide 125µg diluted to 2.5ml with normal saline. Each dose was delivered one hour apart, over six minutes via a nebuliser (IEC 601-1, Medic-Aid, Pagham, UK) driven by air at 5 litres per min. Duplicate one ml samples of arterial blood were taken 10, 15, 20, 30, 45 and 60 minutes after starting drug delivery to determine P_aCO_2 and P_aO_2 and followed at each time point by heart rate measurement. Sixty minutes after starting the first nebulisation the second dose was delivered and measurements repeated over the next hour. Five doses were given in total at hourly intervals and once the final blood sample was taken, the arterial cannula was removed.

4.3.4 Analysis

With twenty patients having salbutamol and assuming a SD of 0.3 kPa for change in P_aCO_2 and P_aO_2, we had >90% power to find a 1 kPa change in P_aCO_2 or P_aO_2 from baseline. The primary endpoint was change in P_aCO_2 from baseline and secondary endpoints were change in P_aO_2 and heart rate.

4.4 RESULTS

In the eighteen months between June 2002 and December 2003, we approached 121 patients, but only ten subjects were suitable and willing to give consent, of which five completed the study. Among the five subjects who were not randomised, two withdrew consent next morning, two were unable to manage overnight without bronchodilators and an arterial cannula could not be inserted in one person. In view of the small numbers we have not carried out any statistical analysis on the data.

Demographic data for the five subjects (two women) who completed the study is shown in table 5.1. The subjects had severe disease (mean FEV_1 28% predicted), were ex-smokers (mean 29 pack years) and used inhaled β_2-agonists on a regular basis. Three had stable ischaemic heart disease. Subject three received ipratropium bromide whilst the other four received salbutamol.

4.4.1 Changes with salbutamol

4.4.1a Partial pressure of arterial carbon dioxide

Individual changes in P_aCO_2 with increasing doses of salbutamol are shown in figure 5.1. There were small fluctuations in P_aCO_2 over the five-hour study period. No subject showed a clear dose related increase in P_aCO_2, nor was any single dose associated with a change in the P_aCO_2 of more than 1 kPa. Subject five, who had the highest baseline P_aCO_2 of 7.1 kPa, showed a short-lived increase in P_aCO_2 after the highest doses of salbutamol (5 mg) at 180 and 240 minutes. Subject one had short-lived increases in P_aCO_2 after four of the five doses of salbutamol.

4.4.1b Partial pressure of arterial oxygen

Individual changes in P_aO_2 with increasing doses of salbutamol are shown in figure 5.2. The subjects experienced a fall in P_aO_2 after most doses of salbutamol, but the magnitude of change does not appear to be greater when higher doses were delivered. Although three subjects (one, four and five) had a fall in P_aO_2 of over 1 kPa after some doses, the decrease was not sustained.

4.4.1c Heart rate

Individual changes in heart rate with increasing doses of salbutamol are shown in figure 5.3. All four subjects had a rise in heart rate over the five-

hour study period (area under the time response curve). Changes were modest however, although subject two had a short period of sinus tachycardia sixty minutes after the first dose of salbutamol.

4.4.2 Changes with ipratropium bromide.

Subject three received ipratropium and there were only modest, inconsistent effects on P_aCO_2 and heart rate. The P_aO_2 fell transiently after each dose of ipratropium bromide.

	Sex	Age	FEV_1 (L)	FEV_1/ FVC (%)	Baseline $PaCO_2$	Baseline PaO_2	Pack yrs	Regular β_2-agonists
1	M	70	0.7	56	5.6	11.72	24	Salbutamol
2	M	78	0.65	30	5.37	9.34	40	Salbutamol Salmeterol
3	M	75	0.8	40	5.26	10.17	35	Salmeterol
4	F	67	0.7	47	4.89	9.95	21	Salbutamol Salmeterol
5	F	76	0.6	48	7.1	9.99	25	Salbutamol Salmeterol

Table 4.1: Demographic data for the five subjects

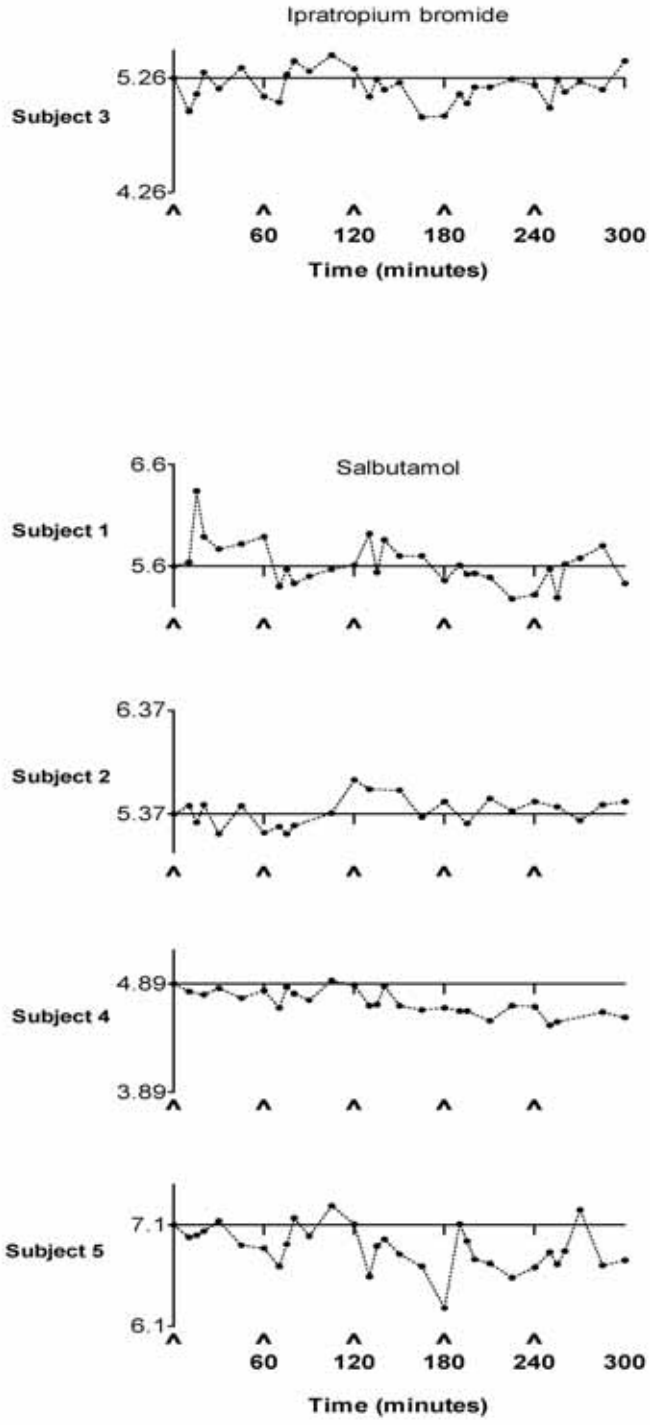

Figure 4.1: Individual changes in P_aCO_2 (kPa) after five doses (^) of bronchodilator.

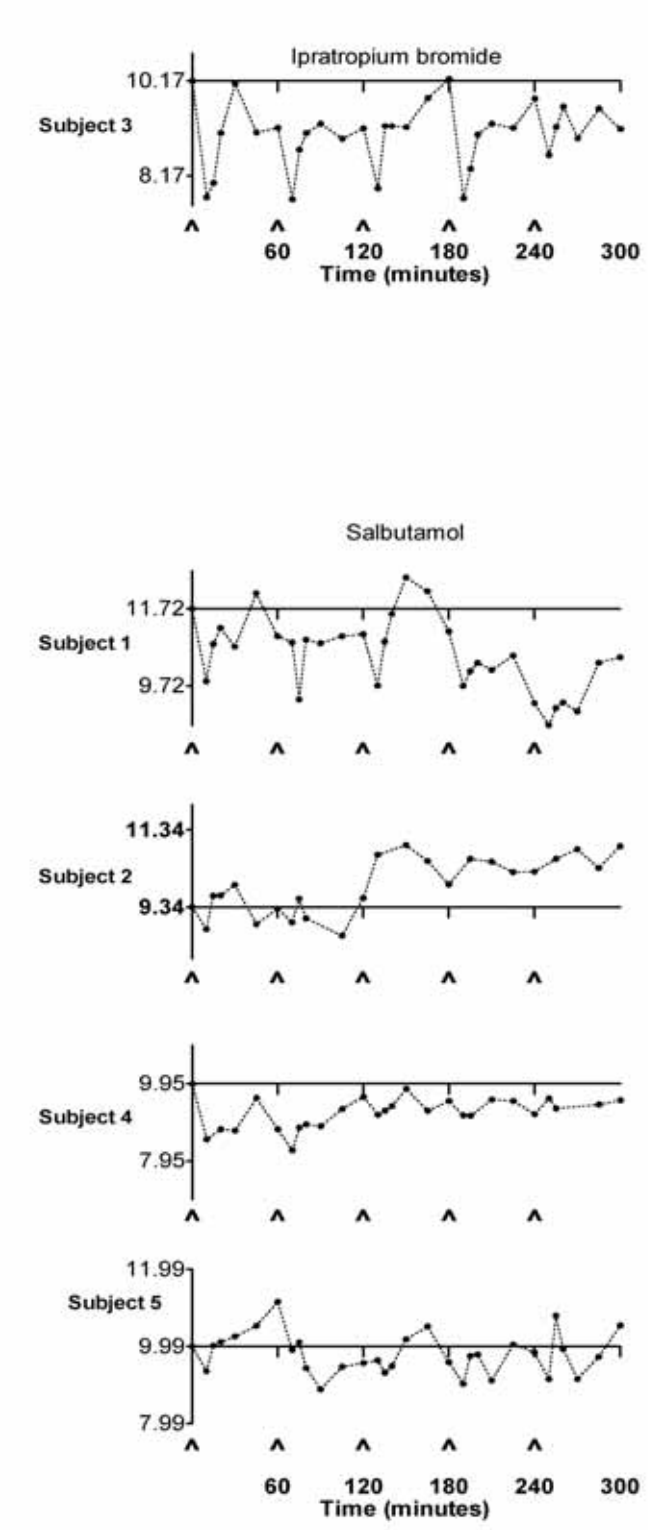

Figure 4.2: Individual changes in P_aO_2 (kPa) after five doses (^) of bronchodilator.

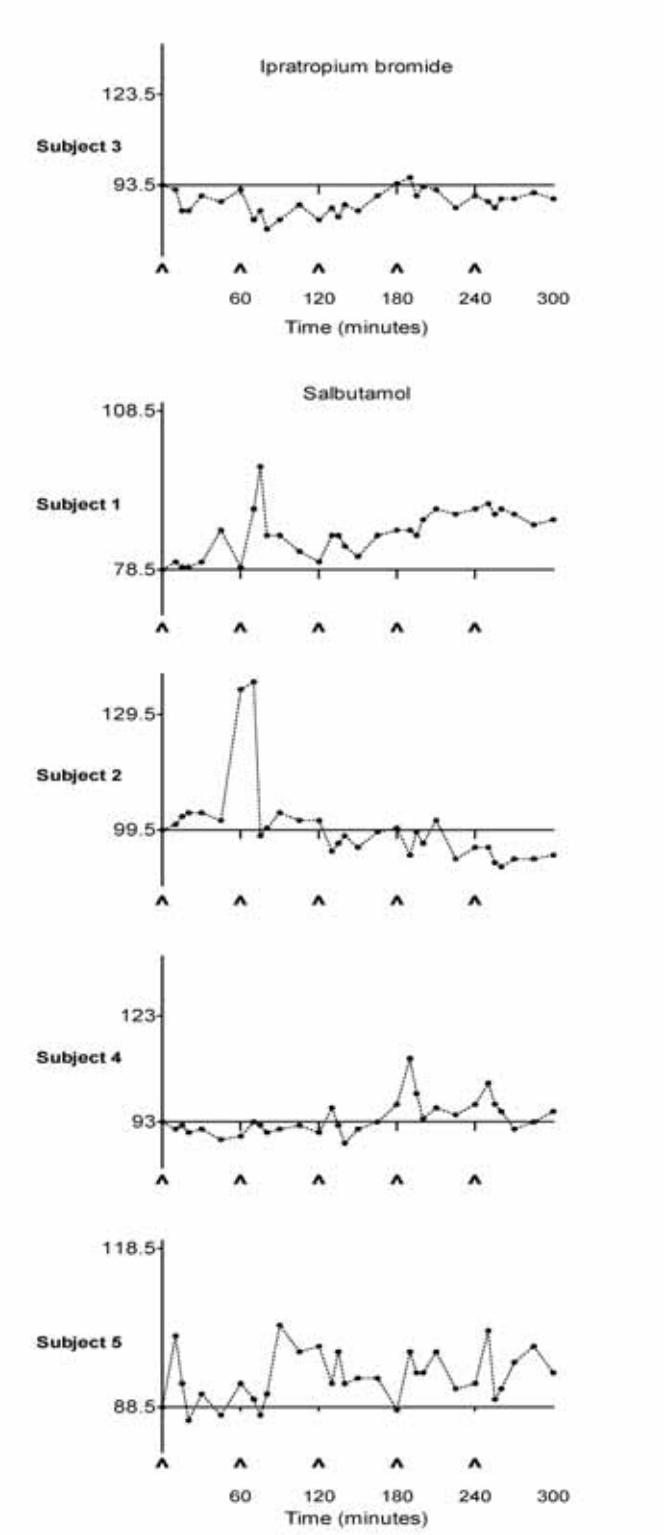

Figure 4.3: Individual changes in heart rate (bpm) after five doses (^) of bronchodilator.

4.5 DISCUSSION

This study was designed to address an important clinical question, namely can high doses of nebulised salbutamol have a detrimental effect on arterial carbon dioxide tension and worsen respiratory failure in subjects admitted with an acute exacerbation of COPD?

The study was carefully planned and the admission rate of patients with COPD to Nottingham City Hospital (15 per month, Royal College of Physicians Audit, 2001) appeared to offer a ready supply of potential candidates. I made daily visits to the medical admissions ward and respiratory base wards to identify appropriate subjects, I enrolled the hospital COPD nurses to highlight or identify admissions from known patients and asked the junior medical staff to liaise with members of the research team when potential study subjects were admitted.

We were however only able to recruit five patients over eighteen months. The main barriers to recruitment were that patients;

a) Were too well.

Some patients seen on the admissions unit with a mild exacerbation were deemed to be too well for hospital admission and had an early supported discharge.

b) Were too unwell.

A proportion of patients were too unwell to participate in the study. Some individuals required non-invasive ventilation; others were unable to manage for long periods without bronchodilators and some felt too ill to consider taking part in a research study.

c) Had other co-morbidities.

The presence of other medical conditions or the potential that other events had precipitated the admission led to many patients being excluded. Evidence of pneumonia on the chest radiograph, the need for the admitting team to exclude a myocardial infarction and co-existing left ventricular failure were common reasons for exclusion.

d) Had issues with consent.

Consent provided another source of difficulty, with some subjects having a degree of confusion and being unable to give informed consent. Others were unwilling to give consent because of concerns about the study duration, the need to abstain from bronchodilators overnight and/or the need for arterial cannulation.

Although our data are insufficient for statistical analysis, some conclusions can be drawn from the results we obtained. Changes in $PaCO_2$ were small and there was no evidence of a dose response relationship between salbutamol and P_aCO_2. The subject with the highest baseline P_aCO_2 had a transient increase in P_aCO_2 after the 5mg doses of salbutamol. Since the increase in $PaCO_2$ was small and only seen in one person, the significance is uncertain. The change is in keeping with our hypothesis and would support further investigation in patients with chronic type 2 respiratory failure.

An initial fall in PaO_2 after salbutamol has been demonstrated before and attributed to an increase in ventilation/perfusion mismatching. The magnitude of the changes in PaO_2 we saw is similar to that seen previously in subjects with COPD [280].

High dose salbutamol caused a modest increase in heart rate among the four subjects. The absence of a larger increase in heart rate may represent

tolerance to the systemic effects of the drug, since all participants used β_2-agonists on a regular basis.

The hypothesis that salbutamol can cause an increase in $PaCO_2$ when delivered to patients with COPD during an acute exacerbation is based on the presumption that high dose β_2-agonists will produce a rise in carbon dioxide production and flux of CO_2 to the lungs. The absence of a consistent rise in $PaCO_2$ after salbutamol suggests that the increase in VCO_2 was not as large as expected or that patients are able to increase ventilation in response to the rise in VCO_2 during an acute exacerbation.

The increase in VCO_2 after salbutamol may be less marked in patients who inhale β_2-agonists on a regular basis, due to the development of tolerance to the systemic effects of the drug. This is supported by the modest changes in heart rate. Patients admitted acutely with a severe exacerbation of COPD who present with hypoxaemia and hypercapnia may be at particular risk of developing worsening respiratory failure with salbutamol if they are β_2-agonist-naïve.

Subject 5 had the highest baseline partial pressure of arterial carbon dioxide and the increase in $PaCO_2$ that they experienced after the highest doses of salbutamol offers some support to our hypothesis.

4.6 CONCLUSION

Semi-invasive studies in an acute exacerbation of COPD are very difficult to conduct. We were only able to recruit five subjects over an eighteen-month period, of which four were randomized to receive a series of doses of nebulised salbutamol. There was no evidence of a dose response effect of salbutamol on P_aCO_2 in these subjects.

Chapter Five

Effects of salbutamol on arterial blood gases in patients with stable hypercapnic Chronic Obstructive Pulmonary Disease

5.1 AIMS

In view of the problems we encountered recruiting subjects during an acute exacerbation, this study was designed to examine the effect of nebulised salbutamol on arterial blood gas tensions in patients with stable COPD and chronic or intermittent hypercapnia. As in chapter five, we tested the hypothesis that high dose salbutamol could cause a rise in the partial pressure of arterial carbon dioxide and worsen respiratory failure among some subjects with severe COPD.

5.2 INTRODUCTION

Patients with COPD and long-standing hypercapnia have chronic alveolar hypoventilation. If high dose salbutamol causes an increase in carbon dioxide production, these subjects may not be able to increase ventilation in response to the flux of carbon dioxide to the lungs and the $PaCO_2$ may rise further (see section 4.2).

We wished to examine the effect of high dose nebulised salbutamol in patients with severe stable COPD and chronic hypercapnia. We chose to give a low dose of salbutamol as the control rather than normal saline to reduce the possibility that patients would require treatment to relieve breathlessness during the study and to try to avoid bronchoconstriction from inhalation of normal saline [380].

We report the changes in arterial blood gas tensions from a crossover study in which fourteen patients with stable COPD, documented hypercapnia and limited reversibility received 10mg or 400µg nebulised salbutamol on separate days, both given in two doses an hour apart.

5.3 SUBJECTS AND METHODS

5.3.1 Subjects

Patients aged 40 to 85 with a clinical diagnosis of COPD were recruited if they had a forced expiratory volume in one second (FEV_1) below 50% predicted and a FEV_1/forced vital capacity (FVC) ratio of less than 70%. Subjects had to have an increase in FEV_1 of not more than 200ml and 15% ten minutes after inhaling 200μg salbutamol. Subjects were required to have had an arterial carbon dioxide tension (P_aCO_2) of greater than 6 kPa on two occasions when clinically stable or have developed hypercapnia (P_aCO_2 > 6kPa) during assessment for long term oxygen.

Exclusion criteria were other causes of respiratory failure, an additional unstable medical condition, an acute exacerbation of COPD requiring oral corticosteroids within the last four weeks and regular medication with a β_2-adrenoceptor antagonist or anticoagulant. Nottingham City Hospital Research Ethics Committee approved the study and written informed consent was obtained at least 24 hours before the first study day.

5.3.2 Measurements

P_aCO_2, P_aO_2 and pH were measured using standard electrodes on a blood gas analyser (Rapidlab 840, Bayer, Leverkusen, Germany), which was calibrated daily (See section 4.3.2).

The electrocardiograph was recorded on a central monitor (Dynascope DS-5100E, Fukuda Denshi, Japan) and heart rate was calculated as mean of six consecutive beats.

5.3.3 Protocol

This was a randomised, double blind, crossover study performed on two non-consecutive days, not more than five days apart. Subjects were required

to withhold short-acting bronchodilators for at least ten hours, and theophylline, tiotropium and long acting β_2-agonists for at least 24 hours before each study day; inhaled and oral corticosteroids and non-pulmonary medications were taken as usual. Subjects using home oxygen discontinued this 30 minutes before leaving home. After an early breakfast without caffeine-containing beverages, subjects came to the hospital by taxi, were escorted to the laboratory by wheelchair and rested in a reclining chair for ten minutes. Three chest leads and the fingertip pulse oximeter were attached and Alan's test performed on the radial and ulnar arteries to confirm the adequacy of local circulation. A cannula (FloSwitch, Becton Dickinson, Swindon, UK) was inserted into the radial artery under local anaesthetic and attached to the transducer. Two one ml blood samples were taken sequentially from the arterial cannula to measure pH, P_aO_2 and P_aCO_2 (mean value recorded) and these were repeated at ten-minute intervals until consecutive mean P_aO_2 and P_aCO_2 readings were within 0.5 kPa. Consecutive heart rate readings also had to be within 5 beats per minute, without an increase in rate. Baseline values for all variables were taken as the mean of the last two results.

On each day the patient was given salbutamol sulphate (Steri-Neb, Baker Norton, UK), either two doses of 5mg in 2.5ml an hour apart or two doses of 200μg diluted to 2.5ml with normal saline an hour apart, with the order determined by computer-generated random sequence. Each dose was delivered over six minutes via a nebuliser (IEC 601-1, Medic-Aid, Pagham, UK) driven by air at 5 litres per min. Duplicate one ml samples of arterial blood were taken 10, 15, 20, 30, 45 and 60 minutes after starting the first dose of drug to determine P_aCO_2 and P_aO_2 and followed at each time point

by heart rate, blood pressure and oxygen saturation measurements in that order. Sixty minutes after starting the first nebulisation the second identical dose of salbutamol was delivered and the measurements repeated over the next hour. Subjects received the alternative dose of salbutamol on the second study day, using an identical protocol, except that the cannula was placed in the contralateral radial artery.

The primary endpoint was the difference in change in P_aCO_2 between the two study days. 14 patients provided > 90% power to find a mean difference of 0.5 $kPa.h^{-1}$, assuming a standard deviation of 0.2 $kPa.h^{-1}$. Secondary outcome measures were differences in change in P_aO_2 and heart rate between the two study days.

5.3.4 Analysis

Change in mean P_aCO_2, P_aO_2 and heart rate for each dose of salbutamol was plotted against time and area under the time-response curves (AUC for 0-120 minutes) calculated by trapezoid integration. T-tests were conducted to exclude carry-over and period effects between baseline readings [381] and paired t-tests to determine differences in AUC measurements between doses. Within subject change in P_aCO_2 (AUC) following high dose salbutamol was related to change in P_aO_2 (AUC) and heart rate (AUC) and to baseline measures of P_aCO_2, P_aO_2 and heart rate using Pearson's correlation. Analyses were carried out using the statistical software program, SPSS version 11 (SPSS inc, Chicago, USA), with statistical significance accepted at $p<0.05$. I conducted all the statistical analysis after advice from Dr Sarah Lewis

5.4 RESULTS

Of the fifteen subjects who agreed to participate, one was withdrawn after the first study day following a fall at home. Mean demographic data for the fourteen patients (three women) who completed the study is found in table 5.1. Twelve patients had had a P_aCO_2 above 6 kPa on two previous occasions and two had developed hypercapnia in response to oxygen (2 L/min) during assessment for long-term domiciliary treatment.

Baseline measurements

Baseline P_aO_2 ranged from 6.0 to 10.1 kPa (mean 7.9 kPa) and showed a negative correlation with baseline P_aCO_2 ($r = -0.6$, $p = 0.02$) and a positive correlation with baseline FEV_1 ($r = 0.6$, $p = 0.02$). The baseline P_aCO_2 ranged from 5.31 to 8.3 kPa (mean 7.0 kPa), the baseline FEV_1 ranged from 0.26 to 1.1 L (mean 0.71 L (27 % predicted)) and cigarette consumption was high (mean 53 pack years).

Comparison of the two study days

There was no carry-over or period effect for the two study treatments.

5.4.1 Mean changes with salbutamol (figure 5.1, table 5.2)

Mean P_aCO_2 for the fourteen patients fell gradually over the study period following both high and low doses of salbutamol. There was no difference between the study days for AUC P_aCO_2 (0.03 $kPa.h^{-1}$; 95% CI 0.02,0.04, $p = 0.57$). The mean fall in P_aCO_2 after 120 minutes was 0.32 and 0.29 kPa for the high and low doses of salbutamol respectively.

There was a progressive fall in mean P_aO_2 on both study days, with a greater fall after high dose salbutamol. The difference between study days for AUC P_aO_2 was significant (0.1 kPa.h^{-1}; 95% CI 0, 0.2, p=0.04). The reduction at 120 minutes was 0.57 and 0.24 kPa for the high and low doses respectively.

There was a greater rise in mean heart rate with the high dose compared to the low dose (difference in AUC HR 1.1 bpm.h^{-1}; 95% CI 0.4, 1.8, p=0.005); the heart rate increase at 120 minutes in the two groups was 4.9 and 1.1 beats per minute.

5.4.2 Within subject changes with high dose salbutamol (figure 5.2)

The change from baseline P_aCO_2 120 minutes after high dose salbutamol ranged from + 0.8 kPa to - 0.82 kPa, with 11 of the 14 subjects showing a fall in P_aCO_2. Patients showing a rise in P_aCO_2 were more likely to have a lower baseline P_aCO_2 and a smaller rise in heart rate with salbutamol i.e. P_aCO_2 AUC showed a negative correlation with baseline P_aCO_2 (r = - 0.6, p = 0.04) and heart rate AUC (r = - 0.6, p = 0.02). There was no significant correlation between P_aCO_2 AUC and P_aO_2 AUC (r = 0.2, p = 0.44) or other baseline measures (P_aO_2 r = 0, FEV_1 r = 0.4, reversibility r = 0).

Patients with the largest falls in PaO_2 had higher baseline FEV_1 and P_aO_2 values and a greater rise in heart rate; ie P_aO_2 AUC showed a negative correlation with FEV_1 (r = - 0.6, p = 0.04), baseline P_aO_2 (r = -0.7, p = 0.01) and heart rate AUC (r = - 0.53, p = 0.05).

Age (years)	66 (7)
FEV_1 (L)	0.71 (0.3)
BMI (kg m^{-2})	28 (6)
Cigarette consumption (pack years)	53 (34)
Reversibility (ml)	109 (57)
P_aCO_2 (kPa)	7.9 (1.0)
P_aO_2 (kPa)	7.2 (1.3)
Number of subjects taking respiratory medication	
-short-acting β_2-agonists	13
-long-acting β_2-agonists	9
-inhaled corticosteroids	9
-short-acting anti-muscarinics	9
-long-acting anti-muscarinic	0
-oral theophylline	2
-home oxygen	8

Table 5.1: Mean (SD) baseline demographic data for the fourteen subjects.

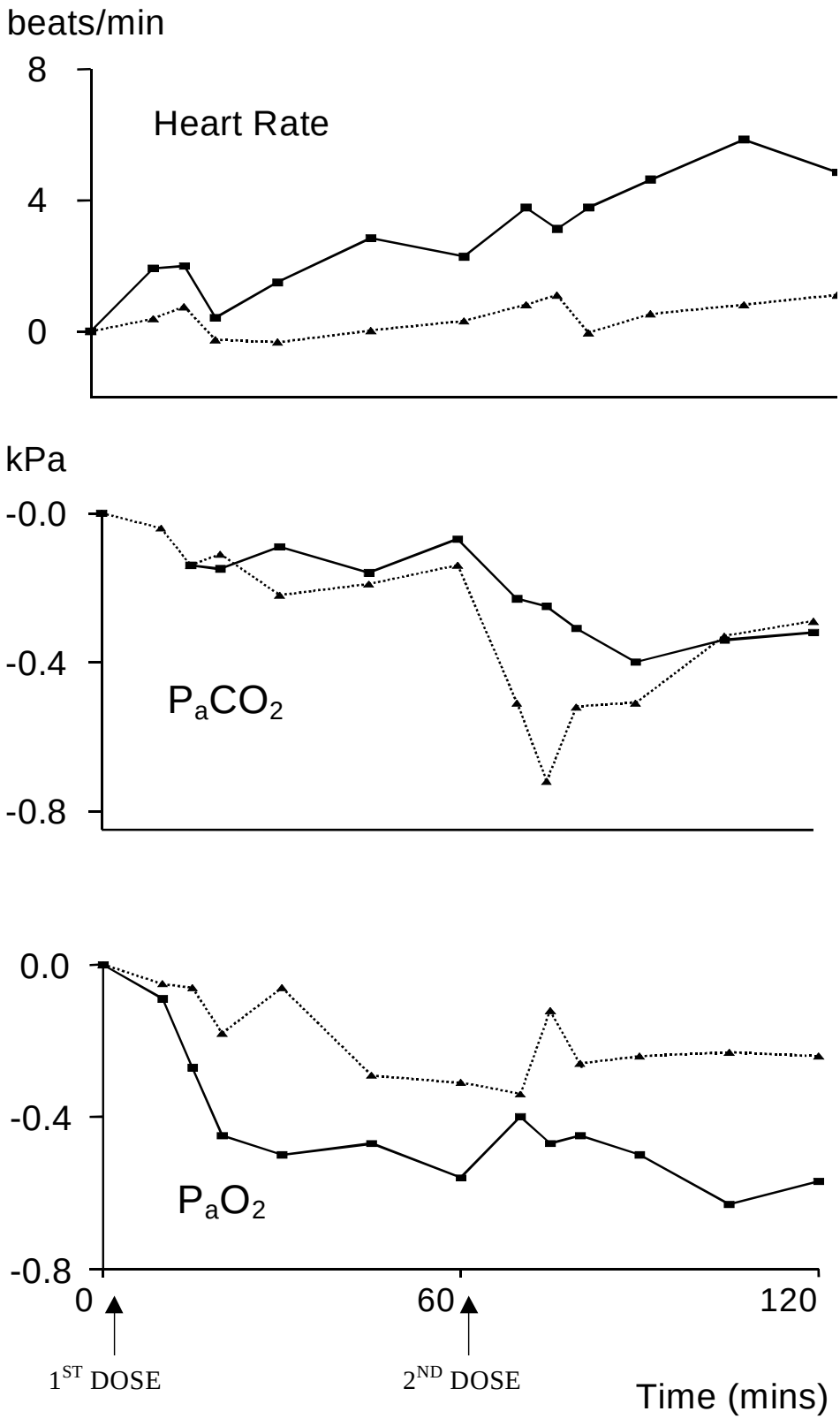

Figure 5.1: Mean change in heart rate, arterial carbon dioxide (P_aCO_2) and oxygen (P_aO_2) tension over 120 minutes after high (■—■) and low dose (▲•••▲) nebulised salbutamol (n=14).

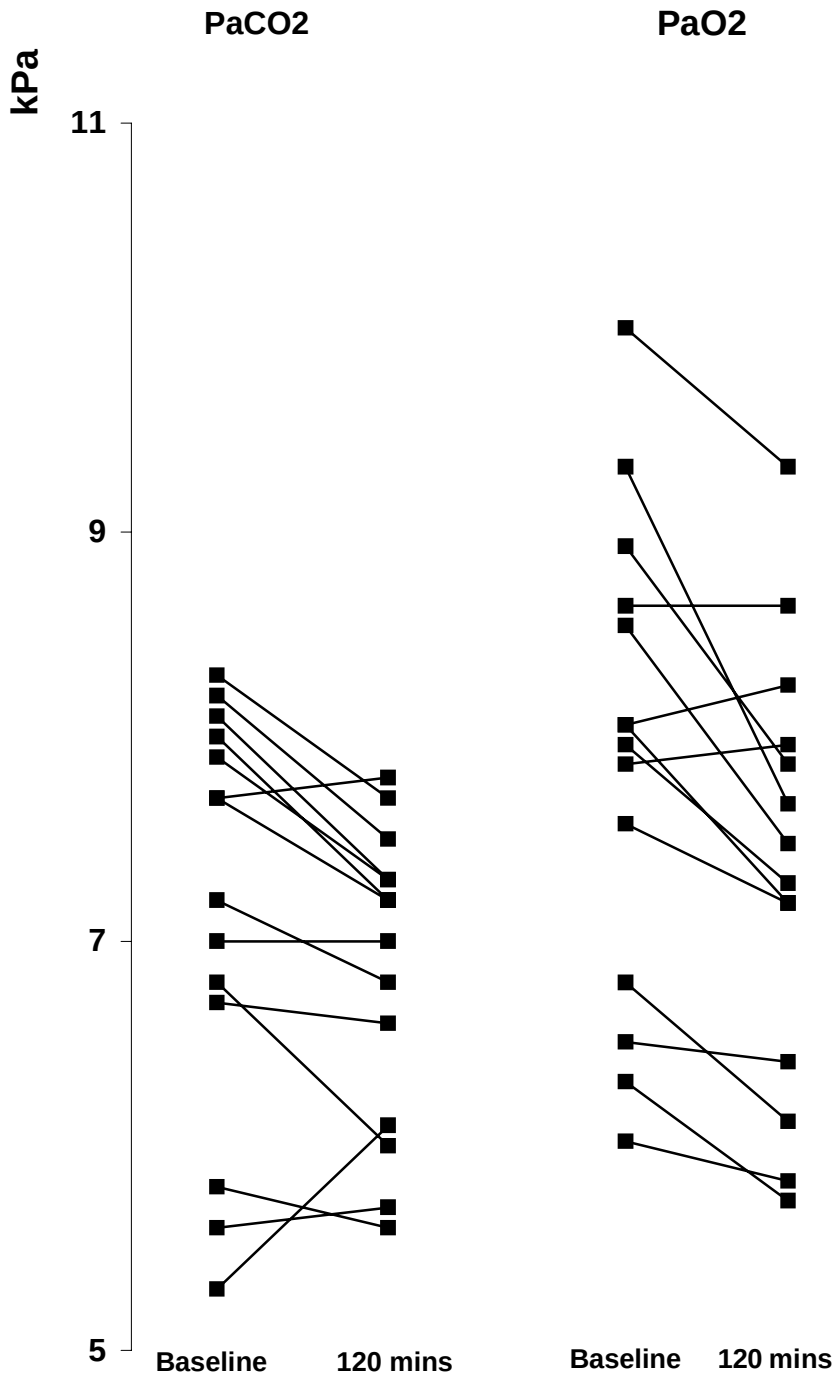

Figure 5.2: The effect of high dose salbutamol on P_aCO_2 and P_aO_2 for each patient (n=14), comparing baseline reading with the reading at 120 minutes post-dose.

P_aCO_2 AUC	
Difference (high v low)	0.03 $kPa.h^{-1}$ (0.02, 0.04), p = 0.57
P_aO_2 AUC	
Difference (high v low)	0.1 $kPa.h^{-1}$ (0, 0.2), p = 0.04
Heart rate AUC	
Difference (high v low)	1.1 $bpm.h^{-1}$ (0.4, 1.8), p = 0.005

Table 5.2: Between dose comparisons of mean AUC for P_aCO_2, P_aO_2 and heart rate.

5.5 DISCUSSION

We examined the effects of high and low dose nebulised salbutamol on arterial blood gas tensions in patients with stable COPD who had limited bronchodilator reversibility and either resting hypercapnia or a raised P_aCO_2 in response to oxygen therapy. The higher dose of salbutamol produced a greater fall in mean P_aO_2 and increase in heart rate, although the differences between the high and low doses were small. Neither dose of salbutamol caused a rise in mean P_aCO_2, although three subjects showed a small increase in P_aCO_2 with high dose salbutamol.

β_2-agonists have reduced P_aO_2 in previous studies in patients with less severe COPD and the changes were attributed to an increase in ventilation/perfusion mismatching [280,382,383]. Although these studies have not shown a rise in P_aCO_2, other studies have documented an acute increase in CO_2 output of over 25% in response to modest salbutamol doses in normal subjects and patients with asthma [281,384]. This increase in VCO_2 has been attributed to increased CO_2 flux to the lungs, due to increased cardiac output, and increased CO_2 production as metabolic rate and skeletal muscle tremor increase. We hypothesized that P_aCO_2 might rise following higher doses of salbutamol in patients with severe COPD, hypercapnia and limited bronchodilator reversibility due to an inability to increase ventilation in response to an increase in VCO_2. After the difficulties we experienced studying patients during an acute exacerbation, we chose to test the hypothesis on patients with severe COPD in a stable condition in whom previous episodes of hypercapnia suggests limited ability to further increase ventilation.

The mean P_aCO_2 in our patients fell after high dose salbutamol, suggesting that either VCO_2 did not rise for most subjects or that ventilation increased appropriately in response to an increase in VCO_2 despite severe airflow obstruction and hypercapnia. We did not measure VCO_2 since it was important that patients were as relaxed as possible. All but one of our subjects was taking regular β_2-agonists and they may therefore have developed tolerance, thus reducing the increase in tremor and cardiac output following salbutamol. Tolerance has been shown for the increase in CO_2 production with β_2-agonists in normal subjects [385] and it would also explain the relatively small increase in heart rate following salbutamol in our study.

There was some variation in change in P_aCO_2 in response to the high dose of salbutamol and a rise in P_aCO_2 was seen in three of the 14 subjects. Contrary to our expectations, patients showing a rise in P_aCO_2 were more likely to have had low baseline P_aCO_2 values and they also had a smaller increase in heart rate. This could reflect relaxation during the study although we went to some lengths to ensure that subjects were relaxed and that baseline measurements were stable before giving salbutamol. Nevertheless patients who were less relaxed would have a lower P_aCO_2 and higher heart rate prior to treatment, and these would tend to increase and decrease respectively during the study. We considered whether salbutamol might potentiate the effects of hypoxaemia on chemoreceptor stimulation and also contemplated the direct effect of salbutamol on respiratory drive. β-adrenoceptors may exist on central chemoreceptors and there is evidence that while β_2-agonists can increase respiratory drive, β-antagonists can have the opposite effect [386,387].

The changes in the partial pressure of arterial carbon dioxide 120 minutes after 10 mg nebulised salbutamol were small (maximum 0.8 kPa) and the rise in $PaCO_2$ seen in the three subjects was unlikely to produce a clinically significant adverse outcome. Some of the change in $PaCO_2$ we found can be explained by regression to the mean and by subjects not being completely relaxed at the start of the study. There will always be some variability in the results as a consequence of the repeatability of blood gas measurements. This was minimised by using the same machine for the whole study, which was calibrated every thirty minutes and had a quoted agreement between $PaCO_2$ results of 0.09 kPa in healthy subjects and hypoxaemic patients.

Change in P_aCO_2 did not correlate with either baseline P_aO_2 or change in P_aO_2 however, which may reflect differences between patients in their ability to increase ventilation, in the extent to which tolerance to β_2-agonists had developed and/or the presence of different β_2-adrenoceptor polymorphisms within the group.

5.6 CONCLUSION

Whatever the mechanism behind these changes, our findings confirm that 10mg nebulised salbutamol reduces P_aO_2 and suggest that an increase in P_aCO_2 following a high dose of salbutamol is uncommon in patients with stable severe COPD who take β_2-agonists regularly.

Chapter Six

Systemic and bronchodilator effects of formoterol in Chronic Obstructive Pulmonary Disease: a dose response study

6.1 AIMS

This study was designed to determine whether higher doses of inhaled formoterol produce systemic adverse effects that outweigh the limited bronchodilator benefit seen in subjects with COPD. We performed this study to determine whether high doses were well tolerated and to establish whether formoterol may have the potential to be used 'as required' by symptomatic patients with COPD.

6.2 INTRODUCTION

Bronchodilators are widely used by patients with symptomatic chronic obstructive pulmonary disease (COPD). Short-acting agents are used for symptom relief, but long-acting β_2-agonists have additional benefits [344,356] and management algorithms recommend their introduction if symptoms persist [388].

Formoterol, a selective β_2-agonist with a twelve-hour duration of action at conventional doses, has been shown to improve lung function and reduce symptoms when inhaled twice daily by patients with COPD [356,365]. It has a rapid onset of bronchodilatation within five minutes, similar to that of short-acting β_2-agonists [229,389] and when used "as-required" by patients with asthma, was more effective than terbutaline, in terms of lung function and prolonging the time to the first severe exacerbation [390].

A similar strategy has been suggested for patients with COPD [391], but the balance of beneficial and adverse effects may be different since additional bronchodilatation with higher doses may be minimal in these patients whilst the potential for adverse effects will continue to increase. In a previous study in subjects with COPD, subjects preferred a modest dose of inhaled

salbutamol (1mg) rather than the highest dose (4mg), presumably because the lower dose offered the best balance between beneficial and adverse effects [253]. The main safety concern with high dose formoterol relates to cardiovascular adverse effects. This is an important consideration among patients with COPD, who may have co-existing ischaemic heart disease and hypoxaemia, both risk factors for cardiac arrythmia.

We therefore carried out a randomised, crossover, dose-response study to compare the efficacy, systemic effects and subject satisfaction following single doses of inhaled formoterol (6, 12, 24 and 48μg) and placebo in twenty patients with symptomatic COPD.

6.3 SUBJECTS AND METHODS

6.3.1 Subjects

Subjects aged 40 to 85 with a clinical diagnosis of COPD were recruited if they had exertional breathlessness, limited reversibility to inhaled salbutamol, a forced expiratory volume in one second (FEV_1) below 70% predicted and a FEV_1/forced vital capacity (FVC) ratio of less than 70%. Patient characteristics on entry to the study are shown in table 6.1. Subjects were excluded if they were current smokers [392] or had an exhaled carbon monoxide level above ten parts per million on study days (Smokerlyzer, Bedfont Scientific, UK), had experienced an acute exacerbation of COPD within the last four weeks, a myocardial infarction or unstable angina in the last three months, had an unstable medical condition or were taking β-adrenergic antagonists. Nottingham City Hospital Research Ethics Committee approved the study and written informed consent was obtained from each subject at least 24 hours before the first study day.

Subject	Age	Sex	FEV_1 (L)	FEV_1 % pred	FEV_1/ FVC	β_2-agonists	Other respiratory medications
1	72	M	0.75	25	44	Formoterol Salbutamol	Budesonide Ipratropium
2	75	F	1.05	58	62	Salbutamol	
3	54	F	1.4	51	54	Salmeterol Salbutamol	Fluticasone Ipratropium
4	59	M	1.64	48	52	Salmeterol Salbutamol	Fluticasone Ipratropium
5	75	F	0.88	49	53	Salbutamol	Ipratropium
6	67	M	0.5	18	28	Salbutamol	Ipratropium
7	71	M	0.74	30	44	Salbutamol	Beclomethasone Ipratropium
8	62	F	1.22	53	53	Formoterol Salbutamol	Budesonide Ipratropium
9	77	M	0.82	31	32	Salmeterol Salbutamol	Fluticasone Ipratropium
10	71	M	0.95	51	51	Salbutamol	
11	66	M	1.4	44	49	Salmeterol Salbutamol	Fluticasone Ipratropium Theophylline
12	64	M	1.95	56	65	Formoterol Terbutaline	Budesonide
13	63	F	0.95	41	44	Salmeterol Salbutamol	Fluticasone Tiotropium
14	60	M	2.65	69	63		Tiotropium
15	62	F	1.37	59	58	Salmeterol Salbutamol	Fluticasone Ipratropium
16	60	M	0.75	23	25	Formoterol Salbutamol	Budesonide Tiotropium
17	77	M	1.4	55	50	Salmeterol Terbutaline	Fluticasone Ipratropium Theophylline
18	75	M	1.28	42	49	Salmeterol	Fluticasone Ipratropium
19	52	M	2.52	68	53	Terbutaline	Tiotropium
20	67	M	2.11	63	54	Salmeterol	Ipratropium

Table 6.1: Subject characteristics on entry into formoterol dose-response study.

6.3.2 Measurements

a) Oxygen saturation

Oxygen saturation was measured using a fingertip pulse oximeter (Minolta Pulsox-7, Tokyo, Japan).

b) Cardiovascular measures

The electrocardiograph was recorded by three-lead ambulatory monitor (DMS 300-7, Numed Cardiac Diagnostics, Sheffield, UK) and heart rate was calculated as the mean of ten consecutive beats. Blood pressure measurements were taken by electronic sphygmomanometry (HEM-705CP, Omron, Japan).

c) Dyspnoea

Dyspnoea was measured using a 100 millimetre visual analogue scale (VAS) ranging from “no breathlessness” to “worst possible breathlessness”.

d) Tremor

Tremor was measured in two ways. Subjective tremor was measured using a 100 millimetre visual analogue scale (VAS) ranging from “no tremor” to “worst possible tremor”. Tremor amplitude was measured for one minute, using an accelerometer (Bruel and Kjaer 4367, Naerum, Denmark) fitted to the terminal phalanx of the right middle finger with the forearm supported and the hand outstretched. The waveform was amplified and filtered to remove frequencies above 50 Hz, before five-second samples were subjected to Fourier analysis to calculate acceleration (Pulse Platform, Bruel and Kjaer, Naerum, Denmark). The machine was calibrated prior to each study day by measuring the tremor amplitude of the accelerometer at rest.

e) FEV_1

Spirometry was performed with the patient seated using a dry bellows spirometer (Vitalograph, Buckingham, UK), taking the better of two successive measurements of FEV_1 within 100ml. Each spirometer was calibrated once every two months with a one-litre Vitalograph® syringe; six litres of air were injected into the spirometer in one litre increments and the observed readings were required to be within +/- 0.05 litres of the volume inserted. In addition each spirometer was checked for air leaks once a fortnight by attaching a one-way valve to the mouthpiece, instilling air and ensuring that the recording pen did not drift downwards over the following two minutes.

f) Plasma potassium

Plasma potassium concentration was measured by flame photometry (Olympus AU5000, Olympus Optical Co. Ltd, Eastleigh, UK).

g) Six-minute walk test

A six-minute walk test was performed according to American Thoracic Society guidelines with standardised verbal encouragement [393] and breathlessness was measured on a Borg scale before and after exercise. Subjects carried out two six-minute familiarisation walk tests before randomisation [394].

h) Satisfaction

Overall satisfaction with each drug/dose was measured on a 200 mm visual analogue scale from “completely dissatisfied, side effects outweigh any benefit” to “completely satisfied, benefit outweighs any side effects”.

6.3.3 Protocol

This was a randomised, double blind, crossover study performed on five days within a two-week period and with each visit commencing at the same time of day. Subjects were required to withhold short-acting bronchodilators for at least ten hours and theophylline, tiotropium and long-acting β_2-agonists for at least 24 hours before each study day. Inhaled corticosteroids and non-pulmonary medications were taken as usual unless they were deemed likely to affect any of the study outcomes. After an early breakfast with a maximum of one cup of tea or coffee, subjects attended the laboratory at 0800 and rested in a comfortable chair for twenty minutes. After checking exhaled carbon monoxide levels, the ambulatory cardiac monitor was attached and baseline measures of S_pO_2, heart rate, blood pressure, breathlessness, tremor (accelerometer and VAS) and FEV_1 were taken. Blood was then taken for plasma potassium measurement and followed by a six-minute walk test, with pre- and post-test measures of breathlessness. After a ten-minute rest, subjects inhaled formoterol (6, 12, 24 or 48 μg) or placebo, with the dose and order determined by randomised computer generated sequence. On each day and under independent supervision, subjects took four inhalations at one-minute intervals from identical Turbohalers®. Three inhalers were used, containing placebo, formoterol 6 μg or 12 μg, with each device being removed from the subject between inhalations in order to ensure blinding. Subjects remained seated for the next four hours with repeat measures of oxygen saturation, heart rate, blood pressure, breathlessness, tremor and FEV_1 in that order at 30, 60, 120, 180 and 240 minutes post inhalation. Every 60 minutes, subjects were asked, “Have you felt any adverse effects that may relate to inhaling the

medication". At four hours, a further blood sample was taken for plasma potassium assay and the subject was asked to record overall satisfaction with the treatment. The six-minute walk test was then repeated.

We were most interested in the patients overall satisfaction with each treatment, as a balance between beneficial and adverse effects, but with no data on which to base the power calculation, we randomised twenty subjects to provide 80% power to detect a 160ml change in FEV_1 [395]. Twenty subjects would also allow us to assess systemic effects of interest [396].

6.3.4 Analysis

The area under the curve for all variables measured at intervals over the four hours was calculated for each subject and treatment. Shapiro-Wilks testing was performed to confirm that the results were normally distributed ($p>0.05$ for all tests). The difference between placebo and formoterol was assessed using repeated measures ANOVA for individual AUC values and the dose-response assessed by statistical significance of the linear contrast with increasing formoterol dose. Change in plasma potassium and six-minute walk distance between baseline and four hours after treatment was assessed using repeated measures ANOVA, followed by post test analysis to assess for linear trend with increasing dose. The difference in mean ectopic beat rate between placebo and each formoterol dose and the dose-response relationship was assessed by repeated measures ANOVA. Mean satisfaction scores for each formoterol dose and placebo were compared using a t-test. Statistical analyses were performed using SPSS Version 12 (SPSS Inc, Chicago, USA) after advice from Dr Sarah Lewis, and statistical significance was accepted as $p<0.05$.

6.4 RESULTS

All twenty subjects (six women) completed the study. Their mean age was 66 years, mean cigarette consumption was 42 pack years, BMI 26 kg/m^2 and FEV_1 1.32 L (47% predicted) with a mean increase in FEV_1 of 33 mL (range 0-160 ml) following salbutamol 200μg.

Thirteen of the twenty participants used inhaled corticosteroids and only four used tiotropium bromide. Nineteen subjects used inhaled β_2-agonists on a daily basis, with thirteen taking a long-acting β_2-agonist twice-daily and six further subjects taking short-acting β_2-agonists four times a day. Eleven subjects used both short- and long-acting β_2-agonists.

Data were available for all time points, except for three plasma potassium levels due to blood clotting. There were no significant differences in baseline FEV_1 values between study days and no crossover effects.

6.4.1 Spirometry (figures 6.1 and 6.3, table 6.2)

FEV_1 increased from baseline with all doses of formoterol and the effect was sustained over four hours. AUC FEV_1 was higher after all doses of formoterol compared to placebo and showed a significant dose-response relationship with increasing doses of formoterol. The largest AUC FEV_1 (626.6 ml.h) was seen with the highest dose of formoterol and reflects a mean increase in FEV_1 of around 157 ml across the four hour time period. There was no correlation between the AUC FEV_1 and baseline FEV_1 ($r = 0.7$, $p = 0.2$).

6.4.2 Oxygen saturation (figures 6.2 and 6.4, table 6.2)

There was an initial fall in mean SpO_2 after each dose of formoterol with the maximum fall occurring between 30 and 60 minutes. The mean fall from baseline was less than 1% after all doses however, and there was no difference between placebo and any formoterol dose and no dose-response relationship for AUC S_pO_2.

6.4.3 Breathlessness (figures 6.1 and 6.3, table 6.2)

All doses of formoterol and placebo were associated with a fall in dyspnoea score and this was sustained over the four hours. There was no difference between placebo and any formoterol dose and no dose related effect of formoterol.

6.4.4 Cardiovascular responses (figures 6.1, 6.2, 6.3, 6.4, 6.5, table 7.2)

There was an initial fall in mean heart rate after the three lowest doses of formoterol, but a small rise with the highest dose and AUC heart rate for the 48 μg dose was greater than that for placebo ($p = 0.02$). There was no difference between placebo and other formoterol doses and no dose response relationship.

Systolic blood pressure fell initially following all formoterol doses and placebo and then increased gradually whilst diastolic blood pressure showed a small rise over the four hours. There were no difference between placebo and any formoterol dose and no dose response relationship for either systolic or diastolic blood pressure.

There was no difference between any formoterol dose and placebo and no dose response relationship for mean hourly rate of supraventicular or

ventricular ectopics, although formoterol 48 μg was associated with the highest ectopic rate.

6.4.5 Tremor (figures 6.1, 6.2, 6.3 and 6.4, table 6.2)

Tremor measured by accelerometer increased with the two highest formoterol doses compared to placebo and AUC tremor showed a dose-response effect. Tremor measured by VAS was more variable. Although there were no differences between individual doses and placebo, a dose response relationship was evident.

6.4.6 Walking distances (figure 6.5)

Changes in six-minute walk distance were trivial, with no difference between placebo and any formoterol dose and no dose-response relationship. The maximum change in six-minute walk distance between placebo and any dose of formoterol was around fifteen metres, which is less than 5% of the mean baseline walk distance of 360 metres.

6.4.7 Exertional dyspnoea (figure 6.6)

Formoterol produced no change in pre- or post-walk Borg dyspnoea scores compared to placebo and no dose response relationship was present.

6.4.8 Plasma potassium (figure 6.5)

At four hours mean plasma potassium had changed by less than 0.1 mmol from baseline for all doses, with no difference between placebo and individual formoterol doses and no dose-response effect.

6.4.9 Satisfaction scores (figure 6.5)

Each formoterol dose produced higher satisfaction scores than placebo, but with no dose-response relationship.

6.4.10 Adverse events

No serious adverse effects were reported during the study. Two individuals reported headache after placebo and formoterol 6 μg respectively and one reported light-headedness two hours after formoterol 6 μg.

6.5 DISCUSSION

In subjects with moderately severe, symptomatic COPD, formoterol produced a dose dependent increase in FEV_1 without a corresponding improvement in dyspnoea or walk distance. Tremor also increased in a dose dependent manner whilst heart rate only increased significantly after the highest formoterol dose. Formoterol had no significant effect on plasma potassium, blood pressure, oxygen saturation or ectopic beat rate. All doses of formoterol produced higher satisfaction scores than placebo, although no dose response relationship was evident.

Satisfaction with any treatment depends on a balance of beneficial and adverse effects. This is particularly relevant in COPD where the largely irreversible nature of the disorder will limit the beneficial effects seen with higher drug doses whilst adverse effects can continue to increase. The increase in FEV_1 in this study was small and of similar magnitude to that seen in other studies in subjects with COPD [397], but was not associated with a dose related decrease in dyspnoea scores or an increase in exercise tolerance. Formoterol [365] and salmeterol [351] have failed to produce a dose related effect on dyspnoea or walk distance in previous longer term

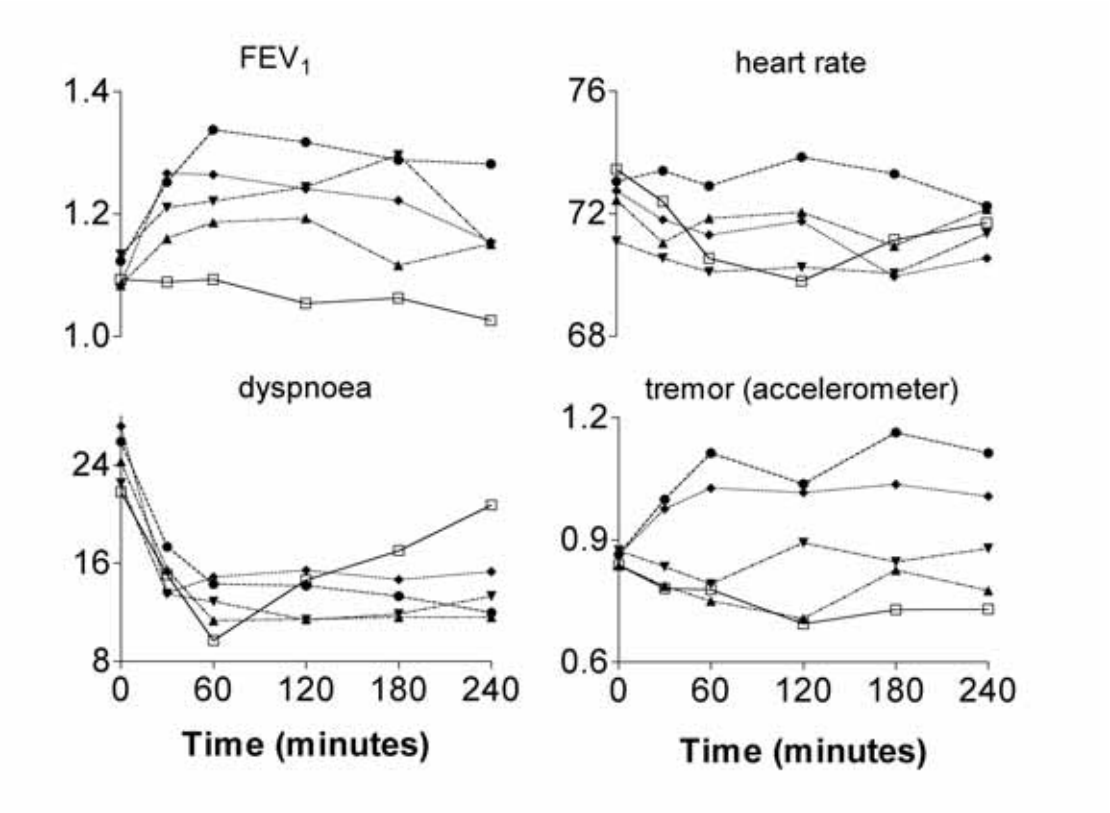

Figure 6.1: Mean values over 240 minutes for FEV_1(L), dyspnoea VAS (mm), heart rate (bpm) and tremor acceleration (m/s/s) after formoterol 6 (▲), 12 (▼), 24 (♦) and 48 (●) µg and placebo (□).

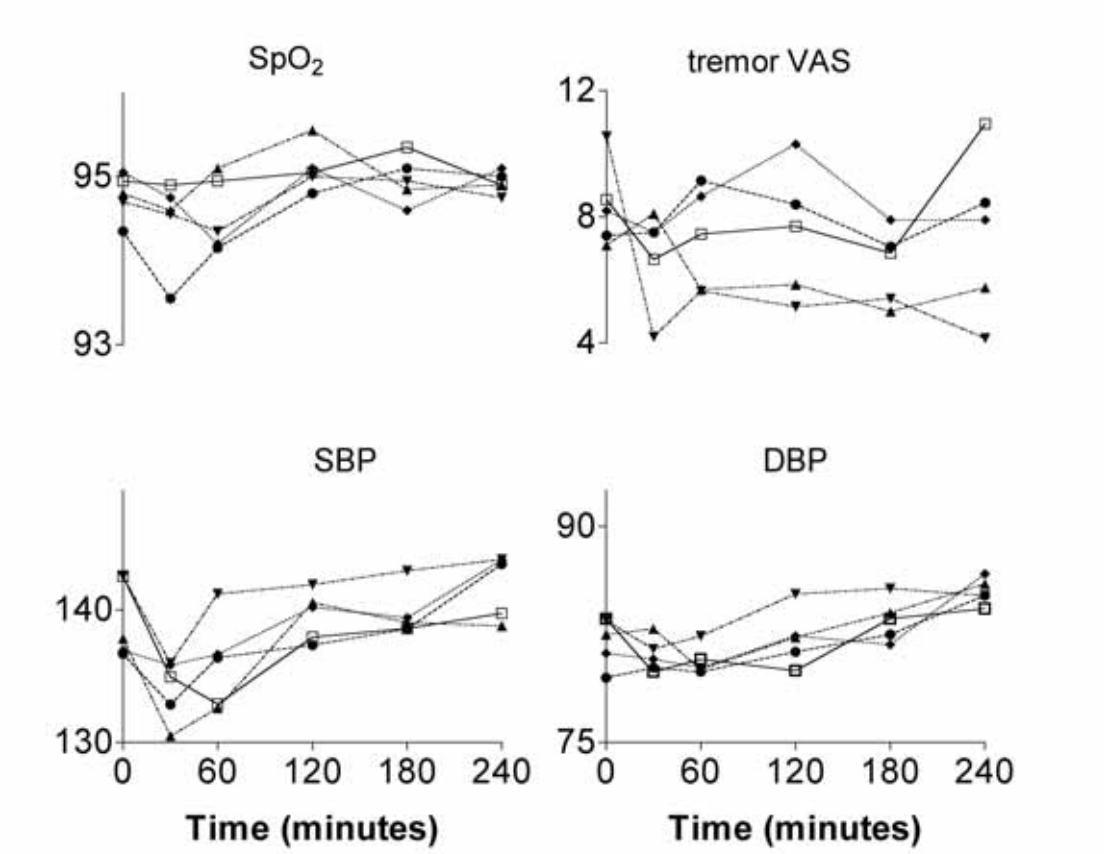

Figure 6.2: Mean values over 240 minutes for SpO2 (%), tremor VAS (mm), systolic and diastolic blood pressure (mmHg) after formoterol 6 (▲), 12 (▼), 24 (♦) and 48 (●) µg and placebo (□).

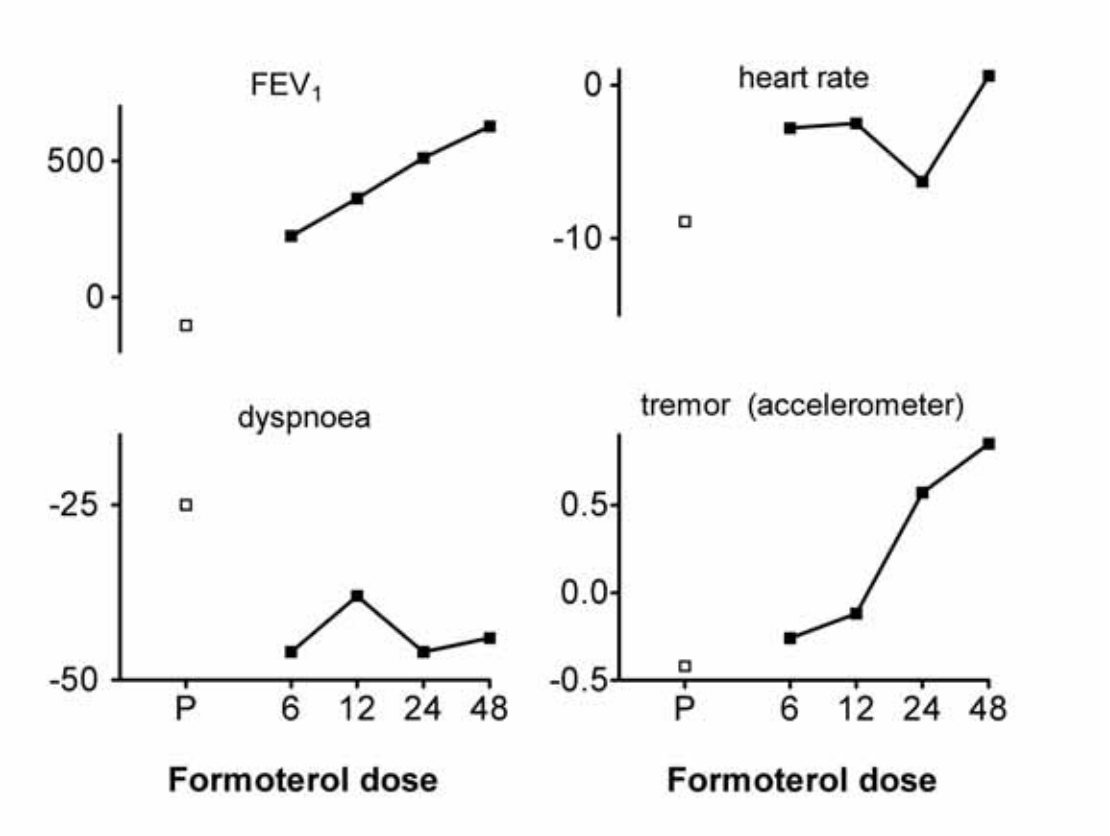

Figure 6.3: The relationship between log dose formoterol (μg) and mean area under the curve for FEV_1 (ml.h), dyspnoea VAS (mm.h), heart rate (bpm.h) and tremor acceleration (m/s/s.h) with placebo (P) shown for comparison (□).

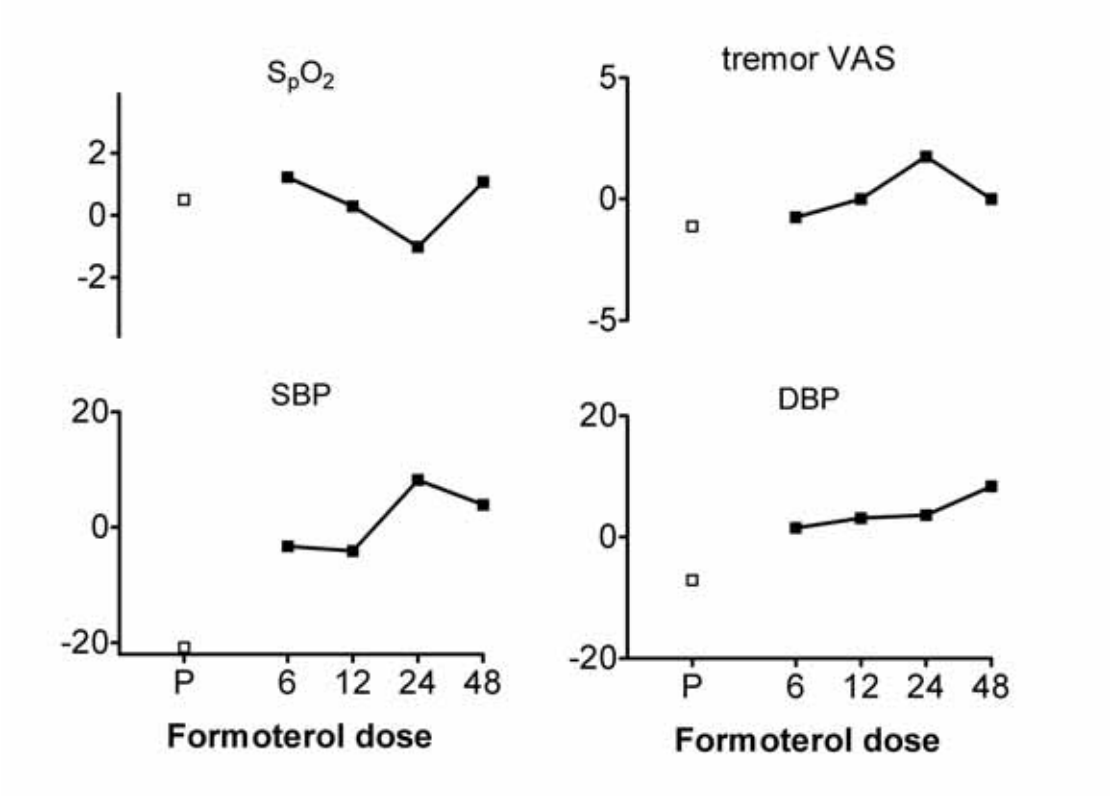

Figure 6.4: The relationship between log dose formoterol (µg) and mean area under the curve for SpO2 (%.h), tremor VAS (mm.h), systolic and diastolic blood pressure (mmHg.h) with placebo (P) shown for comparison (□).

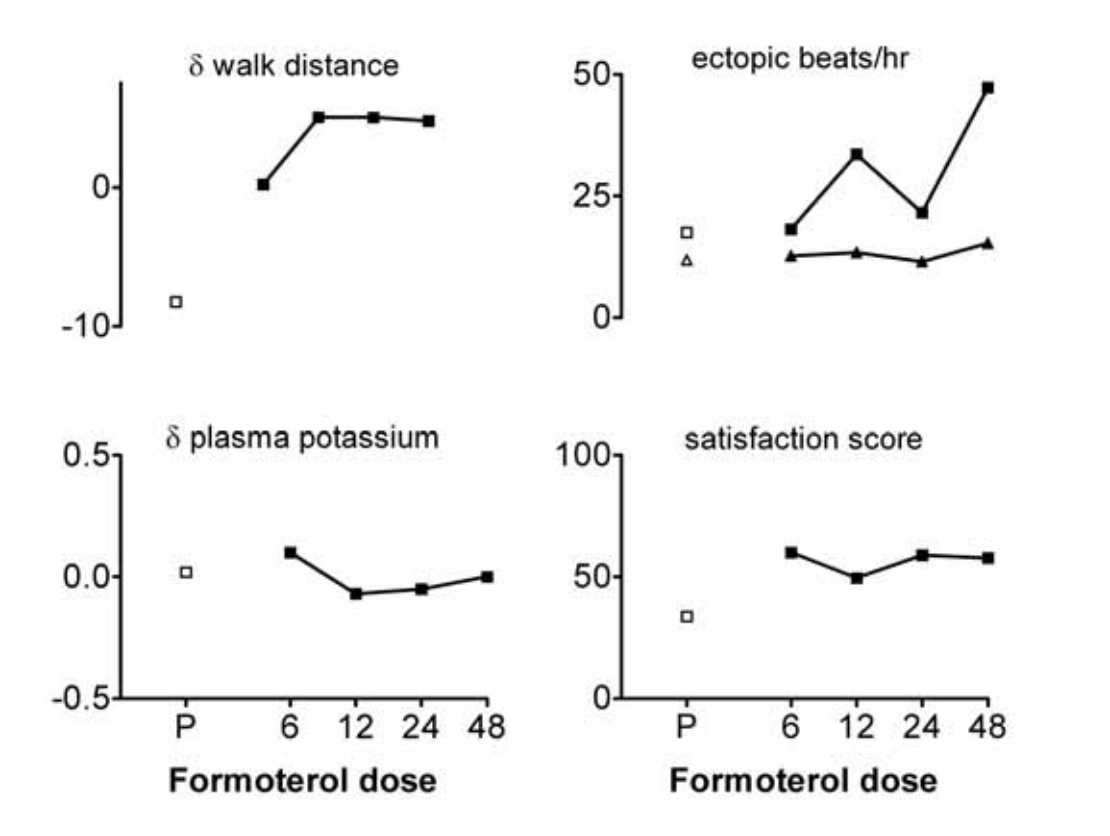

Figure 6.5: The relationship between log dose formoterol (μg) and change in six-minute walk distance (m), change in plasma potassium concentration (mmol/l), mean rate of ventricular (▲) and supraventricular (■) ectopic beats and mean satisfaction scores (mm), with placebo (P) shown for comparison (□).

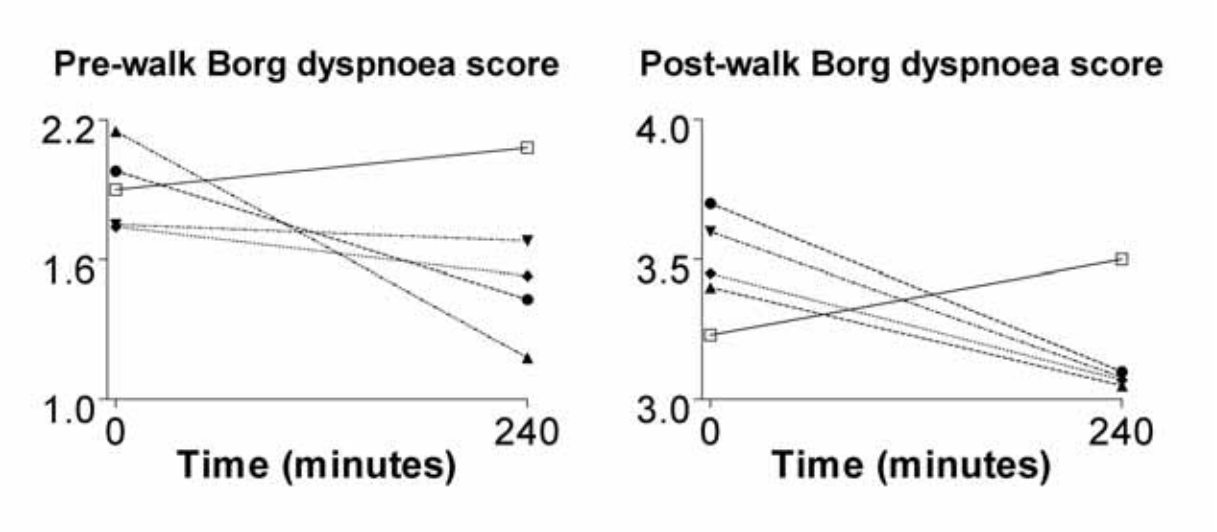

Figure 6.6: The relationship between formoterol 6 (▲), 12 (▼), 24 (♦) and 48 (•) µg and placebo (□) and the pre- and post-walk Borg dyspnoea scores.

	AUC values							
	FEV_1 (ml.h)	**S_pO_2** (%.h)	**Dyspnoea** (mm.h)	**HR** (bpm.h)	**Tremor** (m/s/s.h)	**VAS tremor** (mm.h)	**SBP** (mmHg.h)	**DBP** (mmHg.h)
Placebo	- 104.3	0.5	- 25	- 8.9	- 0.42	- 3.2	- 21	- 6.7
6 µg	225.3†	1.2	- 46.0	- 2.8	- 0.26	- 4.6	- 3.3	1.5
12 µg	362†	0.3	- 38.2	- 2.5	- 0.12	- 20.7	- 4.1	3.1
24 µg	510.8†	- 1.0	- 46.2	- 6.3	0.57†	0.6	8.2	3.6
48 µg	626.6†	1.1	- 44.3	0.6†	0.85†	1.5	3.9	8.4
p value	0.04✱	0.69	0.98	0.65	<0.001✱	0.04✱	0.6	0.68

✱ p = <0.05 for dose response trend

† p = <0.05 for individual formoterol dose versus placebo

Table 6.2: Mean area under the time response curve (AUC) for FEV_1 and the systemic measurements made at intervals over 240 minutes for each formoterol dose.

studies, suggesting that these may be less sensitive outcome measures for evaluating the dose response effect of long-acting β_2-agonists and reflecting the relatively small increases in FEV_1 seen in patients with COPD.

The adverse systemic effects of β_2-agonists are well known, but in this study only tremor and heart rate increased significantly with high doses of formoterol. Moderate doses of formoterol have been shown to increase heart rate, systolic blood pressure and QT interval, and to reduce plasma potassium and diastolic blood pressure in healthy volunteers [264,398,399]. The effects have been less marked in subjects with asthma [399-404], perhaps reflecting the development of tolerance to β_2-agonists for systemic effects [267,405,406]. Tolerance could explain the limited systemic effects and lack of reported adverse effects in our study since most subjects were using β_2-agonists regularly.

We summarized our findings as mean values, and as area under the curve for the measurements that were made on several occasions. Outliers may not be apparent with this approach but could be important in relation to adverse effects. Checking individual data showed that the data was homogenous and that there were no worrying outlying results. We limited the study to four hours so that responses would not be affected by meals, as in previous studies. The greatest pharmacodynamic changes are known to occur within four hours so it is unlikely that we missed additional effects after this time period [264].

The dose response curve produced by the accelerometer suggests that this may a better way than the visual analogue scale to determine the presence of tremor. Subjects did not perceive the symptoms to be troublesome or significant. Again, since most individuals used β_2-agonists on a daily basis,

tolerance may have limited the physiological tremor and the tremor produced may almost be considered normal by those who experience the symptom on a daily basis.

Rather than seeing the bronchodilator response plateau with high doses, there was a dose-related rise in AUC $FEV_{1.}$ The increase in FEV_1 and the limited systemic effects would support the suggestion that inhaled formoterol may have a role as rescue medication in COPD. However, concerns do exist about the adverse effects produced by high dose β_2-agonists, particularly among patients with pre-existing cardiovascular disease. As discussed in chapter four, a small study of subjects with COPD who had had a cardiac arrhythmia suggested that formoterol 12 μg offered a higher safety margin than the 24 μg dose since it had less effect on heart rate, plasma potassium, supraventricular and ventricular premature beats [377]. There was a trend towards increased ectopic beats with higher doses in our study, but a larger long-term study would be needed to fully assess the safety of higher doses in these patients. Adverse cardiovascular effects and dysrhythmias are particularly important for patients with COPD due to the increased incidence of cigarette smoking and risk factors such as hypoxaemia, hypokalaemia and prolonged QT interval [405], which are likely to increase further during an exacerbation [299].

Assessing the balance between beneficial and adverse effects is difficult and asking about overall satisfaction with the drug after each study day was an attempt to address the patients' perception of this balance. Satisfaction scores are difficult to validate but formoterol produced greater subject satisfaction than placebo in keeping with its effect on FEV_1. A similar approach had face validity in a previous study that compared different doses

of salbutamol in subjects with COPD and produced a bell shaped response for satisfaction, in that subjects preferred a modest dose of salbutamol (1mg) to both placebo or the highest dose of salbutamol (4mg), presumably because the 1mg dose offered the best balance between beneficial and adverse effects [253]. Satisfaction improved with all doses of formoterol in our study however, suggesting that the perceived adverse effects of formoterol 48 μg did not exceed the perceived benefit.

6.6 CONCLUSION

In our subjects with moderate to severe COPD, single doses of inhaled formoterol produced a dose-dependent improvement in FEV_1. The lack of serious systemic adverse effects with high doses was reassuring and suggests that further studies designed to explore the role of formoterol as a rescue medication in COPD are warranted.

Chapter Seven

Conclusions

7.1 DISCUSSION

Determining the optimum use of inhaled β_2-agonists in chronic obstructive pulmonary disease is important. COPD is very common, worldwide prevalence is increasing and β_2-agonists are widely used. Patients are often elderly and often have co-morbidities, including an increased incidence of the cardiovascular diseases associated with cigarette smoking. COPD is progressive and much of the pathology is irreversible, with limited reversibility to bronchodilators being a distinguishing feature of the disease. This means that high doses of inhaled β_2-agonists could produce adverse systemic effects that outweigh the limited beneficial bronchodilator effects in some patients with COPD.

The adverse systemic effect profile of inhaled β_2-agonists is well known and includes headaches, agitation, tachycardia, palpitations, muscle cramps, tremor, a reduction in serum potassium and a fall in the partial pressure of arterial oxygen. Although the adverse effects are generally mild and well tolerated, there are concerns that a fall in P_aO_2 and serum potassium and an increase in heart rate could precipitate a serious cardiac event in some patients with COPD, particularly if the patient already has hypoxaemia, hypokalaemia or cardiac problems. The risk of a serious adverse event may be increased further if the patient is taking drugs that can stimulate the myocardium (theophyllines) or cause hypokalaemia (diuretics and corticosteroids) as these patients often are.

Concerns about the safety of using regular short-acting inhaled β_2-agonists in the management of asthma have been present for some time and more recently these concerns have extended to long-acting β_2-agonists. A large-scale surveillance study comparing the safety of regular inhaled salmeterol

with placebo was halted prematurely after an increase in asthma-related deaths and life-threatening experiences was identified amongst subjects receiving salmeterol [297]. Concerns also exist about the safety of relatively high doses, after Mann et al found that the 24 μg twice-daily dose of formoterol was associated with more frequent serious asthma exacerbations than placebo, an effect not seen with the 12 μg twice-daily dose [376]. Although widely used in the management of COPD, few studies have concentrated on the safety of inhaled β_2-agonists in these patients. Placebo-controlled studies that examined the efficacy of regular short-acting inhaled β_2-agonists in COPD did not report an increase in adverse effects with β_2-agonists, but each study only examined a low (200 μg qds) dose of salbutamol. The placebo-controlled studies that examined the efficacy of formoterol and salmeterol in COPD generally found a beneficial effect on lung function and symptoms without an increase in adverse systemic effects (Chapter Four). The three studies in this thesis have examined aspects of the safety of higher doses of inhaled β_2-agonists used in the treatment of patients with COPD.

In the management of COPD, the highest doses of inhaled β_2-agonists are given as rescue medication during an acute exacerbation and similar doses are given on a regular basis as part of the domicilary care of some patients with severe COPD. These may be two situations when high doses of β_2-agonists are particularly hazardous since both are associated with more severe hypoxaemia, which heightens the cardiovascular risk with β_2-agonist use. If inhaled β_2-agonists produce a further fall in PaO_2 and a reduction in plasma potassium the potential hazard may become clinically important.

The reduction in potassium level can be less marked after chronic use of an inhaled β_2-agonist in COPD, a situation where tachyphylaxis could be helpful for the individual patient.

Inhaled β_2-agonists increase carbon dioxide output. A number of mechanisms have been suggested, including an increase in carbon dioxide flux to the lungs as a result of the increase in cardiac output and an increase in CO_2 production as tremor increases [379]. The rise in carbon dioxide output was demonstrated to be over 20% after healthy subjects inhaled salbutamol at a dose of 1.2 mg [281]. Since subjects increase the work of breathing in response to the metabolic demands during acute exacerbations of COPD we hypothesized that some patients would be unable to increase ventilation in response to a β_2-agonist mediated rise in CO flux to the lungs, causing a rise in $PaCO_2$ and worsening respiratory failure. The work of breathing of subjects with severe COPD at rest is much higher than age matched healthy subjects, but the increase in VCO_2 after inhaled β_2-agonists would be less metabolically demanding than moderate exercise. For a β_2-agonist to cause a rise in $PaCO_2$ via the proposed mechanism, the subject would need to be approaching their physiological limit and unable to increase their work any further, for example during a severe exacerbation.

In the first study (chapter 5) we tried to examine the effects of high dose salbutamol on arterial blood gas tensions during an acute exacerbation of COPD. Due to major problems with recruitment, the study was abandoned and instead we examined the effect of high dose salbutamol on arterial blood gas tensions among fourteen patients with stable severe COPD and chronic or intermittent hypercapnia (chapter 6).

Our main findings in the five patients studied during an exacerbation were:

- There was no evidence that high dose nebulised salbutamol had a dose response effect on arterial carbon dioxide (P_aCO_2) or oxygen tension (P_aO_2).
- The individual with the highest baseline P_aCO_2 had a transient rise in P_aCO_2 after the highest doses of salbutamol.
- Semi-invasive studies during an acute exacerbation of COPD are difficult to conduct.

Our main findings in the fourteen patients with COPD and evidence of a raised P_aCO_2 were:

- High dose salbutamol caused a small fall in mean P_aCO_2 and mean P_aO_2.
- Three individuals had a small rise in P_aCO_2 120 minutes after 10mg salbutamol.
- A rise in P_aCO_2 after 10 mg salbutamol was associated with a low baseline P_aCO_2.

Thus there was no evidence of a sustained or consistent rise in P_aCO_2 after high dose nebulised salbutamol in either study and no evidence that salbutamol worsened respiratory failure to any extent in most of the subjects we studied with moderate to severe COPD. The fact that most subjects had no increase in P_aCO_2 suggests that either there was no major increase in CO_2 flux to the lungs after inhaled salbutamol or most patients were able to increase ventilation in response to the CO_2 flux. We have considered the likely mechanisms underlying the mean findings in our studies and assessed why three subjects had a small rise in P_aCO_2 in the second study.

1) The increase in VCO_2 was too small to cause an increase in P_aCO_2.

As described earlier there was a mean rise in VCO_2 of more than 20% when healthy subjects inhaled a modest dose of salbutamol [281]. The study had demonstrated a dose related increase in VCO_2 and we had anticipated that the higher doses of nebulised salbutamol would produce a similar, or greater, increase in carbon dioxide output in subjects with COPD. Possible reasons for a lower increase in VCO_2 include:

a) Transient increase in VCO_2

The rise in VCO_2 may not have been sustained at a sufficiently high level for long enough to cause a rise in P_aCO_2 in our patients. VCO_2 was found to be at its peak five minutes after inhalation of salbutamol by Amoroso et al [281]. Although VCO_2 was still high 60 minutes after inhalation, the increase was only 10% higher than baseline for the last 30 minutes.

b) Downregulation of β_2-adrenoceptors

Regular use of inhaled β_2-agonists can lead to downregulation of systemic response to β_2-agonists [267,406,407]. Most patients in studies one and two were taking inhaled β_2-agonists regularly prior to being studied, so the effect of salbutamol on VCO_2 may have been lower than that demonstrated by Amoroso et al [281] The absence of a large rise in heart rate in both studies suggests that the increase in cardiac output was modest thus reducing any increased flux of CO_2 to the lungs. Downregulation of tremor would lead to a smaller increase in metabolic rate and reduce the anticipated increase in carbon dioxide production. In keeping with downregulation, Wilson et al showed that the acute effect of a single dose of salbutamol on VCO_2 was attenuated by the regular inhalation of β_2-agonists for two weeks [385].

c) β_2-adrenoceptor polymorphisms

Little is known about the clinical relevance of β_2-receptor polymorphisms in COPD. The polymorphisms may affect downregulation and could explain some of the variation in the VCO_2 response to high dose inhaled salbutamol.

2) Most subjects can increase ventilation in response to a rise in VCO_2.

Most subjects did not have a rise in P_aCO_2 after high dose salbutamol even when they are experiencing the increased metabolic demands of an acute exacerbation of COPD. The majority of patients with COPD and a history of hypercapnia (evidence of chronic alveolar hypoventilation) did not have a rise in P_aCO_2 after high dose salbutamol either. This suggests that most subjects with COPD have the ventilatory reserve to respond to a flux of CO_2 to the lungs, even if they have severe disease, chronic hypoventilation or are experiencing an acute exacerbation.

The fact that three individuals had a small rise in P_aCO_2 after high dose salbutamol in the second study could provide some support for our hypothesis or could reflect heterogeneity of biological response. The association between a low baseline P_aCO_2 and a subsequent rise in P_aCO_2 could be due to regression to the mean or anxiety at the start of the study causing hyperventilation and an inappropriately low baseline P_aCO_2 for that patient. The rise in P_aCO_2 might then be due to the patient relaxing over the two hours, rather than a salbutamol induced rise in carbon dioxide production. This would be consistent with the rise in P_aCO_2 being associated with a smaller rise in heart rate (which would tend to fall as the individual became less anxious).

The results from the two studies were broadly reassuring. We cannot exclude the possibility that a more marked or prolonged rise in P_aCO_2 may occur with higher doses of β_2-agonists e.g. if high doses are delivered via

'back-to-back' nebulisers at frequent intervals, or during a more severe exacerbation, or in patients with higher P_aCO_2 tensions. Individuals who have not taken regular inhaled β_2-agonists may also be at risk of a larger rise in P_aCO_2 after high dose β_2-agonists since these patients would not have the downregulation of systemic effects seen after regular use of inhaled β_2-agonists.

Inhaled β_2-agonists reduce P_aO_2 in subjects with COPD by causing vasodilatation in poorly ventilated parts of the diseased lung and increasing V/Q mismatch. A recent small study found that the detrimental effect of salbutamol on P_aO_2 was not present during an acute exacerbation of COPD. Polverino et al examined the effect of salbutamol 5mg on gas exchange during an acute exacerbation of COPD and repeated the investigations three months later. Salbutamol had no effect on the mean P_aO_2, alveolar-arterial PO_2 gradient or P_aCO_2 during an exacerbation, but did reduce the mean P_aO_2 and increase the mean (A-a) gradient when the same subjects were studied three months after the exacerbation [408].

The authors proposed a number of possible explanations for this unexpected finding, suggesting that the vasodilator response to salbutamol may be less marked during acute severe hypoxia, the effect of salbutamol on inflammatory mediator release may reduce vasodilatation and/or the bronchodilators administered earlier in the admission had already caused vasodilatation.

During the acute exacerbation part of the study, 30 minutes after salbutamol there was a mean rise in cardiac output of 12% and a mean rise in heart rate of 10%, without a corresponding rise in P_aCO_2. Although the effect of salbutamol on VCO_2 was measured, the results were not reported in the

paper [408]. The finding that salbutamol has a different effect on P_aO_2 during an acute exacerbation of COPD is contrary to our results and merits further attention.

The inhaled long-acting β_2-agonists formoterol and salmeterol are used twice-daily as maintenance therapy by subjects with COPD and they have been shown to reduce symptoms and improve lung function [248]. Formoterol has the advantage of producing rapid bronchodilatation, with a similar onset of action as the short-acting β_2-agonists. This has led to the idea that inhaled formoterol could have a dual role in the management of COPD, with the potential to be used 'as required' to relieve symptoms as well as being used twice daily as maintenance therapy. Using formoterol 'as required' may lead to inhalation of considerably higher doses of formoterol than are used with maintenance therapy. We hypothesized that the adverse systemic effects would counter the limited beneficial bronchodilator effects if subjects with COPD use formoterol in high doses.

The third study (chapter 7), a dose-response study of inhaled formoterol in twenty subjects with COPD, examined the systemic and bronchodilator effects and patient satisfaction with a range of formoterol doses up to 48 μg. We assessed whether high dose inhaled formoterol was well tolerated by subjects with COPD and tried to determine whether the higher doses produced systemic adverse effects that might outweigh the beneficial bronchodilator effects. The main findings were:

- Inhaled formoterol caused a dose-related increase in FEV_1.
- There was no associated improvement in breathlessness scores, walking distance or patient satisfaction.

- The systemic adverse effects associated with inhaled formoterol were modest and did not compromise patient satisfaction, even in high doses.

The dose-related improvement in FEV_1 did not translate into an improvement in exercise tolerance or symptoms, presumably because the improvements in FEV_1 were too small. Previous studies have concentrated on lung function as a marker of efficacy in COPD and obtained similar results to this study [310,356]. Our findings suggest that FEV_1 alone may not be a good way to measure the benefit of a drug in COPD and future studies need to concentrate on outcome measures that are clinically relevant [409-411].

The systemic effects after single doses of inhaled formoterol were modest and even the 48 μg dose was well tolerated. All but one subject used inhaled β_2-agonists on a regular basis and are likely to have developed tolerance to the extra-pulmonary effects of formoterol.

Satisfaction scores did not increase in a dose-related manner, but were similar with all doses of formoterol, and higher than the scores produced by placebo. We had expected high dose formoterol to produce adverse systemic effects that would outweigh the beneficial bronchodilator effects and hence compromise satisfaction. The absence of a decline in satisfaction with high doses is interesting and although the scale has not been fully validated, this approach provides a different way of assessing the efficacy of treatments in COPD and merits further attention.

The dose response effect on FEV_1 and lack of adverse effects suggest that further studies are justified to determine whether inhaled formoterol may have a role as rescue medication in the management of subjects with COPD,

despite the lack of effect on exercise tolerance and symptoms. We examined the effect of single inhaled doses of formoterol and there may be different outcomes if high doses are taken in quick succession. If formoterol were used 'as required' it would be important to establish a ceiling dose for a 24-hour period to reduce the likelihood of using excessive doses. Our data covers patients who were taking β_2-agonists regularly and the results cannot be extrapolated to subjects who do not use β_2-agonists on a regular basis and who would not have developed tolerance to the systemic effects of inhaled β_2-agonists.

This thesis has discussed three studies designed to determine whether the systemic adverse effects produced by high doses inhaled β_2-agonists could outweigh the beneficial bronchodilator effects when subjects with COPD are in certain clinical situations. All three studies demonstrated that the systemic effects produced by high dose inhaled β_2-agonists were generally modest and well tolerated in patients who use β_2-agonists regularly. Individuals using inhaled β_2-agonists on a regular basis are likely to develop downregulation of β_2-receptors which could allow high doses to be administered without producing excessive adverse systemic effects.

7.2 UNANSWERED QUESTIONS

1) Although our results were broadly reassuring there is still a question as to whether high dose nebulised β_2-agonists could increase P_aCO_2 and precipitate respiratory acidosis in some patients with COPD. This may only be clinically relevant when a subject approaching their physiological extreme (during a severe exacerbation with type two respiratory failure) is exposed to very high doses of β_2-agonist in quick succession.

2) Further work is required to assess whether inhaled formoterol is a safe and effective treatment when used 'as required' in the management of COPD.

3) Although inhaled β_2-agonists are generally safe in recommended doses when used by subjects with COPD, some individuals may be at particular risk of developing adverse effects with high doses, including subjects with certain β_2-adrenoceptor polymorphisms and those who are β_2-agonist naive.

APPENDIX 1

Nottingham City Hospital NHS
NHS Trust

Please ask for: Dr Derek Pearson

Ref: EC02/40

16 May 2002

Dr C Whale
Research Fellow
Division of Respiratory Medicine
Nottingham City Hospital

Research Ethics Committee
Hucknall Road
Nottingham
NG5 1PB

Tel: 0115 969 1169 ext 45860
Fax: 0115 9627784
e-mail: dpearson@ncht.trent.nhs.uk
Minicom: 0115 962 7749
www.ncht.org.uk

Dear Dr Whale

Re: Determining the optimum dose of nebulised salbutamol in acute exacerbations of chronic obstructive pulmonary disease. Ref: EC02/40

Thank you for your letter dated 11 April 2002 answering the queries raised by the committee and for enclosing a revised patient information sheet, consent form and protocol.

I can now give this study officer approval and this will be reported to the full committee at the next meeting to be held on 24 June 2002.

Approval is given on the following understanding:

- Approval is granted for 3 years from the date of this letter. If you fail to start the research within this time you will have to re-apply for further approval.
- It is the responsibility of the investigator to notify the committee immediately of any information received by him/her or of which he/she becomes available, which would cast doubt upon or alter any information contained in the original application or a later amendment application, which would raise questions about the safety and/or continued conduct of the research.
- Patient information stored on computer must be handled in accordance with the Data Protection Act 1998 and local policies and procedures relating to the use of computer held data.
- All research must be conducted throughout according to good clinical research practice standards.
- All serious or unexpected adverse events and adverse drug reactions which may affect the conduct and the continuation of the study must be reviewed by the lead researcher and reported to the committee. Please use the attached pro-forma when submitting adverse event reports.

- All protocol amendments must be referred to the committee for further review and approved prior to implementation except where the welfare of the subject is paramount.

- All research which is discontinued temporarily or permanently should be reported to the committee.

- The committee requests the researcher to provide details of the progress of the research at least annually and details of its conclusion and outcome.

- If you intend to undertake this research at Queen's Medical Centre (QMC) as well approval must be sought from the QMC Research Ethics Committee by submitting one copy of the documentation and a copy of this approval letter to the Honorary Secretary of the QMC Research Ethics Committee.

- The meeting of the committee which considered your application was quorate according to the constitution of the committee.

- The membership of the committee is attached. It is against the policy of the committee to identify which members were present when your submission was approved.

Yours sincerely

Mr M Shehata
Chairman
City Hospital Research Ethics Committee

The NHS in Nottingham
1 Standard Court
Park Row
Nottingham
NG1 6GN

Tel: 0115 912 3344
Fax: 0115 912 3300
DX709521, Nottingham 13

Please ask for: Dr Derek Pearson

Ref: **EC02/154**

18th November 2002

Dr C Whale
Division of Respiratory Medicine
Clinical Sciences Building
City Hospital
Nottingham
NG5 1PB

Dear Dr Whale

Re: Study to determine the effect of high dose nebulised salbutamol on stable hypercapnaeic chronic obstructive pulmonary disease in patients. EC02/154.

Thank you for your letter dated 18th October enclosing the amended patient information sheet and consent form for the above study.

I can now give this study officer approval.

Approval is given on the following understanding:

- Approval is granted for 3 years from the date of this letter. If you fail to start the research within this time you will have to re-apply for further approval.
- It is the responsibility of the investigator to notify the committee immediately of any information received by him/her or of which he/she becomes available, which would cast doubt upon or alter any information contained in the original application or a later amendment application, which would raise questions about the safety and/or continued conduct of the research.
- Patient information stored on computer must be handled in accordance with the Data Protection Act 1998 and local policies and procedures relating to the use of computer held data.
- All research must be conducted throughout according to good clinical research practice standards.
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- All research that is discontinued temporarily or permanently should be reported to the committee.

- The committee requests the researcher to provide details of the progress of the research at least annually and details of its conclusion and outcome.

- The meeting of the committee, which considered your application, was quorate according to the constitution of the committee.

- The membership of the committee is attached. It is against the policy of the committee to identify which members were present when your submission was approved.

Yours sincerely

Dr D Pearson
Chairman
Nottingham Ethics Committee 1

Trent Health Authority NHS

Please ask for:
Linda Ellis, Administrator
Ext: 49435. E-mail linda.ellis@rushcliffe-pct.nhs.uk

Nottingham Local Research Ethics Committee 1
1 Standard Court
Park Row
Nottingham
NG1 6GN

Tel: 0115 912 3344
Fax: 0115 912 3300

REC **C1090316**
(please quote this number on all correspondence)

31 October 2003

Dr C Whale
Clinical Research Fellow
Divison of Repiratory Medicine
Faculty of Medicine and Health Sciences
Clinical Sciences Building
NCH

Dear Dr Whale

Re: **A Study of the systemic, metabolic and bronchodilator dose-response effects of formoterol in chronic obstructive pulmonary disease**

The Chair of the Nottingham Research Ethics Committee 1 has considered the amendments submitted in response to the Committee's earlier review of your application on 14 October 2003 as set out in our letter dated 23 October 2003 . The documents considered were as follows:

. Application Form
. Protocol Version 2 dated October 2003
. Patient Information Sheet Version 2 dated October 2003
. Consent Form
. CV for Dr C Whale

The members of the Committee present agreed that there is no objection on ethical grounds to the proposed study. On behalf of the Committee I am, therefore, happy, to give full approval for this study on the understanding that you will follow the conditions set out below:

1. The Project must be started within three years of the date on which REC approval is given.

2. You must not start your project in any institution until you have received written approval from their R&D department. You should have submitted your original application to the R&D office and parallel reviews will have been taking place. Approval should therefore be imminent.

If your study is to take place in any of the following units then you do not need further ethical approval but you do need R&D approval.

- Queen's Medical Centre
- Nottingham City Hospital
- Nottingham Primary Care Trusts
- Mental Health Care Trust

If your study is to take place in units outside of Nottingham but still within the boundaries of the Strategic Health Authority, then you do not need further full ethical approval. You will however need your study approved by the R&D unit of the institution concerned and an assessment of 'locality issues.' These 'locality issues' (such as appropriate status of research aspects of local research subjects, information sheets) are usually addressed and reviewed by the local ethical committee and you should clarify this point with the administrator of your local REC. These reviews should take place quickly.

3. You must not deviate from, or make changes to, the protocol without prior written approval of the REC, except where this is necessary to eliminate immediate hazards to research participants or when change involves only logistical or administrative aspects of the research. In such cases the REC should be informed within seven days of the implementation of the change.

4. You complete and return the standard progress report form to the REC one-year from the date on this letter and thereafter on an annual basis. This form should also be used to notify the REC when your research is completed and in this case should be sent to this REC within three months of completion.

5. If you decide to terminate this research prematurely you send a report to this REC within 15 days, indicating the reason for the early termination.

6. You advice the REC of any unusual or unsuspected results that raise questions about the safety of the research.

Yours sincerely

Dr D Pearson/Mrs L Ellis
Chair/Administrator
Nottingham Research Ethics Committee 1

cc Research and Development

APPENDIX 2

Sample patient letter

Dear

We are trying to improve and develop the treatments available for patients who have Chronic Obstructive Pulmonary Disease (COPD), and we have designed a number of studies to try and improve our understanding of the treatment of this condition.
I am writing to you because it would appear that you would be ideally suited to participate in one of our studies. If you could spare the time we would be grateful if you could come to City Hospital to see our research fellow, Dr Chris Whale, who would tell you more about the study and explain what it involves.

Dr Whale will give you a call in the next few days to see if you would be interested. I have enclosed a copy of an information sheet about the study for your interest. We would be happy to arrange a taxi to pick you up and take you home to reduce any inconvenience.

Best Wishes

Yours sincerely,

Prof AE Tattersfield
Professor of Respiratory Medicine

Sample GP letter

Dear Dr

RE: Name:
Address:
DOB:

The above named patient has agreed to help with a research project we are carrying out here at City Hospital looking at the effects of bronchodilators on blood gases in patients admitted with an acute exacerbation of Chronic Obstructive Pumonary Disease. There is some evidence to suggest that high doses of nebulised salbutamol may have some detrimental effects on oxygen consumption and carbon dioxide production, and this is what we are interested in.

The study will take place on one morning as soon as possible after being admitted with an acute exacerbation of COPD. It will involve having five nebulised doses of Ventolin or Atrovent, given in a double blinded fashion. On completion of the study, the patient will revert to their normal treatment, and follow up will be arranged as normal.

If you have any further queries regarding the trial please do not hesitate to contact me on 0115 9691169 ext 34757.

Many thanks,

Dr Chris Whale
Clinical Research Fellow
Department of Respiratory Medicine
Nottingham University

Information Sheet for Adults

Determining the optimum dose of nebulised salbutamol in acute exacerbations of Chronic Obstructive Pulmonary Disease.

Professor AE Tattersfield, Dr TW Harrison, Dr CI Whale, Dr R Mahajan, Dr MP Sovani.

1. Invitation to take part in the study

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you want to take part.

2. What is the purpose of the study?

The overall aim of this work is to try and find out which dose of salbutamol (Ventolin) given by nebuliser to people with your condition is most helpful.

3. Why have I been chosen to take part in the study?

You have been admitted to hospital because you have an exacerbation or flare up of your lung problem, which is known as COPD (Chronic Obstructive Pulmonary Disease). In this situation, it is usual to give certain drugs by nebuliser to help the airways open up. The drug that is usually given is called salbutamol (Ventolin), often with another drug called ipratropium (Atrovent). It is common to give quite a high dose of the nebulised Ventolin, although there is some evidence that patients like you might be better on a lower dose. We are carrying out a study therefore to try and find out which dose of Ventolin is best for patients in your condition. We are asking whether you would consider helping us with this study.

4. Do I have to take part?

This is a research study and you do not have to take part. Whether you take part or not is voluntary. You may refuse to take part or stop at any time. This would not affect the care you would receive in any way. You should read all the information before deciding whether you wish to take part.

5. What will happen to me if I take part?

If you agree to take part in the study it will take place over six hours on one morning. All your treatment would continue as usual, apart from your nebulised drugs (Ventolin, and Atrovent if it has been prescribed); these will be stopped at 10 pm on the day before. On the morning of the study day we will give you a series of nebulisers,

every hour from 8am until Noon. The nebuliser would deliver either Ventolin, in slowly increasing doses similar to the ones you would normally receive, or repeated small doses of Atrovent.
We will measure the oxygen and carbon dioxide levels in the blood before and for one hour following each of the nebulisers. So that we can do this accurately and don't have to do repeat blood tests, we would put a cannula into a small artery in the wrist. This would be inserted in the morning and would stay in place until around 1pm. Insertion of the cannula may be a little uncomfortable, but we will use some local anaesthetic to reduce any pain. After a nebuliser we will take 10 samples of blood over the course of each hour. Each sample will be a small volume, so that during the study we will take 125 mL of blood in total. The cannula will be attached to a monitor that will measure your heart rate. We will ask you to breath oxygen through a mask and rest on the bed during the study period.
We will ask if you have noticed any side effects from the nebuliser and we will also ask you to mark a scale to say how breathless you are at intervals before and after each of the nebulisers.

6. What do I have to do?

If you consent to take part in the study we would probably do the study tomorrow. A cannula will be inserted into the wrist at 7.30am, and over the course of the day blood samples will be taken from it. Your nebuliser treatment will be given every hour from 8am until noon. You will be asked to breathe oxygen through a mask during the study period. All other drugs, except those given by nebuliser, will be given as normal. Once the study has ended, you will return to your normal medication.
We will arrange for a light breakfast at 7am and then ask you to abstain from eating and drinking (apart from water) during the study.

7. What is the drug that is being tested?

The main study drug is called salbutamol (Ventolin). It is routinely used worldwide for the treatment of COPD, at the same doses as you will receive. It is a drug that aims to open up your airways since you are short of breath.
The other drug ipratropium bromide (Atrovent) is also used routinely throughout the world. It is another drug to open up the airways, but it works in a different way to Ventolin.

8. Are there other ways of treating my condition?

Treatment options are quite limited in this condition, but this study will not prevent you receiving the other medications that are available such as antibiotics.

9. What are the side effects of the study treatment?

Ventolin can cause some twitchiness and palpitations and we will be asking you about these during the study. These usually settle gradually over half an hour to an hour. Atrovent may cause some dryness of the mouth.

10. What are the possible disadvantages and risks of taking part?

Salbutamol has been shown to be a very safe drug. If, however, you experience unpleasant side effects at any point you may wish to stop participating in the study.
We would not normally insert an arterial cannula in your wrist although they are used in patients with severe breathing problems to allow oxygen and carbon dioxide levels to be monitored closely. Complications from an arterial cannula are rare although infections, bleeding and obstruction of the artery have occurred very rarely in ill patients requiring the cannula to be in place for many days. Since the cannula will only remain in place for 6 hours, we do not expect any complications. The cannula will be inserted by an experienced doctor who will monitor it carefully during the study and take measures to minimise any potential risk of complications. It is possible, but unlikely, that a further cannula may be needed if there is a problem with the first one.

11. What are the possible benefits of taking part?

It is unlikely that taking part in this study will directly help your medical condition. The information we get from this study may help us improve future treatment for patients with your condition.

12. What if new information becomes available?

Since Ventolin has been used very widely for a long time it is unlikely that new information will become available during the course of the study. Should new information become available, the study doctor will tell you about it, and will discuss whether you wish to remain in the study.
If the doctor decides that it is in your best interests to withdraw you from the study they will explain the reasons for this and arrange for your care to continue.

13. What happens when the research study stops?

At the end of the study period around 1pm, you will return to your normal medications, which is likely to include Ventolin and Atrovent.

14. What if something goes wrong?

You should inform the study doctor of any side effects you experience. You may withdraw from the study at any time you wish. Your medical care will not be affected if you do this. Your doctor

will withdraw you from the studies if they feel it is in your best interest.
If taking part in the study harms you, there are no special compensation arrangements. If you are harmed because someone has made a mistake, then you may be able to take legal action but you may have to pay for it. If you want to complain about the way you have been approached or treated during this study, the normal National Health Service complaints mechanisms may be available to you.

15. Will my taking part in this study be kept confidential?

All information collected about you will be kept strictly confidential. Any information from the study will have your name removed so you cannot be identified and your name will not be disclosed outside the hospital or appear in any study results unless we are obliged to do so by law.
Your GP will be told that you have taken part in the study.

16. What will happen to the results of the research study?

Findings will be published in a medical research journal. Of course, this would be anonymous.

17. Who is organising and funding the research?

This is being organised and funded through Nottingham University Division of Respiratory Medicine.

18. Who has reviewed this study?

Two independent doctors have reviewed this study.

19. Contact for further information?

If you would like further information, please contact the following person:
Name: Dr Chris Whale
Telephone Number: 0115 9691169 ext 34757

20. What do I do now?

If there are any questions, or there is anything you do not understand, then please ask the study doctor. Please take your time to decide if you want to take part. You are free to discuss the study with your family or friends before you make your decision.
If all your questions have been answered and you agree to take part in the study, you will be asked to sign a consent form. You will be given a copy of this information and a copy of the consent form for you to keep.

If you agree to take part, you can still change your mind at any time, even if you have started the study.

We would like to thank you for taking the time to read this information.

Patient Information Sheet

Study to determine the effect of high dose nebulised salbutamol on stable hypercapnic chronic obstructive pulmonary disease patients.

Professor AE Tattersfield, Dt TW Harrison, Dr CI Whale, Dr R Mahajan, Dr MP Sovani

1. Invitation to take part in the study

You are being invited to take part in a research study. Before you make a decision it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you want to take part.

2. What is the purpose of the study?

Breathlessness is a major problem for people with Chronic Obstructive Pulmonary Disease (COPD) and it can lead to an increase in carbon dioxide levels in the blood. Nebulised drugs are given to try to open up the airways and the most commonly administered drug, salbutamol (Ventolin), is often given at high doses, although there is some evidence that patients like you might be better on a lower dose. We are carrying out a study to determine whether high or low doses of Ventolin are better for patients with COPD. We are asking whether you would consider helping us with this study which will involve visiting the hospital for about 3 hours on two mornings.

3. Why have I been chosen to take part in the study?

We aim to study 14 people, like yourself, who are known to have COPD and who have some increase in their blood carbon dioxide levels.

4. Do I have to take part?

This is a research study and you do not have to take part. Whether you take part or not is voluntary. You may refuse to take part or withdraw at any time. This would not affect the care you would receive in any way. You should read all the information before deciding whether you wish to take part.

5. What will happen to me if I take part?

If you agree to take part in the study it will take place over two three-hour periods, on separate mornings, within a few days of each other.
On each study day, after a light breakfast at home and before you have taken any of your morning inhalers or nebulisers, we will arrange for a taxi to bring you to hospital.

After a short rest, we will attach leads to your chest and place aprobe on your fingertip, to measure your heart rate and oxygen levels. We will ask you to remain on bed rest during each study day. We will then place a small cannula into an artery in the wrist to measure oxygen and carbon dioxide levels more accurately. This avoids us having to use a needle each time further samples are required. The cannula stays in place for around three hours and although insertion of the cannula may be a little uncomfortable, we will use local anaesthetic to reduce any pain.
When the blood tests are stable, we will give you two doses of nebulised Ventolin an hour apart, taking 6 blood samples over the course of the hour following each drug. Each sample will be a small volume, so that on each study day we will take about an egg cup full of blood (a maximum of 50 mL). The cannula will be connected to a monitor to allow measurement of blood pressure throughout the study. On one study day you will receive two low doses of salbutamol and on the other day two higher doses. Neither you, nor the doctor, will know which dose you are receiving on a particular day. During the study we will ask you whether you have experienced any side effects and we will ask you to rate your degree of breathlessness using a simple scale. The cannula will be removed about two and a half to three hours after it was inserted, and we will arrange a taxi to take you home.

6. What do I have to do?

If you consent to take part in the study we would arrange for the two visits to take place on convenient days.
For each study day we will ask you to avoid caffeine-containing food and drinks from midnight, and avoid taking certain inhalers and nebulisers prior to each visit. On the study day, you can have a light breakfast at around 8am before coming to the hospital and we will then ask you to abstain from eating and drinking (apart from water) during the study. You will receive two doses of nebulised medication, an hour apart and all your other drugs, except for those that open up the airways, will be taken as normal. When the study has ended, you will continue your normal medication.

7. What is the drug that is being tested?

Salbutamol (Ventolin) is routinely used worldwide for the treatment of COPD at the same doses that you will receive in the study. It is a drug that aims to prevent breathlessness and open up your airways when you are short of breath.
On one study day you will receive the higher doses of salbutamol twice, and on the other day you will receive the low dose twice.

8. What are the side effects of the study treatment?

Ventolin can cause some twitchiness and palpitations and we will be asking you about these during the study. These usually settle gradually over half an hour to an hour and many people are not troubled at all.

9. What are the possible disadvantages and risks of taking part?

Salbutamol has been shown to be a very safe drug. If, however, you experience unpleasant side effects at any point you may wish to stop participating in the study. Arterial cannulae are used in patients with severe breathing problems to allow oxygen and carbon dioxide levels to be monitored closely. Complications from an arterial cannula are rare, although infections, bleeding and obstruction of the artery have occurred in seriously ill patients who required the cannula to be in place for many days. Since the cannula will only remain in place for 3 hours, we do not expect any complications. The cannula will be inserted by an experienced doctor, who will monitor it carefully during the study, taking measures to minimise the risk of complications. It is possible, but unlikely, that a further cannula may be needed if there is a problem with the first one.

10. What are the possible benefits of taking part?

The study may not be of direct benefit to you. It is unlikely that taking part in this study will directly help your medical condition. The information we get from this study may help to improve future treatment for patients with COPD.

11. What if new information becomes available?

Since Ventolin has been widely used for a long time it is unlikely that new information will become available during the course of the study.

12. What happens when the research study stops?

At the end of the study period and between the two study days you will return to your normal medications.

13. What if something goes wrong?

If you want to complain about the way you have been approached or treated during this study, the normal National Health Service complaints mechanism is available to you.
You should inform the study doctor of any side effects you experience. You may withdraw from the study at any time you wish and your medical care will not be affected. Your doctor will withdraw you from the studies if they feel it is in your best interest.
If taking part in the study harms you, there are no special compensation arrangements. If you are harmed because someone has made a mistake, then you can take legal action but you may have to pay for it.

14. Will my taking part in this study be kept comfidential?

All information collected about you will be kept strictly confidential. Any information from the study will have your name removed so you cannot be identified and your name will not be disclosed outside the hospital or appear in any study results unless we are obliged to do so by law.
Your GP will be told that you have taken part in the study.

15. What will happen to the results of the research study?

Study results will be published in a medical research journal without mentioning the names of participants.

16. Who is organising and funding the research?

This is being organised and funded through Nottingham University Division of Respirtory Medicine.

17. Who has reviewed this study?

Two independent doctors have reviewed this study.

18. Contact for further information?

If you would like further information, please contact the following person:
Name: Dr Chris Whale
Telephone Number: 0115 840 4757

19. What do I do now?

If there are any questions, or there is anything you do not understand, then please ask the study doctor. Please take your time to decide if you want to take part. You are free to discuss the study with your family or friends before you make your decision.

If all your questionshave been answered and you agree to take part in the study, you will be asked to sign a consent form. You will be given a copy of this information sheet and a copy of the consent form to keep.

If you agree to take part, you can still change your mind at any time,even if you have started the study.

We would like to thank you for taking the time to read this information.

PATIENT INFORMATION SHEET

The effect of different doses of formoterol in patients with COPD

(A study of the systemic, metabolic and bronchodilator dose-response effects of inhaled formoterol in chronic obstructive pulmonary disease.)

1. Invitation paragraph

We are inviting you to take part in a research study. Before you decide whether to take part or not, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

2. What is the purpose of the study?

The aim is to assess the effects of four different doses of formoterol, a drug used to open up the airways of patients with COPD. We aim to determine which dose of formoterol provides the best balance between beneficial and adverse effects. We will do this by monitoring the general effects of the drug, and how it affects your lung function.

3. Why have I been chosen?

You have been chosen because you have chronic obstructive pulmonary disease. We aim to study 20 patients in total.

4. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the care you receive in any way.

5. What will happen to me if I take part?

The study will take place on five separate mornings over two weeks. On each morning we will arrange for a taxi to pick you up and bring you to Nottingham City Hospital for 8am. You can have a light breakfast before attending if you wish.
Each morning will be similar, and will start with a 20-minute rest. We will then check your lung function (simple blowing tests), take a small blood sample (2ml), check your heart rate and blood pressure, measure any tremor and take a reading of your oxygen level with a fingertip probe. We will then measure the distance you can walk.
After a further 20-minute rest period, we will ask you to inhale a dose of formoterol or placebo from a device called a Turbohaler. You will take four inhalations from designated inhalers, and they will give you placebo (a dummy drug with no active ingredients) or one of the four doses of formoterol (6, 12, 24 or 48μg). To reduce bias, you will not know which dose you will receive on a given day. The schedule for doses will be determined by computer.
We will then ask you to rest on a comfortable chair, and will take measurements at set intervals. At 30, 60, 120, 180 and 240 minutes after inhaling the drug, we will repeat the measurements of lung function, tremor, heart rate and oxygen saturation. Also after 240 minutes, we will ask how you feel, then repeat the blood test and six-minute walk test.

Once this is done, the study day is complete and we will arrange for a taxi to take you home, at around 1pm.

6. What do I have to do?

The study would not interfere with your daily life. However, we would ask you to limit consumption of tea or coffee to one cup only prior to attending on the morning of each visit. The study will not affect your regular medications, although we may require you to abstain from taking some of your normal inhalers prior to attending on each of the five mornings.

7. What is the drug or procedure that is being tested?

Inhaled formoterol is widely used to open airways in patients with asthma and COPD.
We aim to assess the effects of different doses of formoterol in COPD via a Turbohaler. We plan to assess the effect of different doses of formoterol on your heart rate, breathing tests, oxygen levels, tremor and walking distance.
We will ask you to inhale a dose of formoterol or placebo (dummy drug) on each of the five study days. For the study period (less than 2 weeks), you will be given a small card (like a credit card) with details of the trial, which you will be asked to carry at all times.

8. What are the alternatives for diagnosis or treatment?

The study will not interfere with other medications you need to take.

9. What are the side effects of taking part?

Formoterol is a β-agonist like Ventolin but with a longer action. It can cause certain recognised side effects, the main ones are tremor and more occasionally palpitations, muscle cramps and headaches.

10. What are the possible disadvantages and risks of taking part?

Formoterol is considered to be a safe drug, and the side effects (tremor, fast heart rate, muscle cramps and headaches) are well recognised. We will monitor these effects at intervals on each study day and ask you to tell us if you experience any discomfort during the visits. The highest dose (48μg) of formoterol may cause some of these side effects to be more noticeable than you have experienced before with your Ventolin inhaler.
We will take two blood samples on each study day, which may be a little uncomfortable and you will experience some breathlessness during the walk tests.
A doctor will be present throughout each visit.

11. What are the possible benefits of taking part?

This study is not intended to provide benefit to individual patients.

The information we get from this study may help us to treat future patients with COPD better.

12. What if the new information becomes available?

Sometimes during the course of a research project, new information becomes available about the drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

13. What happens when the research study stops?

The drug may be available to you after completing the study, at the discretion of your consultant and general practitioner.

14. What if something goes wrong?

If you are harmed in any way by taking part in this research project, there are no special compensation arrangements. If you are harmed due to individual negligence, then you may have grounds for a legal action but you may have to pay for it.

If you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

15. Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

If you agree, your own GP will be notified of your participation in the trial.

16. What will happen to the results of the research study?

We would aim to publish the results anonymously in a respiratory medicine journal.
We will let you know the findings once the study is completed.

17. Who is organising and funding the research?

University of Nottingham, Division of Respiratory Medicine.

19. Contact for further information

Dr Chris Whale
0115 8404757
chris.whale@nottingham.ac.uk

Many thanks for taking the time to read this information sheet

APPENDIX 3

Modified Borg scale for perceived dyspnoea

Scale	Severity
0	No breathlessness at all
0.5	Very very slight (just noticeable)
1	Very slight
2	Slight breathlessness
3	Moderate
4	Somewhat severe
5	Severe breathlessness
6	
7	Very severe breathlessness
8	
9	Very very severe (almost maximum)
10	Maximum

Protocol for six minute walk test

"The object of this test is to walk as far as possible for six minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able.

You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you. Please watch the way I turn without hesitation."

Walk one lap. Walk and pivot around a cone briskly.

"Are you ready to do that? I am going to use this borad to keep ttack of the number of laps you complete. I will tick it each time you turn around at this starting line.

Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but dobn't run or jog.

Start now, or whenever you are ready."

1st minute "You are doing well. You have 5 minutes to go."

2nd minute "Keep up the good work. You have 4 minutes to go."

3rd minute "You are doing well. You are halfway done."

4th minute "Keep up the good work. You only have 2 minutes left."

5th minute "You are doing well. You only have 1 minute to go."

5.45 "In a moment I'm going to tell you to stop. When I do, just stop right where you are and I will come to you."

6th minute "Stop."

Walk over to the patient. Take a chair if needed. Mark the spot on the floor.

Congratulate and offer a glass of water.

APPENDIX 4

Raw data from Chapter five

Effects of salbutamol on arterial blood gases in patients with stable hypercapnic Chronic Obstructive Pulmonary Disease

	Time (minutes)												
	0	10	15	20	30	45	60	70	75	80	90	105	120
1	5.31	5.29	5.32	5.32	6.02	5.91	5.95	5.46	5.84	5.7	5.87	6.05	6.11
2	7.58	7.27	7.4	7.3	7.26	7.01	6.75	7.08	7	7.05	6.97	6.69	7.18
3	7.93	8.7	8.04	7.88	8.11	8.21	8.08	8.34	8.08	7.99	7.86	8.19	8.02
4	6.45	6.36	6.49	6.39	6.59	6.12	6.36	6.33	6.29	6.47	6.31	6.24	6.39
5	8.57	8.59	8.12	8.12	7.4	7.4	7.24	7.63	7.61	7.23	7.04	8.11	7.54
6	8.74	8.47	8.05	8.48	8.64	7.99	8.37	8.28	8.75	8.02	8.03	7.91	8.68
7	9.04	8.4	8.13	8.3	7.91	8.1	8.01	8.08	7.95	7.92	8.02	8.13	7.92
8	7.96	7.42	7.58	7.21	7.27	7.86	7.3	7.62	7.56	7.26	7.77	7.24	7.31
9	10.05	9.74	10.52	9.32	9	9.01	9.34	9.25	9.72	9.8	9.34	9.07	9.42
10	6.25	5.98	5.94	5.99	5.96	6.12	5.87	5.92	5.93	5.78	5.85	5.69	5.66
11	6.03	7.26	6.26	6.41	6.11	6.39	6.35	6.51	6.39	6.51	6.22	5.81	5.75
12	6.75	6.42	6.79	6.79	6.6	6.76	6.55	6.25	6.23	6.65	6.68	6.03	6.1
13	8.07	7.29	7.14	6.94	6.88	6.99	6.6	6.9	7.18	7.24	7.08	7.18	7.18
14	8.3	8.41	8.23	8.19	8.11	7.96	8.03	8.08	8.02	8.05	8.25	8.12	8.26

Change in $PaCO_2$ (kPa) from baseline to 120 minutes after 10 mg salbutamol.

	Time (minutes)												
	0	10	15	20	30	45	60	70	75	80	90	105	120
1	9.4	9.49	8.69	7.58	8.27	9.53	7.86	9.19	7.8	8.87	8.58	7.92	7.93
2	8.17	8.42	7.97	7.95	7.69	7.83	7.89	7.9	7.67	7.53	7.31	7.62	7.46
3	7.18	6.56	6.94	6.93	7.06	6.96	7.12	6.91	6.8	6.81	6.67	6.64	6.8
4	7.76	7.85	7.77	7.76	7.59	7.86	7.9	7.89	7.81	7.74	7.56	7.85	7.82
5	7.91	7.74	7.58	7.42	7.56	7.47	7.9	7.5	7.55	7.47	7.52	7.19	7.32
6	5.59	5.72	5.46	5.42	5.52	5.63	5.48	5.29	5.31	5.2	5.33	5.15	5.68
7	7.71	7.98	7.12	7.07	7.31	7.25	7.22	7.19	7.2	7.24	6.92	7.05	7.22
8	7.97	7.82	7.84	7.83	7.92	7.75	7.72	7.53	7.4	7.6	7.31	7.37	7.15
9	5.82	5.75	5.43	5.71	5.76	5.71	5.82	5.54	5.49	5.49	5.42	5.55	5.58
10	8.1	8.7	8.55	8.57	7.87	7.68	7.83	7.49	7.63	7.56	7.29	7.55	7.3
11	6.97	7.06	7.07	7.06	7.31	7.08	7.29	7.25	7.11	7.11	6.97	6.9	6.97
12	8.3	8.36	8.24	8.17	8.19	8.07	8.02	8.1	8	7.83	7.61	8	7.7
13	6.81	6.49	6.48	6.35	6.42	6.18	6.3	6.4	6.24	6.15	6.07	6.04	6.01
14	6.69	6.66	6.52	6.53	6.74	6.63	6.79	6.5	6.62	6.46	6.74	6.54	6.55

Change in PaO_2 (kPa) from baseline to 120 minutes after 10 mg salbutamol

	Time (minutes)												
	0	10	15	20	30	45	60	70	75	80	90	105	120
1	75	79	74	73	72	75	72	77	74	74	73	75	72
2	106	106	103	105	108	106	110	105	108	110	110	110	110
3	87.5	84	91	84	85	88	88	90	93	92	90	91	91
4	76.5	79	77	79	78	84	78	79	80	81	81	82	85
5	65.5	72	68	69	71	73	77	77	81	8	85	83	84
6	74	76	78	78	78	76	75	80	78	76	81	83	78
7	80.5	93	94	84	85	86	94	95	93	95	95	93	94
8	65	66	63	64	64	70	64	74	67	70	68	70	73
9	109	109	110	108	112	110	113	109	113	113	112	113	113
10	87	90	89	89	88	90	90	92	90	90	91	93	82
11	93	93	93	91	95	98	95	91	90	94	92	97	97
12	80.5	80	81	78	78	79	77	78	77	78	81	85	81
13	70	74	75	75	79	79	80	81	82	82	86	83	87
14	91	91	91	89	90	92	90	90	90	91	93	93	96

Change in heart rate (bpm) from baseline to 120 minutes after 10 mg salbutamol

Raw data from Chapter six

Systemic and bronchodilator effects of formoterol in Chronic Obstructive Pulmonary Disease: a dose response study

	Time (minutes)					
Dose (mcg)	0	30	60	120	180	240
0	0.86	0.79	0.7	0.69	0.58	0.65
0	1.2	1.18	1.15	1.3	1.22	1.17
0	1.04	1.2	1.04	1.1	1.06	1.18
0	1.2	1.27	1.36	1.1	1.21	1.03
0	0.92	0.84	0.91	0.82	0.87	0.82
0	0.64	0.52	0.5	0.44	0.5	0.52
0	0.54	0.57	0.51	0.5	0.52	0.5
0	0.98	0.96	0.89	0.86	0.84	0.75
0	0.74	0.71	0.71	0.69	0.68	0.68
0	0.75	0.79	0.76	0.75	0.83	0.9
0	0.97	0.95	1.01	0.98	1.03	0.93
0	1.76	1.49	1.6	1.3	1.39	1.15
0	0.42	0.55	0.71	0.71	0.7	0.7
0	2.52	2.63	2.6	2.7	2.67	2.58
0	1.01	1.02	1.09	1.12	1.12	1.15
0	0.6	0.54	0.48	0.42	0.39	0.35
0	1.16	1.31	1.3	1.24	1.33	1.18
0	0.7	0.82	0.74	0.8	0.78	0.72
0	1.94	1.93	1.95	1.9	1.94	1.94
0	1.9	1.7	1.84	1.66	1.6	1.62
6	0.78	0.84	0.79	0.75	0.66	0.61
6	1.21	1.05	1.2	1.33	1.25	1.25
6	1.06	1.24	1.3	1.32	1.23	1.1
6	0.98	1.31	1.3	1.31	1.25	1
6	0.82	0.9	0.88	0.89	0.85	0.75
6	0.72	0.68	0.7	0.65	0.65	0.61
6	0.61	0.59	0.66	0.67	0.67	0.65
6	0.87	0.9	0.93	0.95	0.99	0.85
6	0.75	0.73	0.75	0.71	0.74	0.72
6	0.82	0.77	0.87	1	0.77	1.01
6	1.15	1.15	1.2	0.93	1.17	1.17
6	1.35	1.75	2	1.78	2.02	1.98
6	0.51	0.74	0.8	0.69	0.75	0.7
6	2.51	2.72	2.7	2.77	2.7	2.74
6	1.03	1.14	1.1	1.14	1.1	1.23
6	0.73	0.64	0.7	0.66	0.6	0.55
6	1.32	1.29	1.2	1.58	1.42	1.35
6	0.77	0.86	0.8	0.83	0.84	0.83
6	2.06	2.2	2.17	2.12	2.01	2.19
6	1.62	1.69	1.66	1.78	1.65	1.72
12	0.98	0.8	0.93	0.82	0.81	0.67
12	1.03	1.15	1.21	1.15	1.23	1.27
12	1.2	1.5	1.55	1.42	1.5	1.43
12	1.65	1.69	1.48	1.65	1.35	1.01
12	0.92	0.93	0.85	0.92	0.84	0.84
12	0.62	0.61	0.61	0.6	0.61	0.62
12	0.67	0.6	0.61	0.58	0.67	0.55
12	1.07	1.05	1.1	1.13	1.2	1.01

12	0.74	0.75	0.74	0.76	0.8	0.75
12	0.83	1	1.02	1.02	1.05	0.95
12	1.17	1.34	1.36	1.33	1.29	1.21
12	1.25	1.19	1.12	1.68	1.2	1.15
12	0.52	0.93	1	0.95	0.81	1.02
12	2.5	2.62	2.68	2.69	2.69	2.61
12	1.04	1.2	1.26	1.25	1.29	1.29
12	0.7	0.57	0.66	0.6	0.57	0.54
12	1.2	1.44	1.3	1.43	1.42	1.49
12	0.84	0.9	0.93	0.9	0.93	0.79
12	2.04	2.09	2.12	2.14	2.09	2.09
12	1.7	1.85	1.88	1.85	1.57	1.7
24	0.97	0.95	0.96	0.88	0.78	0.71
24	0.83	1	1.21	1.2	1.23	1.2
24	1.29	1.56	1.53	1.4	1.26	1.42
24	1.28	1.38	1.4	1.33	1.3	1.1
24	0.87	0.95	0.8	0.85	0.76	0.65
24	0.56	0.64	0.62	0.65	0.6	0.59
24	0.62	0.6	0.65	0.67	0.57	0.64
24	0.78	0.88	0.9	0.86	0.96	0.8
24	0.76	0.85	0.87	0.8	0.89	0.83
24	0.88	1.21	1.03	0.99	1.08	1.14
24	1	1.18	1.11	1.2	1.18	1.05
24	1.54	1.87	1.86	1.42	1.46	1.1
24	0.58	1.1	1.09	1.23	0.93	1.01
24	2.49	2.69	2.68	2.75	2.8	2.73
24	1.1	1.15	1.2	1.19	1.32	1.21
24	0.65	0.75	0.71	0.66	0.64	0.58
24	1.21	1.53	1.64	1.68	1.64	1.53
24	0.79	0.94	0.92	0.91	0.8	0.87
24	2.01	2.15	2.15	2.14	2.15	2.11
24	1.47	1.94	1.95	2.01	2.09	1.81
48	0.8	0.95	1.05	1.05	0.92	0.73
48	1.12	1.14	1.27	1.25	1.21	1.37
48	1.11	1.42	1.42	1.43	1.33	1.21
48	1.51	1.56	1.65	1.54	1.36	1.4
48	0.77	0.91	0.9	0.92	0.93	0.82
48	0.66	0.65	0.71	0.69	0.72	0.7
48	0.72	0.81	0.8	0.65	0.76	0.71
48	1	1.01	1.11	1.15	1.07	1.12
48	0.93	0.89	0.93	0.93	0.91	0.93
48	0.82	0.99	1.2	1.08	1.07	1.11
48	1.11	1.23	1.45	1.53	1.42	1.48
48	1.52	1.62	1.52	1.61	1.71	1.7
48	0.46	0.95	1.12	1.14	0.94	1.15
48	2.64	2.77	2.8	2.85	2.89	2.86
48	0.98	1.14	1.26	1.27	1.3	1.25
48	0.6	0.7	0.75	0.66	0.65	0.64
48	1.04	1.02	1.45	1.24	1.38	1.35
48	1.01	1.03	1.08	0.96	1	0.92
48	2.12	2.17	2.25	2.25	2.12	2.12
48	1.67	2.08	2.01	2.14	2.06	2.06

Change in FEV_1 (L) over 240 minutes after a range of doses of inhaled formoterol

	Time (minutes)					
Dose (mcg)						
0	77	75	80	79	80	88
0	74	76	72	80	69	73
0	80	74	76	76	86	80
0	75	73	74	74	71	75
0	65	61	64	65	68	70
0	77	76	71	68	72	66
0	82	76	75	77	76	76
0	60	68	59	56	65	62
0	75	75	73	72	80	77
0	75	83	87	87	85	70
0	70	75	70	61	70	76
0	59	61	60	58	61	60
0	75	69	68	69	64	66
0	84	86	86	85	82	80
0	65	67	60	58	61	58
0	82	84	84	76	77	80
0	63	58	56	54	55	53
0	58	59	54	57	62	70
0	93	87	82	82	78	82
0	80	65	60	62	61	72
6	74	72	72	71	72	74
6	77	79	73	79	73	70
6	72	76	78	90	82	82
6	80	76	77	76	75	87
6	62	64	64	66	67	71
6	64	65	62	64	67	66
6	80	73	72	72	72	76
6	64	65	58	62	62	66
6	72	70	72	80	83	81
6	74	76	80	68	83	83
6	70	71	76	69	76	73
6	59	62	66	59	62	61
6	72	65	66	67	61	63
6	88	85	89	90	88	84
6	73	65	66	73	61	63
6	83	85	80	76	79	75
6	62	55	58	53	53	55
6	60	59	58	68	66	64
6	91	91	104	84	74	85
6	72	67	66	74	63	64
12	73	70	72	76	72	75
12	70	81	78	69	66	70
12	79	79	79	85	78	94
12	78	75	73	76	77	73
12	61	62	67	66	72	76
12	64	64	63	62	67	58
12	86	78	77	79	84	85
12	61	60	58	57	59	57
12	65	71	76	72	75	75
12	71	75	71	77	70	75
12	70	72	70	77	67	75

12	60	60	62	59	64	60
12	72	65	65	68	64	64
12	87	84	80	76	81	76
12	70	72	72	69	64	71
12	84	82	82	84	82	77
12	60	56	56	54	53	53
12	57	57	56	56	58	64
12	86	87	81	79	82	86
12	68	61	64	64	66	63
24	82	79	73	78	79	79
24	79	80	77	68	70	67
24	89	85	84	85	83	82
24	73	71	76	71	74	79
24	70	68	65	65	68	71
24	64	71	63	89	63	70
24	82	81	75	73	75	75
24	58	63	64	60	64	55
24	75	68	75	77	69	75
24	74	77	72	70	79	75
24	70	66	72	79	75	71
24	62	62	64	62	60	64
24	71	71	66	66	63	67
24	85	76	81	77	78	84
24	62	68	70	64	65	63
24	78	81	77	79	78	76
24	58	56	61	56	53	55
24	62	56	56	60	60	56
24	95	91	92	96	81	82
24	66	66	63	60	62	65
48	75	81	82	83	80	85
48	75	79	76	72	72	65
48	89	85	79	86	82	81
48	76	78	74	75	75	78
48	62	64	66	66	70	72
48	64	63	70	81	81	84
48	74	72	74	73	74	71
48	59	61	61	63	59	59
48	82	82	80	79	80	80
48	64	79	85	82	85	84
48	68	67	70	73	67	67
48	60	59	64	66	66	62
48	75	72	70	76	75	71
48	85	93	89	93	92	87
48	67	70	65	63	68	64
48	82	82	83	75	75	71
48	65	61	58	59	59	57
48	71	60	63	66	60	60
48	90	94	85	84	82	80
48	78	66	64	62	64	67

Change in heart rate (bpm) over 240 minutes after a range of doses of inhaled formoterol

	Time (minutes)					
Dose (mcg)						
0	92	94	92	92	94	92
0	94	96	94	96	96	95
0	93	96	96	92	95	93
0	99	98	98	99	97	98
0	94	92	95	94	93	92
0	93	92	92	92	93	91
0	94	93	93	95	94	94
0	95	95	96	95	95	96
0	96	96	96	97	97	96
0	96	95	95	93	94	94
0	96	96	95	95	95	95
0	94	93	94	95	95	95
0	95	95	95	95	96	96
0	95	95	97	97	97	96
0	97	96	97	97	98	98
0	96	96	96	96	97	96
0	95	94	94	95	94	95
0	97	97	97	95	96	96
0	95	94	94	96	97	94
0	93	95	93	95	94	96
6	93	93	94	93	94	94
6	94	91	95	95	95	95
6	93	94	96	99	91	94
6	97	97	97	98	96	97
6	94	94	95	95	94	91
6	93	91	92	94	94	90
6	95	95	94	95	94	95
6	95	93	94	94	95	95
6	96	97	95	96	95	95
6	97	96	97	96	96	95
6	94	96	95	94	95	95
6	94	93	94	95	94	95
6	95	95	96	97	96	96
6	96	95	97	97	96	96
6	95	97	97	97	97	98
6	94	95	95	95	93	95
6	95	94	95	95	94	95
6	95	96	96	96	96	96
6	96	95	95	95	95	95
6	95	95	93	95	97	96
12	93	93	93	93	93	93
12	94	92	94	94	93	92
12	93	96	92	95	92	92
12	95	95	94	95	96	95
12	89	92	94	94	93	91
12	92	91	93	92	93	94
12	94	94	92	93	94	94
12	96	96	93	94	95	96
12	97	96	95	96	96	97
12	95	95	94	96	95	95
12	96	95	96	95	96	96

12	95	94	95	96	95	94
12	96	96	96	97	99	97
12	96	97	96	97	97	96
12	96	97	95	96	97	96
12	95	92	94	94	94	93
12	95	94	95	94	95	96
12	97	96	96	96	96	97
12	95	94	95	96	94	95
12	95	96	95	97	96	96
24	93	94	94	95	94	94
24	93	94	93	92	92	93
24	92	96	95	92	93	95
24	98	98	97	98	98	97
24	95	96	95	95	94	94
24	93	90	90	92	91	94
24	94	92	93	94	94	95
24	96	93	94	95	95	95
24	97	95	94	97	95	96
24	96	96	94	95	94	95
24	95	95	94	95	94	95
24	95	93	94	95	94	94
24	96	97	95	96	97	97
24	94	95	96	98	97	97
24	96	96	95	97	95	95
24	96	95	93	94	93	93
24	94	92	93	93	95	95
24	97	96	96	97	96	97
24	95	95	94	96	96	95
24	96	97	95	96	95	96
48	91	92	90	93	94	94
48	93	91	93	94	93	94
48	91	91	93	93	93	96
48	97	95	94	96	96	97
48	94	93	95	93	94	94
48	94	92	90	93	94	94
48	94	92	93	92	94	94
48	95	92	94	94	95	95
48	97	94	96	95	96	96
48	96	94	95	96	96	94
48	96	96	96	97	95	95
48	92	93	94	95	94	95
48	96	96	96	96	96	96
48	94	93	95	95	96	97
48	94	94	95	96	98	96
48	93	97	97	96	96	95
48	95	93	93	94	94	93
48	96	96	95	97	96	95
48	95	94	96	96	95	95
48	94	93	93	95	97	95

Change in SpO_2 (%) over 240 minutes after a range of doses of inhaled formoterol

	Time (minutes)					
Dose (mcg)						
0	36	14	12	8	13	80
0	13	13	7	10	12	6
0	0	0	0	0	0	0
0	2	1	2	2	2	4
0	7	9	7	8	9	17
0	7	7	7	10	9	7
0	14	9	9	10	19	19
0	1	2	29	38	36	49
0	1	1	1	1	1	1
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	17	17	8	10	10	9
0	8	6	2	2	1	1
0	4	6	4	3	2	3
0	0	0	0	0	0	0
0	30	34	43	37	10	14
0	24	8	9	11	8	6
0	4	3	7	0	0	0
0	0	1	0	2	2	0
0	3	2	2	2	3	3
6	15	12	15	12	11	14
6	15	14	11	12	16	16
6	0	0	0	0	0	0
6	5	5	4	3	3	4
6	6	8	12	10	10	12
6	11	12	8	10	8	8
6	11	5	7	4	6	4
6	17	28	7	8	4	11
6	1	2	1	1	1	1
6	0	0	0	0	0	0
6	0	0	0	0	0	0
6	2	3	2	2	2	3
6	8	4	5	4	1	2
6	3	4	2	2	2	3
6	0	0	0	0	0	0
6	33	36	23	38	28	29
6	12	12	14	7	6	5
6	0	15	0	0	1	0
6	1	0	1	2	0	1
6	2	2	2	2	1	2
12	19	10	7	10	5	7
12	67	15	14	15	12	16
12	0	0	0	0	0	0
12	2	3	3	3	4	2
12	8	7	8	13	18	7
12	18	10	9	11	11	13
12	18	8	16	11	13	9
12	22	5	12	9	15	8
12	2	2	2	2	2	2
12	0	0	0	0	0	0
12	0	0	12	0	0	0
12	4	4	4	5	3	0

12	1	2	2	1	1	0
12	3	2	4	2	4	2
12	0	0	0	1	0	0
12	2	3	8	10	10	10
12	19	9	8	6	6	4
12	24	0	1	0	1	1
12	0	1	0	1	1	0
12	2	3	3	3	2	2
24	11	12	11	13	10	8
24	52	34	49	75	73	83
24	0	0	0	0	12	0
24	2	3	2	3	2	2
24	9	4	8	4	1	4
24	10	12	14	13	11	11
24	5	5	6	7	5	7
24	43	31	40	48	6	9
24	1	1	1	2	2	1
24	0	3	0	0	0	0
24	0	0	0	0	0	0
24	3	4	3	1	2	2
24	2	8	7	4	2	1
24	2	4	3	4	2	3
24	0	1	0	0	0	0
24	17	20	21	21	20	19
24	5	5	6	7	6	5
24	0	0	0	0	1	1
24	0	1	0	2	1	0
24	2	2	2	2	2	2
48	46	41	62	48	48	42
48	13	15	27	18	16	15
48	0	0	0	0	0	0
48	5	3	3	2	2	3
48	4	6	7	9	5	6
48	11	10	8	10	12	10
48	4	6	3	3	7	8
48	6	15	8	3	1	2
48	1	1	1	2	2	11
48	0	0	0	0	0	0
48	0	0	0	0	0	0
48	3	4	5	3	3	4
48	2	3	14	9	3	25
48	7	4	7	12	2	3
48	0	0	0	0	0	0
48	39	33	28	41	34	31
48	0	6	8	4	4	4
48	3	0	0	0	0	1
48	2	1	0	2	0	1
48	2	2	2	2	2	3

Change in VAS (mm) over 240 minutes after a range of doses of inhaled formoterol

	Time (minutes)					
Dose (mcg)						
0	1.105	1.364	1.096	1.082	0.876	1.2
0	0.259	0.274	0.169	0.35	0.33	0.203
0	0.313	0.29	0.208	0.229	0.167	0.163
0	1.233	0.753	1.128	0.604	0.892	1.295
0	0.488	0.721	0.421	0.367	0.57	0.493
0	1.125	0.712	0.9	0.563	0.446	0.399
0	0.922	0.97	0.999	0.612	0.735	0.968
0	1.324	1.362	1.787	1.489	1.303	1.311
0	0.305	0.204	0.22	0.308	0.266	0.337
0	1.408	1.34	1.588	1.454	1.418	1.276
0	0.765	0.659	0.531	0.747	0.663	0.594
0	1.889	1.363	1.148	1.06	1.548	1.395
0	0.637	0.922	0.575	0.531	0.455	0.359
0	0.327	0.275	0.278	0.23	0.247	0.317
0	0.354	0.344	0.283	0.298	0.298	0.32
0	0.755	0.693	0.893	0.851	0.914	0.797
0	1.432	1.63	1.657	1.61	1.936	1.481
0	1.204	0.987	0.931	0.84	0.846	0.983
0	0.433	0.33	0.316	0.211	0.272	0.24
0	0.476	0.417	0.426	0.444	0.399	0.461
6	1.816	1.26	1.358	0.974	1.195	1.027
6	0.236	0.218	0.207	0.426	0.399	0.236
6	0.289	0.22	0.205	0.25	0.193	0.299
6	0.891	0.607	0.614	0.535	0.722	1.017
6	0.558	0.528	0.424	0.531	0.567	0.429
6	1.224	1.27	0.746	0.842	1.45	1.308
6	0.718	0.736	0.76	1.091	0.72	0.67
6	0.971	1.329	1.213	1.148	1.395	1.306
6	0.251	0.313	0.331	0.288	0.285	0.272
6	1.377	1.267	1.274	1.056	1.476	1.277
6	0.608	0.631	0.592	0.56	0.676	0.685
6	1.516	1.681	1.29	1.381	1.451	1.146
6	0.693	0.918	0.735	0.312	0.964	0.391
6	0.276	0.269	0.268	0.276	0.248	0.33
6	0.391	0.357	0.277	0.405	0.476	0.327
6	0.72	0.566	0.648	0.629	0.794	0.965
6	1.352	1.677	1.965	1.365	1.497	1.606
6	1.582	0.924	1.142	1.201	1.135	1.327
6	0.512	0.41	0.372	0.304	0.263	0.277
6	0.773	0.565	0.586	0.575	0.62	0.634
12	1.381	1.291	1.131	1.046	1.557	0.886
12	0.415	0.401	0.298	0.463	0.518	0.303
12	0.221	0.206	0.292	0.297	0.375	0.642
12	2.2	1.921	1.469	1.419	1.382	1.833
12	0.68	0.41	0.541	0.59	0.634	0.622
12	0.807	0.822	0.904	0.777	0.565	0.982
12	0.701	0.525	0.658	1.207	0.881	0.677
12	1.535	1.385	1.581	1.954	1.441	2.062
12	0.193	0.278	0.259	0.255	0.262	0.342
12	1.125	2.032	1.535	1.282	1.107	1.223
12	0.773	0.689	0.509	0.39	0.468	0.493

12	1.049	0.974	0.732	0.829	1.236	1.106
12	0.793	0.699	0.818	1.493	0.654	0.44
12	0.338	0.318	0.357	0.36	0.271	0.312
12	0.396	0.443	0.42	0.494	0.5	0.396
12	0.802	0.628	0.679	0.721	0.921	0.991
12	1.873	1.445	1.49	1.655	1.801	2.034
12	1.199	1.276	0.951	1.223	1.113	1.149
12	0.324	0.274	0.482	0.51	0.279	0.384
12	0.67	0.671	0.736	0.887	0.957	0.694
24	1.04	1.503	1.351	1.137	1.423	2.11
24	0.273	0.265	0.4	0.435	0.406	0.375
24	0.321	0.639	0.449	0.289	0.443	0.309
24	1.623	1.163	1.369	1.445	1.06	1.362
24	0.587	0.523	0.569	0.675	0.915	0.537
24	1.039	0.589	1.204	1.714	1.153	1.119
24	0.694	0.819	1.095	1.081	1.167	1.535
24	1.365	2.265	1.954	2.2	1.769	1.931
24	0.293	0.26	0.28	0.267	0.232	0.348
24	1.903	1.667	1.613	1.623	1.721	1.176
24	0.749	0.581	0.684	0.703	0.71	0.775
24	1.381	2.177	1.695	1.678	2.092	1.075
24	0.56	0.689	0.908	0.468	1.288	0.486
24	0.285	0.425	0.384	0.407	0.315	0.46
24	0.293	0.44	0.503	0.481	1.047	0.918
24	0.77	0.78	1.049	1.046	0.715	0.902
24	1.915	2.241	2.335	2.274	2.249	2.073
24	1.085	1.162	1.571	1.433	0.935	1.475
24	0.368	0.475	0.346	0.343	0.377	0.369
24	0.745	0.848	0.786	0.628	0.708	0.806
48	1.694	1.733	1.424	1.757	2.154	1.92
48	0.384	0.37	0.322	0.442	0.38	0.276
48	0.239	0.245	0.325	0.268	0.262	0.257
48	1.836	1.754	1.673	1.746	1.972	2.179
48	0.238	0.647	1.086	0.779	0.94	0.529
48	0.912	1.082	1.442	1.02	1.895	1.037
48	0.794	1.601	1.593	1.354	1.18	1.144
48	1.053	1.636	1.978	1.897	1.801	1.723
48	0.247	0.397	0.371	0.507	0.546	0.493
48	0.989	1.093	1.435	1.491	1.75	1.652
48	0.841	0.928	0.778	0.97	1.008	1.048
48	1.427	1.036	1.21	1.474	1.718	2.905
48	1.138	1.358	2.286	1.382	0.588	0.918
48	0.247	0.367	0.376	0.463	0.357	0.41
48	0.349	0.523	0.353	0.368	0.466	0.316
48	0.801	0.965	1.107	1.099	1.3	0.738
48	1.81	2.01	1.881	1.48	2.33	2.4
48	0.994	0.989	1.312	0.99	1.213	0.944
48	0.626	0.53	0.574	0.609	0.457	0.676
48	0.652	0.709	0.728	0.639	0.941	0.686

Change in tremor (accelerometer, m/s/s.h) over 240 minutes after a range of doses of inhaled formoterol

	Time (minutes)					
Dose (mcg)						
0	63.5	76	31	41.5	50	73
0	15	12	5.5	7	10	9.5
0	6	0.5	3	3	23	18
0	67	37	37	40	43	49
0	6.5	8.5	6.5	8	9	10
0	5.5	10.5	15	22	21.5	25
0	24	5	7	13.5	22	20
0	0	0	2.5	3	2.5	58
0	1	1	1	1	1	2
0	19	4	5	2	5	4
0	60	41.5	0	46	45	41
0	17	7.5	2	5	5	6
0	25	9	2.5	2	2	1.5
0	2.5	9.5	2	3	3	3.5
0	4	2	1.5	0	0	0
0	22.5	24	26	30	37	46.5
0	24	20	26	24	21	12.5
0	41	22.5	17.5	37	38	34
0	7	1.5	1	2	1	1
0	26	9.5	2.5	2.5	2	0.5
6	42.5	32	46	42	50	56.5
6	16	14.5	7.5	13	13	15.5
6	38	4.5	1	0.5	2.5	0.5
6	75	43	36	24.5	24	20
6	6	11	9.5	10.5	9.5	12
6	22	6.5	6.5	7	6	6.5
6	3	4	2.5	2.5	7	2
6	15.5	10	1.5	3	1.5	2
6	13	1	1	1	0.5	1
6	39.5	29	14.5	4	8	3
6	42	13	14	30	39	35
6	1.5	1	2	1	1	1
6	35.5	7	5	3	1.5	0.5
6	2.5	1.5	2.5	1.5	1.5	3
6	2	1	0	0	0	0
6	30	36	21	30	27	28.5
6	28	25	28	10	13	6.5
6	61	64	23	41	19	32
6	11.5	4	4	3	6	4.5
6	1.5	1.5	1	1.5	2	2
12	50	54	49	40.5	51	74.5
12	12	22	17.5	15.5	10.5	19
12	65.5	39	23.5	6	0.5	15
12	61	53	51.5	40	48	46.5
12	7.5	14	8	16.5	19	5.5
12	32.5	10.5	9.5	11.5	11	11.5
12	36	2.5	8	6	5.5	7
12	2	1	5	0	0	0
12	3	1	2	1	1	1
12	4.5	3	2.5	1	1	5
12	0	12.5	18	21	33	20
12	4.5	1.5	1.5	4.5	1.5	1.5

12	12.5	0.5	1	1	1	0
12	5.5	2	2	2	2.5	2
12	4	1	0	0	1.5	0
12	48	13	23.5	15	16.5	16
12	29	19	10	13	8.5	8.5
12	59	18	22	32	21	29.5
12	1	0.5	1	0	2	2
12	13	2	2	1	2	2
24	49.5	43	38	48.5	41	63
24	66.5	40	58.5	74	80	80
24	23	1	3	4	11	0.5
24	56	54	45	43.5	41	29
24	13.5	6.5	31	6	3.5	3.5
24	26.5	12	11	11.5	9.5	11.5
24	3.5	7	3	4	3	3
24	48	20	10	14	4.5	3.5
24	1.5	2	1.5	1.5	1.5	1
24	39.5	6	1.5	0	1	1
24	66	0	0	33	26.5	31.5
24	2	2	2	1.5	1.5	1.5
24	21	8.5	15	5	3	2.5
24	2	3	2.5	2	2	3
24	1.5	1.15	0.5	1.5	1.5	0
24	26	22.5	30	26	24	21.5
24	17.5	20.5	16	13	14	14.5
24	65.5	17	24.5	13	21.5	33
24	13	3	2	4.5	1.5	0.5
24	1.5	2.5	2.5	2	2	2
48	83	56.5	57	52.5	52	34
48	15.5	15	15	16.5	18.5	16.5
48	48	23.5	6.5	8	8	7.5
48	77.5	45	55	53	46	48
48	4	7	7	7.5	8	6
48	11.5	11	8.5	10	11.5	7.5
48	7	4	3	3	3	4
48	10	4.5	2	1	0	0
48	1.5	1	1.5	1.5	1.5	1.5
48	13	7	2	1	1.5	2
48	32	25	20	9	20	17.5
48	1	4.5	3	3	2	2
48	46.5	27	10	13.5	2	8
48	23	4.5	6	5	4.5	2.5
48	0	0	0	0	0	0
48	33.5	35.5	29	36	32	28
48	39.5	49	31.5	24	18	17
48	38.5	22	25	34	35	27
48	17.5	2	1.5	2.5	0	7
48	16	3	2.5	2.5	3	3.5

Change in VAS dyspnoea (mm) over 240 minutes after a range of doses of inhaled formoterol

Placebo

Baseline	30 mins	60 mins	120 mins	180 mins	240 mins
202/111	152/91	158/82	153/82	158/93	162/99
151/85	151/90	126/84	128/80	130/80	126/82
98/77	96/77	103/75	99/82	107/76	105/89
122/103	133/77	132/84	134/82	128/99	141/94
158/73	150/81	149/78	159/78	155/83	153/80
158/82	120/60	111/65	118/65	117/68	141/84
145/61	140/66	127/60	138/63	138/63	147/67
145/82	135/71	134/85	127/64	142/79	133/77
129/60	129/71	128/72	143/78	152/75	152/78
164/96	147/94	138/86	139/82	154/92	141/91
125/81	123/80	128/95	133/86	136/84	129/78
189/106	190/105	182/101	199/111	174/120	192/106
108/86	106/59	106/63	111/67	99/73	101/61
135/82	130/88	131/86	124/80	122/82	142/86
142/75	145/78	150/85	157/73	154/72	147/77
134/87	110/81	112/77	124/82	126/81	106/74
122/69	130/71	136/76	136/79	149/79	132/82
176/94	166/99	163/99	178/97	171/102	182/107
113/76	116/74	112/73	110/76	117/75	120/78
135/86	130/91	132/89	149/92	143/93	143/95

6 mcg

Baseline	30 mins	60 mins	120 mins	180 mins	240 mins
157/83	152/86	154/82	164/77	148/100	153/96
154/93	136/84	119/80	137/90	131/85	134/88
116/79	92/73	97/77	98/76	106/78	112/75
118/80	134/101	133/90	149/106	124/73	136/117
148/77	153/83	151/82	160/86	164/95	156/83
118/60	104/63	128/62	129/66	148/85	153/86
157/89	133/62	132/59	137/66	131/67	139/68
145/81	134/81	123/76	142/72	152/76	134/75
152/77	145/66	136/77	152/77	143/76	138/77
122/76	126/89	129/89	131/80	141/88	131/86
124/97	149/101	136/89	150/97	140/87	139/91
154/108	153/107	152/97	166/108	161/107	156/102
99/62	102/61	106/63	107/60	113/64	112/67
116/77	122/93	119/88	132/86	144/89	121/83
131/78	134/75	130/70	142/75	147/74	144/78
110/71	109/76	126/73	106/76	110/81	122/86
150/76	132/83	142/79	154/82	141/88	154/84
161/98	158/96	187/99	181/99	167/95	167/100
182/101	106/90	105/73	122/72	119/74	119/80
143/87	136/88	147/96	152/94	151/97	156/98

12 mcg

Baseline	30 mins	60 mins	120 mins	180 mins	240 mins
152/91	167/77	158/92	157/108	150/100	152/94
133/99	137/91	136/80	134/82	147/88	138/87
158/80	128/76	137/79	101/79	108/75	122/80
135/80	128/86	132/81	141/81	142/82	129/92
154/77	152/76	150/78	159/84	162/88	160/86
138/76	123/69	130/78	144/73	132/70	150/74
153/61	152/64	141/63	142/63	144/65	159/63
123/68	110/72	131/71	134/76	119/73	133/75
129/67	152/69	130/81	149/85	146/77	155/74
145/91	131/94	133/97	155/92	150/89	140/94
141/98	128/89	137/82	149/83	145/88	135/77
161/108	159/102	170/108	154/113	165/110	174/114
111/65	116/75	131/68	117/71	111/80	116/83
141/85	114/77	131/79	130/87	143/90	138/82
132/77	137/79	140/73	151/85	146/79	132/76
112/76	108/84	119/87	107/80	112/77	113/81
150/84	139/83	158/79	158/88	174/101	171/85
208/106	183/95	187/99	181/96	193/104	174/102
133/91	120/84	123/82	130/81	113/82	123/86
142/89	136/87	150/91	145/98	157/96	162/99

24 mcg

Baseline	30 mins	60 mins	120 mins	180 mins	240 mins
153/92	149/93	161/93	153/90	146/89	142/91
159/95	139/87	124/77	140/90	143/90	141/94
99/80	98/82	102/73	99/80	113/86	104/83
132/82	136/77	135/80	147/87	131/83	131/101
136/92	149/87	143/84	141/83	138/81	141/81
138/76	143/81	125/72	152/76	120/70	137/92
148/52	150/68	165/57	153/74	153/69	153/74
189/91	150/80	162/80	163/81	161/83	165/86
119/767	106/62	129/77	132/74	130/64	139/68
130/81	133/89	126/81	128/83	124/83	152/89
121/86	133/85	118/99	129/80	142/88	139/87
192/104	157/91	153/103	156/102	161/92	159/107
137/78	99/57	99/58	108/62	108/66	106/63
120/86	131/82	135/83	141/85	136/87	160/98
136/79	138/78	143/73	152/75	144/79	142/76
98/66	114/77	117/79	125/78	122/80	13181
128/73	142/72	141/74	144/81	144/75	161/80
156/86	171/97	162/88	187/103	192/100	192/106
115/70	142/82	151/85	115/74	121/77	121/75
133/88	137/90	142/87	139/89	159/94	159/102

			48 mcg		
Baseline	30 mins	60 mins	120 mins	180 mins	240 mins
152/76	147/95	145/92	157/98	154/80	153/71
139/87	140/120	120/68	103/71	130/82	121/81
111/72	103/76	105/78	105/83	104/75	104/79
136/81	130/70	131/80	122/79	133/78	152/85
158/78	156/79	153/79	163/85	158/82	153/83
165/83	141/81	129/79	135/66	145/82	145/89
138/64	134/64	148/64	129/67	132/65	149/71
136/81	136/73	139/77	130/76	122/75	164/95
118/66	119/67	125/70	133/75	122/74	160/85
139/86	139/86	129/89	131/81	134/85	140/91
126/78	127/76	141/78	135/85	131/84	135/75
192/103	193/104	199/101	184/107	183/103	192/98
103/81	105/55	114/67	109/65	103/79	106/79
121/80	140/88	131/76	140/81	133/79	152/91
130/67	120/71	130/72	136/76	138/73	120/72
114/76	111/72	123/79	128/76	131/83	116/81
147/81	137/71	122/69	148/73	147/82	142/105
127/77	135/86	154/87	169/90	176/95	179/97
138/79	117/83	137/100	137/100	139/102	123/80
144/93	127/87	153/92	153/91	156/91	163/96

Change in Blood Pressure (mmHg) over 240 minutes after a range of doses of inhaled formoterol

Placebo		6mcg		12mcg		24mcg		48mcg	
Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
3.8	3.8	3.9	4.3	4	4	3.8	4	3.9	4.2
4.6	4.5	4.1	4.5	4.4	4.4	4.1	4.5	4.5	4.5
4.3	4.4	4.5	4.5	4.2	4.1	4.3	3.7	4.7	4.2
4.2	4.2	4.1	4.2	4.3	4.1	4.3	4	4.1	4.1
4.3	3.9	4.1	4.4	4.1	4.1	4.2	3.9	4.3	4.2
4.2	4	4.1	4.2	4.1	4.3	4.3	4	4.3	4.3
4.4	4.6	4.1	4.6	4.6	3.9	4.1	4.7	4.4	4.6
4	4.4	4.3	4.4	3.9	4	4.5	4.1	4.4	4.1
3.6	3.9	4.1	4.3	4	4.2	4	4.3	4	4.2
4.3	4.2	3.5	3.9	4.6	4.1	3.5	3.9	3.6	3.5
4.4	4.4	4.3	3.8	4.5	4.1	4.7	4	4.1	3.9
4.4	4.4	4.2	4.1	4.5	4.5	4.5	4.1	4.2	4.2
4.1	4.1	4.4	4.2	4.1	4.3	4.4	4.2	4.4	4.2
4.3	4.1	4.1	4.1	3.9	4	4	4.5	3.8	4.1
4.5	4.5	4.1	4.1	4.3	4.1	4.1	4.1	4	4.1
4.1	4	4.2	4.1	3.9	4.1	3.9	4.3	4.1	4.3
4.3	4.5	4.1	4.1	4.5	4.1	4.5	4.1	4.1	4.4
4	4.1	4.1	4.2	4.1	4.3	4.2	4.1	4.6	4.6
4	4.1	4.2	4.5	4.2	4.1	4.1	3.9	4.1	4.1

Change in plasma potassium (mmol/l) over 240 minutes after a range of doses of inhaled formoterol

Placebo		6mcg		12mcg		24mcg		48mcg	
Pre	Pre	Pre	Post	Pre	Post	Pre	Post	Pre	Post
122	75	123	65	120	101	120	119	150	128
421	427	417	399	417	408.75	406	401.6	418	418
334	316	333	329	300	321	321	331	317	327
375	390	378	405	390	405	388	405	387	383
291	303	281	285	277	286	291	292	285	281
293	233	298	314	284	307	316	324	300	322
303	273	302	305	313	288	309	307	311	307
397	340	370	372	399	402	354	360	373	380
336	345	327	333	358	360	367	360	367	381
323	331	299	315	308	316	308	312	292.5	302
362	360	355	359	363	363	357	360	368	375
430	426	455	441	440	443	447	451	439	448
411	420	390	423	420	453	435	450	450	462
396	395	410	396	437	445	444	440	425	400
378	368	388	395	391	390	375	378	404	405
387	365	402	375	396	387	386	399	375	383
344	351	333	330	337	351	343	351.5	283	313
344	355	322.5	351	354	354	349	361	360	363
444	450	469	478	405	427	429	449	410	436
466	469	485	472	486	488	480.5	475	482	478

Change in walk distance (m) over 240 minutes after a range of doses of inhaled formoterol

Publications arising from this thesis

A benefit-risk assessment of inhaled long-acting β_2-agonists in the management of obstructive pulmonary disease. Sovani MP, Whale CI, Tattersfield AE. Drug Safety 2004;27(10):689-715.

Effects of rac-albuterol on arterial blood gases in patients with stable hypercapnic chronic obstructive pulmonary disease. Whale CI, Sovani MP, Mortimer KJ, Oborne J, Cooper S, Harrison TW, Tattersfield AE. Br J Clin Pharmacol 2006;62(2):153-157.

Systemic and bronchodilator effects of inhaled rac-formoterol in subjects with chronic obstructive pulmonary disease: a dose response study. Whale CI, Sovani MP, Mortimer KJ, Harrison TW, Tattersfield AE. Br J Clin Pharmacol 2008;65(6):841-847.

Related publications

Poor adherence with inhaled corticosteroids in asthma: can a single inhaler containing budesonide and formoterol help? Sovani MP, Whale CI, Oborne J, Cooper S, Mortimer KJ, Ekstrom T, Tattersfield AE, Harrison TW. Br J Gen Pract 2008;58(546):37-43.

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