SECTION A

Review of the Literature

CHAPTER ONE

1.1 Phospholipids

1.1.1 Introduction

Phospholipids are a class of naturally occurring lipids found in living cells (Lumberg, 1951). They are a group of well-known lipids which have been thoroughly studied in the last three centuries (McMurray, 2004). Although many different phospholipids exist, each has a complex lipid group containing one or more phosphate groups (Szuhaj, 1993). Phospholipids contain fatty acid chains attached to a backbone of either glycerol or sphingosine with a polar group (alcohol or other organic compounds like choline) attached to the phosphate group (Nelson and Cox 2005). The differences in the fatty acid chains, and the differences in the groups attached to the phosphate head is responsible for the rich diversity of phospholipids (Hills, 1988; Nelson and Cox, 2005).

1.1.2 Chemical Structure of Phospholipids

Phospholipids have been described as being "amphipatic" in nature, containing a hydrophilic polar head and hydrophobic fatty acid groups (tails) (Hawgood, 1991). Based on the chemical structure of the backbone, they have been divided into glycero-phospholipids and sphingolipids; with glycerol and sphingosine as their respective backbones (refer to Figures 1.1 and 1.2).

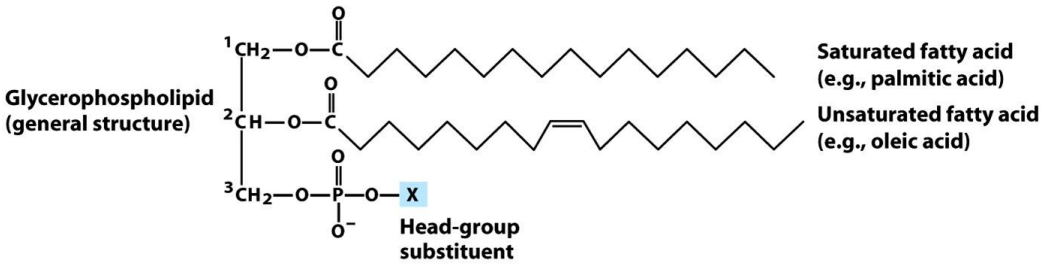

Figure 1.1 – The general structure of a glycerophospholipid (from Nelson and Cox, 2005)

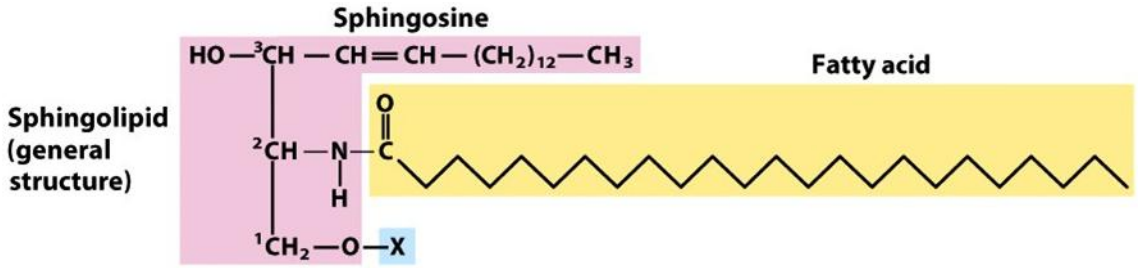

Figure 1.2 – The general structure of a sphingolipid (from Nelson and Cox, 2005)

Glycero-phospholipids (the most abundant of the two classes) is described as a diacylglycerol molecule with a phosphate molecule attached to the third carbon and fatty acid molecules attached to the first and second carbon atoms (α and β positions). The fatty acid molecules could either be saturated (*e.g.* palmitic acid) or unsaturated (*e.g.* oleic acid). Highly polar or charged groups such as choline, ethanolamine, serine, and inositol can be attached to the phosphate group onthe third carbon atom, leading to the formation of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol respectively (Szuhaj, 1993). The combination of the hydrophilic polar ends (charged groups) and the hydrophobic tails (fatty acids) confers the amphipatic nature on phospholipids.

1.1.3 Molecular Features of Phosphatidylcholines

The predominant component of surface active phospholipids, irrespective of where they are found in the body, is phosphatidylcholine (Hills, 2002a). One form of phosphatidylcholine is dipalmitoylphosphatidylcholine (DPPC) which is the most abundant phospholipid in lung surfactant and the most physiologically important glycerol-phospholipid in mammals (Hawgood, 1991; Whitsett, 1991).

The molecular configuration of dipalmitoylphosphatidylcholine is shown in Figure 1.3. There are two saturated fatty acid chains attached to the 1st and 2nd carbon atoms (α and β positions) of the glycerol backbone of the dipalmitoylphosphatidylcholine molecule, and a choline head group is attached to the single phosphate group, which is on the 3rd carbon atom of the glycerol backbone (Whitsett, 1991).

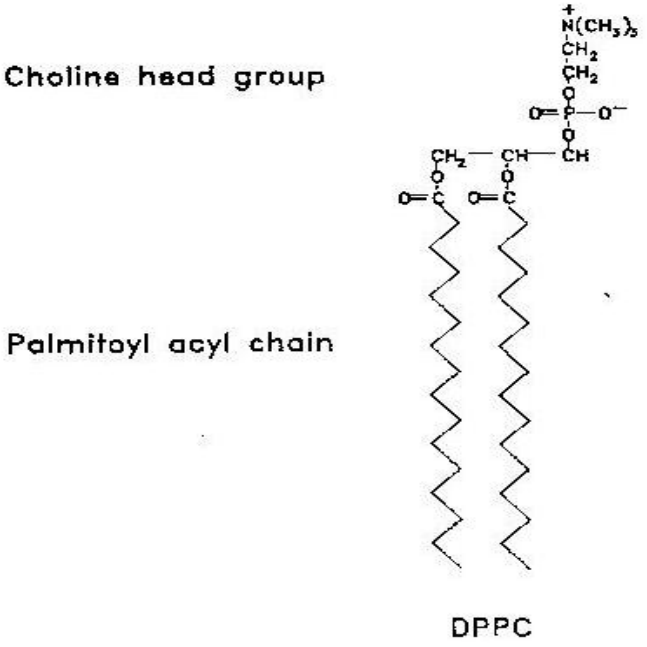

Figure 1.3 – Molecular structure of dipalmitoylphosphatidylcholine (from Whitsett, 1991)

The central phosphate ion on the phosphatidylcholine molecule renders the net charge on the molecule electrically neutral. However, the presence of mobile cations such as Ca^{2+} in the physiological milieu electrically neutralizes the phosphate ions, thus making dipalmitoylphosphatidylcholine effectively cationic (Hills, 2002a). The

terminal quaternary ammonium ion is strongly positively charged and this potentiates strong adsorption on to most biological surfaces that possess a surplus of negatively charged ions or radicals. This quaternary ammonium ion is similar to the one used in industrial cationic surfactants for binding to solid surfaces to provide many useful properties (*e.g*. lubrication and barrier formation) (Hills, 2002a; Hills 2002b).

The dipalmitoylphosphatidylcholine molecule has equal cross-sectional areas of 40 A^2 (Ångstrom^2) at both the polar and non-polar moieties. This feature makes it possible for both ends of the molecules to pack tightly, making them able to effectively reduce the high surface tension of any aqueous environment where they are found, and it also contributes to the ability to effectively form surface barriers when adsorbed (Hills, 2002a). When dipalmitoylphosphatidylcholine molecules bind to solid surfaces, they do so with their quaternary ammonium ions, but the presence of mobile cations which are interspersed between the phosphate groups, pull the dipalmitoylphosphatidylcholine molecules together into a close matrix, thus imparting strong cohesion to the adsorbed monolayer (Hills, 1988). This principle has been much exploited in the physical sciences, in the practice of phosphating, in which a coating layer is formed to preserve steel (Hills, 1988; Hills, 2002a). In addition, the two properties most desired for imparting good boundary lubrication and formation of good barriers are strong adsorption and strong cohesion, and these properties are provided by the molecular configuration of dipalmitoylphosphatidylcholine (Hills 1988; Hills, 2002a).

1.1.4 Self-aggregating Nature of Phospholipids

As amphipatic molecules, phospholipids have low solubility in water. In aqueous environment, rather than existing as individual molecules, phospholipids are found as self-aggregating molecules, forming mono-layers, multi-layers adsorbed unto solid surfaces, and lamellae (Hills, 1988). Their low solubility in water explains why they exist as self-aggregating molecules, which are arranged in such a way that their polar heads are directed towards the polar water molecules, while the non-polar fatty acid tails face the non-polar environment, which could be air or another phospholipids tails (Hawgood, 1991; Hills, 1988).

1.1.5 Mono-layers at Air: Water Interface

Phospholipids form a monomolecular surface film at the air-liquid interface. This happens as a result of the electrostatic interactions and resulting orientation of phospholipids molecules in such milieu. More specifically, electrostatic attraction between adjacent bipolar water molecules pushes the hydrophobic phospholipid tails out of the water at the air:water interface, while the hydrophilic polar end of the phospholipid is retained in the water (Hawgood, 1991; Hills, 1988). The presence of positively charged ions (in particular $Ca^{+2}$and Na^{+}) in the aqueous phase of the interface form electrostatic bridges between the negatively charged ions on adjacent bipolar heads of the phospholipids molecules, thereby tightly packing the fatty acid tails together to give an overall hydrophobic monomolecular film at the water surface (refer to Figure 1.4) (Hills, 1988; Hills, 2002a).

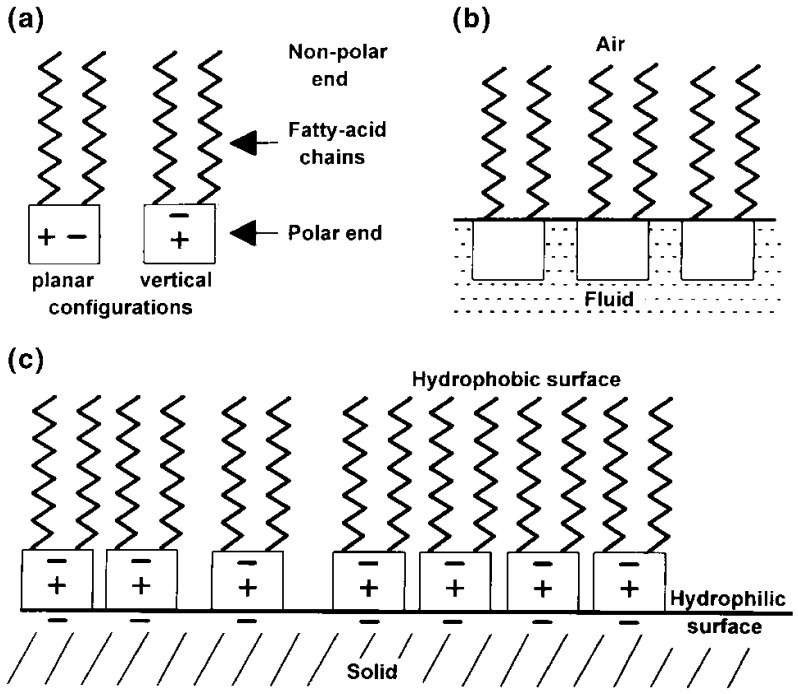

Figure 1.4 – (a) A schematic structure of a saturated phosphatidylcholine (PC) molecule showing polar and non-polar groups (b) orientation of the molecule at liquid–air interface, and (c) how phosphatidylcholine molecule adsorb to a hydrophilic surface to make it hydrophobic (from Hills 2002a).

1.1.6 Adsorption onto Mucosal Surfaces

Phospholipids molecules are absorbed onto mucosal surfaces because of the presence of negatively charged radicals (*e.g.* carbonyl and sulphonyl groups) on the mucosal surfaces (Hills, 1988). This provides an avenue for electrostatic attraction between the negatively charged radicals and the positive groups on the bipolar head of the phospholipids molecules. As a result, phospholipids become adsorbed onto mucosal surfaces with their positively charged heads while the fatty acid tails point outwards (Hills, 1988; Hills, 2002a). As earlier mentioned, the presence of cations (Na^{+}, Ca^{+2}) makes the phospholipids molecules tightly packed together by electrostatic attraction at these surfaces (refer to Figure 1.5).

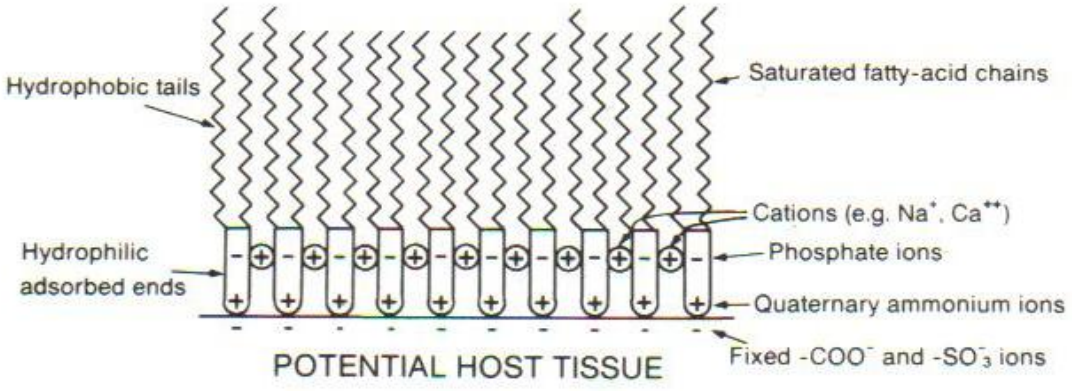

Figure 1.5 - A schematic molecular model showing the adsorption of phospholipids molecules to a negatively charged surface (from Hills, 1988).

1.1.7 Lamellae Formation

Phospholipids tend to form bilayers, each comprising of hydrophilic polar heads at the exterior (facing the polar aqueous environment) and hydrophobic tails facing each other in between two polar heads. This orientation is made possible because of the electrostatic attraction between the bipolar heads of the phospholipids molecules and the bipolar water molecule. The bilayers are arranged repeatedly on top of each other in the aqueous polar environment as a multilayered structure called lamellae with a small water molecule separating each opposing bilayer (refer to Figure 1.6) (Hills, 1988; Hills, 2002a; Hills 2002b).

The lamellated phospholipid structure has been demonstrated in various parts of the human body by electro-microscopy, including the lining of the lung alveolar surfaces, (refer to figure 1.7) (Ueda *et al.*, 1983; Ueda *et al.*, 1985), gastric mucosal lining (Hills, 1988; Ueda *et al.*, 1983; Ueda *et al.*, 1985) and the synovial cavity (Hills, 1989).

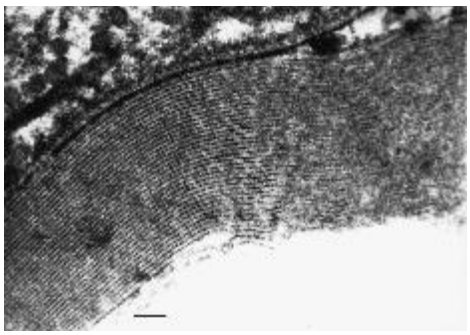

Figure 1.6 - An electron photomicrograph of human bronchial epithelium (mucus free) prepared by the method of Ueda *et al.* (1983) showing the multilamellar lining of adsorbed surface-active phospholipids. The bar represents 50 nm (from Hills, 2002a)

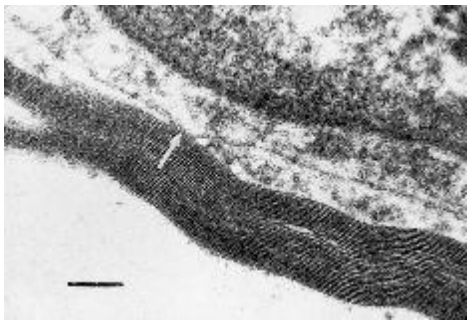

Figure 1.7 - An electron photomicrograph of the alveolar surface of a human lung prepared by the protocol described by Ueda *et al*. (1983), showing oligolamellar lining of surface active phospholipids adsorbed on to the alveolar epithelium. The bar represents 100 nM (from Hills, 2002a)

1.1.8 Physicochemical Properties of Phospholipids

Specifically, phospholipids are able to adhere on to various surfaces (as earlier described) due to their chemical composition, and they impart certain physicochemical properties on such surfaces, making them to exhibit entirely different properties from their original or inherent ability (Hills, 1989; Hills, 2002a).

1.1.8.1 Surface Tension Lowering Effect

At the air: water interface, phospholipids form monolayer as a result of their self-aggregating behavior. This results into the displacement of most or all of the water molecules by the phospholipids tails (fatty acids), and it also lowers the surface tension at water surface (Hawgood, 1991). As described by Hawgood (1991), the surface tension can be decreased from as much as 70N/M to 25N/M or even lower, and if the layer is further compressed, the surface tension can be made to be near zero (Hawgood, 1991). The surface tension lowering effect of phospholipids monolayer is influenced by the presence of positively charged ions or radicals (*e.g.* Ca^{+2}, Na^{+}), and also by temperature (West, 1974). The presence of positively charged moieties usually aids the compression of the phospholipids monolayer into an area that is smaller than which it occupies under equilibrium condition. This makes the phospholipid molecules more tightly packed together, with the resultant exclusion of more water molecules from the surface layer, thereby lowering the surface tension further (Hawgood, 1991).

Phospholipids adsorb on to surfaces when at temperatures above their gel – liquid transition phase (Hawgood, 1991). There is a rapid spread of phospholipid molecules over such surfaces, which allows for rapid monolayer formation across the whole surface, thereby effectively and rapidly reducing the surface tension across the entire liquid surface (Hawgood, 1991; Hills, 1988). Interestingly, as temperature increases from 22 to 37 degrees Celsius, a minimal increase in the surface tension of lung surfactant was recorded by Schurch *et al* (1985).

1.1.8.2 Adhesion

Phospholipids act as adhesives or release agents when applied to certain surfaces such as glass. This is also due to the self-aggregating nature, and the ability to be adsorbed to surfaces. The phospholipid molecules orientate themselves in a way that the polar heads reversibly bind to the surface by electrostatic forces, and the non- polar fatty acid tails faces outwards to form a 'hydrocarbon' layer on the surface (Hills, 1989). This hydrocarbon layer confers an adhesive property on the glass surface (Hills, 1988; Hills, 2002a).

1.1.8.3 Lubrication

Phospholipids serve as lubricants between boundaries and opposing surfaces (Hills, 1988). As explained above, due to their orientation at various surfaces, phospholipids molecules form hydrocarbon exterior which makes the surface slippery, thus markedly reducing frictional forces between two opposing surfaces or boundaries (Hills, 1988; Hills, 1989; Hills, 2002b). Many industrial surfactants with phospholipids as the major constituent have been developed for use as good lamellated-solid lubricants in the field of engineering (Hills, 2002b).

1.1.8.4 Anti-corrosive Protection

The anti-corrosive protective nature of phospholipids is better described at mucosal surfaces. Here, the positively charged bipolar heads of the phospholipids molecules are electrostatically attracted to the negatively charged mucosal surfaces. This results in the formation of a layer of phospholipids which coats the mucosal surface. This coating layer so formed, protects the underlying mucosal surface from corrosion as hydrophilic ions (*e.g.* H^+) are unable to penetrate the tightly packed fatty acid tails which are already formed on the mucosal surface by the orientation of the phospholipids molecules (Hills, 1988). This principle has also been applied in engineering where corrosion of metals has been prevented by use of corrosion inhibitors (Hills, 2002b).

1.1.9 Biological Properties of Phospholipids

The components of surface active phospholipids within the body is much the same, but the proportions differ from one region to another (refer to Table 1.1) (Hills, 2002a). Phospholipids exert a range of biological effects (e.g. surface tension lowering effect, anticorrosive effect, adhesion and lubrication) due to their ability to adhere onto surfaces and alter the physicochemical properties imparted on such surfaces (Hills, 1988). In mammals, high levels of endogenous phospholipids are found in various organs where they play significant roles in body functions (Hills, 1988). Important examples of such mammalian organs and functions include:

- lubrication of the tear film in eyes as a result of phospholipids adsorbing on to the ocular surfaces, which prevents eyelids adhering especially during sleep (Hills, 2002b).

- lubrication of the pleura, pericardium and synovial fluid (joints), which lowers the high frictional forces between these surfaces, thereby reducing possible wear and tear (Hills, 2002b; Ueda *et al.*, 1986).

- an anti-adhesive effect within the Eustachian tubes which prevents the closure of these narrow tubes (Hills, 1984).

- an anticorrosive action in the stomach and lower oesophagus (Hills and Kirkwood, 1989; Hills, 1990; Hills, 1994).

- a surface tension lowering action in the alveoli and the small airways (Hills, 1988; MacKlem *et al.*, 1970). As the principal component of these endogenous surfactants (Goerke and Clements, 1986; Hawgood, 1991; Hills, 1988; Macklem *et al.*, 1970; Whitsett, 1991).

Table 1.1 - Percentage phospholipids distribution (from Hills, 2002a)

Source/Site	Lung lavage extract	Pulmonary lamellar bodies	Peritoneal dialysate	Mucus-free oxyntic 'scraping'	Synovial fluid
Phospholipid/Species	Human	Human	Human	Canine	Human
Phosphatidylcholine	73.0	71.2	81	45	48
Phosphatidylethanolamine	2.6	7.7	4	20	22
Sphingomyelin	3.7	2.2	6.5	12	14
Phosphatidylglycerol	12.4	9.9	–	Trace	–
Phosphatidylserine	3.3	2.3	3.5	10	7.4
Phosphatidylinositol	2.7	3.8	5	9.4	6.5
Lysophosphatidylcholine	–	–	–	35	1.9

1.1.10 Pharmacological Considerations

The important role of endogenous phospholipids especially as surfactants in the body is well established and documented in many literatures. The word "surfactant" in respiratory physiology covers the whole complex of phospholipids, which is the major active component (~67%), others being proteins (8%), neutral lipids (~21%) and minor constituents (~2%). Therefore phospholipids form the principal active component of surfactants, and sometimes these words are used interchangeably (Hills, 2002a). Exogenous phospholipid surfactants have been used therapeutically in some clinical disorders and some of the clinical uses of phospholipids are discussed below:

1.1.10.1 Infant Respiratory Distress Syndrome (IRDS)

Infant respiratory distress syndrome is a clinical condition in which there is complete absence or insufficient amount of lung surfactant in the new born. This is a common clinical condition which occurs especially in premature babies, with the resultant effect of lung collapse and difficulty with breathing, which ultimately leads to death if unattended by clinical intervention (Frerking *et al*., 2001; Whitsett 1991). In infants with respiratory distress syndrome, the mechanism necessary for surfactant production is underdeveloped, although they survive *in utero* because surfactants are not required (Whitsett, 1991), and the respiratory system only resumes function immediately after birth. Also, the lungs are not fully matured or developed until about 36weeks gestation. That is why steroids are given to expectant mothers to help the lung to mature in cases of preterm delivery.

Exogenous surfactant preparations have been used successfully in the treatment of IRDS (Frerking *et al*., 2001), in which affected infants are given exogenous surfactants immediately after birth to prevent infant respiratory distress syndrome. In premature babies, they are given in the early hours after delivery following the identification of signs and symptoms of respiratory distress e.g. dyspnoea, cyanosis and hypoxia (Frerking *et al*., 2001). This so called "Surfactant rescue" has enjoyed outstanding success in reducing infant mortality by 40 -50% since its inception (Speer*et al*., 1992).The various surfactants used for the treatment of respiratory distress in infants can either be from natural sources or from synthetic compound. Survanta ® and Curosurf ® are exogenous phospholipids extracted from bovine and porcine lungs respectively (Hills, 2002a) while Exosurf Neonatal ® is a synthetic compound containing a mixture of tyloxapol, cetyl alcohol (hexadecanol) and colfoscefil palmitate (Frerking *et al*., 2001).

1.1.10.2 Acute Respiratory Distress Syndrome (ARDS)

A similar respiratory disorder to that of infant respiratory distress syndrome is acute respiratory distress syndrome. In this clinical condition, there is profound inflammation of the lung parenchyma, resulting in exudation of fluids into the alveoli, which thus compromise lung function as a result of alveolar flooding and collapse. The overall picture is that of reduced lung compliance, increased work of breathing and gas exchange impairment at the alveolar level (Colledge *et al*., 2006; Gunther *et al*., 2001).

Patients with acute respiratory distress syndrome show significant clinical improvement (in breathing) when treated with exogenous surfactants (Colledge *et al*., 2006; Gunther *et al*., 2001; Hills, 2002a). Application of exogenous surfactants to the lungs provides surfactant rescue in the treatment of acute respiratory distress syndrome, and spreads rapidly over the surface of the incumbent fluid to alleviate hypoxia and help to restore membrane permeability (Colledge *et al*., 2006; Gunther *et al*., 2001; Hills, 2002a). This further assists in a remarkably rapid uptake of fluids from the already flooded airways, thus restoring lung function.

In practice, the clinically available exogenous surfactant formulations are all designed to spread highly insoluble dipamytoilphosphatidylcholine as rapidly as possible over a liquid-air interface in order to reduce surface tension and facilitate water re-uptake in the water-logged lungs, thereby alleviating hypoxia (Hills, 2002a). Most clinically available lung surfactant preparations are derived from animal lung extracts which include endogenous apoproteins to help adsorb the phospholipids at the air: liquid interface, whereas tyloxapol and hexadecanol are used for this purpose in the synthetic lung surfactant Exosurf Neonatal® (Hall *et al*., 1992; Hills, 2002a).

1.1.10.3 Gastric Protection

Three of the most common gastric mucosa barrier breakers are bile-salts, ethanol and non-steroidal anti-inflammatory drugs. These agents chemically disrupt the coating of surface active phospholipids which normally protects the gastric mucosa from corrosive effects of the acidic secretions of the stomach, and it is believed that this effect contributes to the formation of gastric ulcers (Hills, 1988; Hills, 2002b; Lichtenberger, 1995). Exogenous surfactants have been found to offer protection for the gastric mucosa in a number of species and ulcer models in laboratory rats (Hills and Kirkwood, 1989). In addition, Hills and Kirkwood (1989) showed that phospholipids present in bananas offer appreciable protection of the gastric mucosa against acid insult in a dose-dependent manner. Therefore, the application of exogenous surface active phospholipids may be therapeutically beneficial in the treatment of gastric ulcers (Hills, 2002b).

1.1.10.4 Obstructive Sleep Apnoea and Snoring

Obstructive sleep apnoea is a clinical condition characterized by frequent or recurrent collapse of the pharynx, due to the relaxation or closure of upper airway dilator muscles (*e.g.* the palatoglossus and genioglossus) during sleep with resultant obstruction of the upper airways, and associated loud snoring, irrespective of posture. The incidence of obstructive sleep apnoea has been put to about 2-4% of the entire middle age population, (Young *et al*., 2002). Sleeping patients with obstructive sleep

apnoea often arouse transiently to allow the upper airway dilating muscles to re-open the obstructed or closed pharynx, after which they fall back to sleep. This occur many times throughout a night's sleep, so that obstructive sleep apnoea patients experience excessive daytime sleepiness with difficulty in concentrating, impaired work performance, impaired cognitive function, depression and irritability (Colledge *et al*., 2006; Young *et al*., 2002). Importantly, the high daytime sleepiness in these patients is associated with a three-fold risk of road traffic accidents and a nine-fold risk of single vehicle accidents (Colledge *et al*., 2006).

Conventional mainstay treatment of obstructive sleep apnoea is by the use of continuous positive airway pressure (CPAP) which applies continuous positive pressure via a nasal mask to splint open the collapsible pharynx during sleep. Though this treatment option is effective in achieving symptomatic relief, alternative therapeutic considerations have been sought because nasal CPAP is intrusive and has associated side effects such as nasal problems, tight or painful nasal mask, and air leakage (Hoffstein *et al*., 1992).

Various studies have shown that application of exogenous surfactants into the upper airway lumen oppose pharyngeal collapse and facilitate pharyngeal re-opening experimental animals (Kirkness *et al*., 2003a; Miki *et al*., 1992). Similar outcomes have also been observed in human volunteers without obstructive sleep apnoea (Hoffstein *et al*., 1987; Kirkness *et al*., 2003c; Morrell *et al*., 2002; Van der Touw *et al*., 1997; Van der Touw *et al*., 1997; Widdicombe and Davies, 1988).Van der Touw *et al*. (1997) proposed that surface active phospholipids may be useful in therapeutic management of obstructive sleep apnoea. Studies that have examined the effects of instilling surfactants into the upper airways of patients with obstructive sleep apnoea have shown that attenuated pharyngeal collapse, facilitated pharyngeal re-opening, and improved sleep-disordered breathing (Jokic *et al*., 1998; Kirkness *et al*., 2003c; Morrell *et al*., 2002). In addition, Ward *et al* (1998) showed that CPAP requirements were reduced when surfactant is applied to the upper airways of sleeping patients with severe obstructive sleep apnoea.

1.1.10.5 Joint Lubrication

The lubrication provided by synovial fluids at various joints throughout the body has been attributed to the presence of surface active phospholipids in the synovial fluid (Hills, 1988; Hills, 1989; Hills, 2002b). Surface active phospholipids in the synovial fluid adsorb onto the apposing articulating joint surfaces and conferring lubricating capacity onto these surfaces (Hills, 2002b). There is a significant decrease in the content and distribution of phospholipids in arthritic joints as opposed to what is normally present (Hills, 1989; Hills, 2002b). This reduction in phospholipid content has also been described in joints of patients with previous trauma (Hills, 1989; Hills, 2002b). It has therefore been proposed that application of exogenous surfactants onto articulating joint surfaces may result in the improvement of mobility of arthritic joints (Hills, 1989). Clinical trials have reported early promising results, with exogenous application of the phospholipid dipamitoylphosphatidylcholine markedly reducing friction, wear and tear in prosthetic hip joints (Hills, 2002b).

1.1.10.6 Otitis Media

Otitis media is defined as an inflammation of the middle ear, with subcategories including acute otitis media, otitis media with effusion (also called secretory otitis media) and chronic otitis media (Paananen, 2001). Infections in the middle ear are among the most common illnesses during childhood and have become a major socioeconomic problem globally (Karma, 1999). Usually, the inflammation in otitis media starts in the upper airways and then rapidly spreads through the narrow eustachian tube to the middle ear (Ghadiali *et al*., 2002).

Phosphatidylcholine acts to reduce the passive opening pressure of the eustachian tube (Venkatayan *et al*., 2000), and facilitates the opening of the eustachian tube by their surface tension lowering and anti-adhesive (abhesive) effects (Ghadiali *et al*., 2002). In addition, application of exogenous surfactants derived from pig lungs into the eustachian tubes reduces the pressure required to open the eustachian tube in laboratory rats with established ear infections (White *et al*., 1990).

1.1.11 Phospholipid Content of Foods

Good natural food sources of phospholipids include brain, liver, heart, kidney, egg yolk, bone marrow, soy beans and corn kernels. Other rich food sources of phospholipids include oilseeds, shellfish, cereal grains, fish and lean meat (Lundberg, 1959; Shurtleff and Aoyagi, 2007; Weihrauch and Son, 1983). In addition, phospholipids from food sources have been used extensively in the food industry for manufacturing foods such as margarines, bakery items, frostings, non-dairy creamers, ice creams, pan coatings and confectionary products (Weihrauch and Son, 1983).

Hills and Kirkwood (1989) reported that bananas have a relatively high content of highly surface active phospholipids, and these authors have speculated that these phospholipids may have therapeutic potential. In addition, electron microscopy examination of bananas has demonstrated lamellar bodies similar to those found in alveolar rinsing which are the principal sources of lung surfactants (Hills and Kirkwood 1989; Kallarackal *et al*., 1987). However, soy lecithin is a much richer source of natural food phospholipids than bananas (Beare-Rogers et al., 1992; Helmerich and Koehler, 2003; Hurst and Marttin, 1984, Nzai and Proctor, 1999; Shurtleff and Aoyagi, 2007). Indeed, the concentration of phosphatidylcholine in soy lecithin is approximately 100-fold higher than in bananas (Zeisel et al., 2003).

1.1.11.1 Soy Lecithin

Lecithin is a naturally occurring complex mixture of food components which includes a high phospholipid content (Lundberg, 1951). The word lecithin has often been used interchangeably with phosphatidylcholine, but by composition lecithin is a mixture of glycolipids, triglycerides and phospholipids. Major sources of commercial lecithin includes soy beans (the most important source), rapeseed and sunflower (Helmerich and Koehler, 2003) and the composition of these lecithins varies depending on the source from they are derived (Lundberg, 1951). Other sources include peanuts, calf liver, wheat, oat meal and eggs (Shurtleff and Aoyagi, 2007). The form of lecithin prepared from soy beans (*Glycine max*) is called soy lecithin and it has a very high content of phospholipids (Shurtleff and Aoyagi, 2007).

Soy lecithin is used extensively in the food industry, especially as an emulsifier in the manufacture of confectioneries and other food stuffs (Hurst and Marthin, 1984). Lecithin has also been used in the manufacturing of cosmetics products, in pharmaceuticals industry, in the production of coating materials (such as paints, magnetic tape coating, waxes, polishes, wood coatings), and has also been used extensively in plastic and rubber industry, glass and ceramic processing, paper and printing, masonry and asphalt products, petroleum industry, metal processing, pesticides, adhesives, textiles, and leathers (Shurtleff and Aoyagi, 2007). Lecithin is also widely consumed because of its purported health benefits, but most clinical studies are yet to demonstrate clear health benefits from lecithin intake (Shurtleff and Aoyagi 2007).

1.1.11.2 Phospholipid Content in Soy Lecithin

Soy lecithin is a rich source of surface active phospholipids. The three main phospholipids in soy lecithin are phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, all of which are surface active phospholipids (Hurst and Marttin, 1984; Shurtleff and Aoyagi, 2007). Substantial differences exist in the phospholipid composition of soy lecithin reported in the literature. Helmerich and Koehler (2003) reported that the total phospholipids content of soy lecithin was 55.7-57.9 % w/w, whereas Weihrauch and Son (1983) gave a value of 50 % w/w. These differences may reflect natural plant variation and differences in the mode of extraction and the analytical methods used in the determination of the phospholipids content (refer to Table 1.2) (Beare-Rogers *et al.*, 1992; Helmerich and Koehler 2003; Hurst and Marttin, 1984). In addition, refined soy lecithin contains more phospholipids than does unrefined soy lecithin (refer to Table 1.3) (Shurtleff and Aoyagi, 2007).

Table 1.2 - Phospholipid Content of Soy Lecithin

Total (% w/w)	PC (% w/w)	PE (% w/w)	PI (% w/w)	PA (% w/w)	PS (% w/w)	Reference
55.7-57.9	17.5-32.4	7.9-17.3	11.4-16.0	3.7-6.6	<0.8-1.8	Helmerich and Koehler (2003) Table 4
---	11.2-16.4	6.4-13.3	4.4-21.3	---	0	Hurst and Martin (1984) Table 1
---	15.0-34.4	---	---	---	---	Press *et al*. (1981) Tables 1 and 2
50	19.3	8.2	9.6	3.7	0.3	Weihrauch and Son (1983) Table IX

PC - phosphatidylcholine, PE – phospatidylethanolamine, PI – phosphatidylinositol, PA – phosphatidic acid, PS – phosphatidylserine

Table 1.3 - Composition by Weight of Unrefined and Refined Soy Lecithin (from Shurtleff and Aoyagi , 2007)

Phospholipids	Unrefined Lecithin	Refined Lecithin
Phosphatidyl choline	17.5%	23%
Phosphatidyl ethanolamine	15.0%	20%
Phosphatidyl inositol	10.0%	14%
Other Phospholipids	14-18%	-----

1.2 Asthma

Although the primary pathophysiological feature of asthma is episodic and often reversible reductions in the intraluminal diameter of intrathoracic airways (*i.e.* episodic exacerbations of reversible airway obstruction), there is no clear or widely accepted definition of asthma (GINA, 2002). However, the Global Initiative For Asthma (GINA, 2006) has prepared the following description of asthma:

"Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment." (GINA, 2006).

Episodic exacerbations of airflow obstruction in asthma can be provoked by a wide range of triggers such as cigarette smoke, respiratory allergens, upper respiratory tract infections (viruses in particular), cold air, and exercise (Fireman, 2003). Other triggers include dust, acrid fumes, organic materials, emotional stress, and also inadvertent prescription or use of β -adrenoceptor antagonists, aspirin and non-steroidal anti-inflammatory drugs (Davidson and Haslett, 2002). Bronchoconstriction can be induced in the laboratory for clinical and research purposes by a variety of direct and indirectly acting airway challenges to determine the presence of bronchial hyperresponsiveness (Fireman, 2003).

1.2.1 Prevalence of Asthma

Asthma is a common chronic respiratory disorder, and its prevalence has been on the rise over the past few decades in many parts of the world (GINA, 2002). It is not certain whether the rise in the prevalence of bronchial asthma is due to the increased incidence of the disease worldwide or merely because of the increasing or growing world population. Also, there appears to be a major geographical variation in asthma, with countries like Australia, New Zealand and Britain having the highest prevalence while the prevalence is lowest in China and Malaysia (refer to Figure 1.8) (Davidson and Haslett, 2002; Masoli *et al*., 2004).

Bronchial asthma occurs at all ages, but predominantly in early life (early onset). About one-half of cases develop before age 10 (pre-pubertal), while about one-third occur before age 40 (adulthood). The male to female ratio is put at 2:1 in childhood, but this equalizes at around age 30 (1:1) (Kasper *et. al*., 2005).

Asthma is a very common disease which imposes a high economic cost and a high burden on health facilities globally (Masoli *et al*., 2004). For instance, an estimated 4 to 5% of the population of the United States is said to be affected. In 1998, about 10-11 million persons had acute attacks in the US, which resulted in 13.9million outpatient visits, 2million urgent care requests, and 423,000 hospitalizations, with a total cost of approximately $6 billion (Kasper *et al.*, 2005).

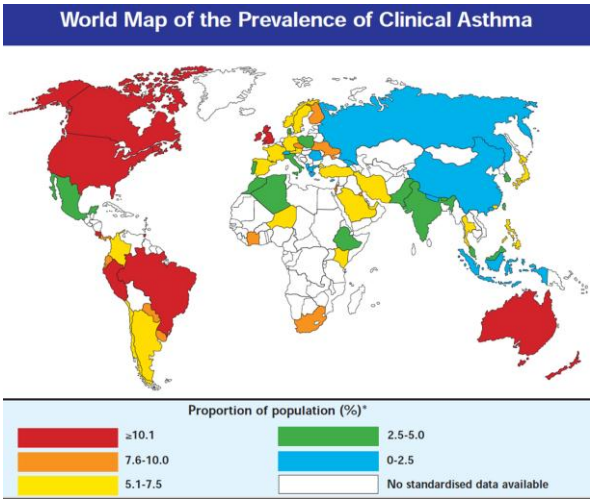

Figure 1.8 – World map showing the distribution and prevalence of asthma (from Masoli *et al*., 2004).

1.2.2 Aetiology and Pathogenesis of Asthma

Despite decades of intensive research, the aetiology of asthma remains only partly understood. There is strong evidence of a familial component in asthma and of significant genetic contributions to the predisposition of developing asthma (Kasper *et. al*., 2005; GINA, 2002). However, the rise in asthma prevalence in many countries in recent decades strongly suggests that environmental factors also play an important role. In particular, inadequate microbial exposure during early childhood (*i.e.* the hygiene hypothesis) is believed to play a preeminent role in the development of asthma and other atopic disorders (Strachan, 1989; Strachan, 1997; Wold, 1998).

Atopy is the single largest recognized risk factor for the development of asthma. A high proportion of asthmatics suffer from other atopic disorders such as hay fever (Corren, 1997). Some studies have reported that not all asthmatics exhibit atopic features (Davidson and Haslett, 2002; Kasper *et. al*., 2005). However, some type of IgE mediated atopic process is typically present in asthmatics. This has been demonstrated by well designed large population based studies that have shown elevated levels of nonspecific total IgE in most asthmatics even when other evidence of atopy (*e.g.* positive skin allergen tests and elevated levels of specific IgE) was absent (Burrows *et al*., 1989; Sunyer *et al*., 1996).

Inflammatory changes in the walls of the intrathoracic airways are evident in newly diagnosed asthmatics (Laitinen *et al*., 1993). Once established, the airway inflammatory processes persist to some degree, even in mild and asymptomatic asthmatics, and amplify when asthma symptoms worsen (Beasley *et al*., 1989; Martin *et al*., 1991). A range of responses contribute to airway inflammation in asthma, including airway smooth muscle contraction (bronchospasm), mucous plugging, and oedema and leukocyte infiltration within the airway wall (Barnes 1996; Bousquet *et al*., 2000). The cell types and mediators involved in the inflammatory process are complex and only incompletely understood. However, it is known that mast cells and eosinophils play important roles and are a source of many pro-inflammatory mediators and, when suitably stimulated, these cells can initiate and amplify a cascade of inflammatory reactions (Barnes, 1989; Barnes, 1996; Bousquet *et al.* 2000; Busse *et al*., 1993).

1.2.3 Measurement and Assessment of Lung Function

Measurement of lung function and arterial blood gases are useful in the clinical evaluation of pulmonary impairment, including grading of the severity of asthmatic exacerbations. For example, during severe exacerbations, the 1 second forced expiratory volume (FEV_1) or peak expiratory flow rate (PEFR) are typically < 40% of predicted while the residual volume may approach 400% of normal and functional residual capacity may double (Kasper *et. al*., 2005). In acute asthmatic exacerbations, hypoxia remains a universal finding when low oxygen tensions of 60-69 mmHg are usually observed as a result of the resulting mismatch between alveolar ventilation and perfusion (GINA, 2002; Kasper *et. al*., 2005).

Lung function is frequently assessed by spirometry, a method by which timed expired and inspired volumes of air are measured by flow sensing spirometers (Johns and Pierce, 2008). This allows rapid and comparatively simple determination and quantification of how quickly and how effectively the lung can be emptied or filled. A spirometric measurement that is widely used to assess the severity of ventilator impairment is the FEV_1 which the volume of air expired the first second of a maximal expiration after a maximal inspiration. Pellegrino *et al*. (2005) has categorized the

severity of lung function impairment into mild ($FEV_1 \geq 70\%$ predicted), moderate (FEV_1 between 60% and 69% predicted) and moderately severe (FEV_1 between 50% and 59% predicted), severe (FEV_1 between 35% and 49% predicted) and very severe ($FEV_1 \leq 35\%$ predicted). Other important forced expiratory indices that are commonly used in the assessment of ventilatory impairment include:

- **MMEF**: This is the average expired flow over the middle half of the forced manoeuvre. It gives an indication of small airways narrowing.

- **PEFR**: This is the maximal expiratory flow rate achieved during a forced expiratory manoeuvre. It occurs very early in the forced expiratory manoeuvre. It is also gives an indication of airflow obstruction or limitation.

- **FVC**: This is the maximum volume of air which can be exhaled or inhaled during a maximally forced manoeuvre. It is also an indication of airflow obstruction and air trapping.

- **FEV_1/FVC**: This is the expression of the FEV_1 as a percentage of FVC. It is a clinically useful index of airflow limitation, (Johns and Pierce, 2008).

1.2.4 Methods of Airway Challenge

Airway hyperresponsiveness is regarded as a hallmark characteristic of asthma (Sverrild *et al*., 2009; Woolcock, 1988). Various methods have been used to provoke airway challenge in asthmatics to determine airway hyperresponsiveness. Examples of airway challenge techniques include direct provocation agents that act on specific receptors to evoke airway obstruction as a result of airway smooth muscle contraction (*e.g*. inhaling aerosols of histamine and methacholine), and various indirect airway challenges that induce airway obstruction through nonspecific means that stimulate

inflammatory cells to release inflammatory mediators (Anderson *et al*., 1997; Haby *et al*., 1995; Scott and Braun, 1991; Sterk *et al*., 1993; Woolcock *et. al*, 1988).

Generally, airway challenges with histamine and methacholine are easy to administer by continuous nebulization of increasing concentrations or discrete cumulative doses from either a breath-actuated or a hand-held nebulizer, and the severity of response to both agonists shows a correlation with the severity of asthma (Woolcock *et. al*., 1988). However, while histamine and methacholine act as provoking stimuli which exert their effect by acting directly on airway receptors to elicit bronchial constriction of airway smooth muscle (Woolcock *et al*., 1988), other stimuli such as hyperventilation with dry air, fog, sulfur dioxide, adenosine, bradykinin and mannitol act indirectly through mediator-releasing cells or possibly by neural stimulation (Woolcock *et. al.,* 1988). Table 1.4 shows examples of direct and indirect bronchial challenge tests according to their main mechanism of action (Schoor, 2005).

Indirect airway challenge tests are believed to be more closely related to natural stimuli that trigger bronchoconstriction in asthmatics (Brannan *et al*., 2009; Leuppi *et al*., 2002). Moreover, bronchoconstriction induced by indirect airway challenges is believed to be indicative of both airway hyperresponsiveness and airway inflammation, and there is supportive evidence that indirect tests such as inhaled mannitol are more specific for currently active asthma and for determining optimal treatment regimens for asthma (Brannan *et al*., 2005; Brannan *et al*., 2009; Koskela *et al*., 2003b; Leuppi *et al*., 2002). In addition, responsiveness to inhaled mannitol has been shown to correlate better with airway inflammatory markers such as the percentage of sputum eosinophils (Porsbjerg *et al*., 2007) and exhaled nitric oxide levels than does responsiveness to methacholine (Porsbjerg *et al*., 2007; Sverrild *et al*., 2010).

Table 1.4 - Classification of bronchial challenge tests according to their main mechanism (from Schoor, 2005)

Direct stimuli	**Indirect stimuli**
Pharmacological stimuli	Pharmacological stimuli
Cholinergic agonists	Adenosine (AMP)
Acetylcholine	Tachykinins (SP, NKA)
Methacholine	Bradykinin
Carbachol	Metabisulphite/SO2
Histamine	Propranolol
Prostaglandin D2/F2a	
Leukotriene C4/D4/E4	
	Physical and physicochemical stimuli
	Exercise
	Isocapnic hyperventilation with (cold) dry air
	Osmotic stimuli
	Hypertonic aerosols (e.g. HS)
	Hypotonic aerosols (e.g. UNDW)
	Hypertonic mannitol dry powder

AMP, adenosine 50-monophosphate; SP, substance P; NKA, neurokinin A; HS, hypertonic saline; UNDW, ultrasonically nebulized distilled water; SO2,

1.2.5 Mannitol Airway Challenge

The discovery of mannitol as a broncho-provocating agent to measure airway hyperresponsiveness has opened up a new area of clinical testing and research in the understanding and diagnosing of asthma and other related airway disorders such as chronic obstructive pulmonary disease (COPD) (Anderson *et. al*., 1997; de Nijs *et. al*., 2011; Koskela *et. al.*, 2005). Mannitol is a naturally occurring sugar-alcohol found in most vegetables with the molecular formula of $C_6H_{14}O_6$. It is neither absorbed by the gastro-intestinal tract nor metabolized to any appreciable extent when injected (Anderson *et. al*., 1997).

Like other non-iso-osmolar substances used in indirect airway challenges, mannitol is believed to produce bronchoconstriction by increasing the osmolarity of the periciliary fluid lining the airway mucosa, resulting in the release of inflammatory mediators by inflammatory airway cells which instruct airway smooth muscle to contract (Anderson *et. al*., 1997; Brannan *et. al*., 2003; Cockcroft, 2006; Koskela *et. al*., 2005; Leuppi *et. al.*, 2002; Spector 2010).

Inhalation of mannitol provokes cough like other osmotic acting agents (Fahy *et. al*., 1995; Koskela *et. al*., 2005; Lowry *et. al*., 1988). The responsible mechanism is not fully understood, although it has been suggested that inhaled mannitol elicits coughing by stimulating airway C-fibre receptors which produces a central cough reflex as a result of the hyperosmolarity of the airway (Koskela *et. al*., 2005). However, mannitol provoked cough is independent of the bronchoconstrictor effect suggesting involvement of different physiological mechanisms (Koskela *et. al*., 2005). Interestingly, the frequency of coughing after mannitol challenge is related to the severity of asthma and decreases with inhaled corticosteroids.

Unlike other direct and indirect provoking stimuli, the mannitol challenge test is performed using a standardized test administration kit which is simple to use, inexpensive, safe and well tolerated by most patients (Anderson *et. al*., 1997; Ballweg, 2012). Side effects such as coughing have been reported (Anderson *et al*., 2009; Barben *et al*., 2011; Brannan *et al*., 2005; Holzer *et al*., 2003; Kersten *et al*., 2009).

Airway challenge with inhaled mannitol requires minimal equipment and the disposable nature of the device and the capsules containing the dry mannitol powder makes it a useful alternative to other methods of performing airway challenges which usually require significant laboratory equipment, cleaning and maintenance (Anderson *et al*., 1997; Ballweg, 2012). In addition, serial airway challenges on separate days have shown that inhaled mannitol is a reproducible test of airway responsiveness (Barben *et al*., 2003; Brannan, 1998) with fewer adverse effects than have been reported with methacholine and histamine (Anderson *et al*, 1997; Anderson *et al*., 2010). Spontaneous recovery following a standardized mannitol airway challenge has also been shown to compare favourably with that following methacholine challenge (Anderson *et al*, 1997, Anderson *et al.*, 2010). Furthermore, airway challenges with inhaled mannitol take less time to perform than do other types of airway challenges (Anderson *et al*., 2009; Barben *et al*., 2011; Kersten *et al*., 2009).

1.2.5.1 Sensitivity and Specificity of Inhaled Mannitol

Airway hyperresponsiveness has been regarded as a hallmark of asthma (Sverrild *et al*., 2009; Woolcock, 1988), and exercise has previously been recognized as one airway provocation method that will induce significant airflow obstruction in almost all asthmatics (Jones *et al*., 1963). In clinical research, it has been shown that inhaled mannitol is a useful practical means of assessing bronchial hyperresponsiveness, with its use correlating closely and somewhat equivalent to the airway response following inhaled adenosine monophosphate (Currie *et al*., 2003) and methacholine airway challenge (Anderson *et al*., 2009). Inhaled mannitol has also been shown to compare well with exercise induced bronchoconstriction (Barben 2011; Kersten *et al*., 2009) and it could be a useful alternative to eucapnic voluntary hyperapnea used for identifying exercise induced asthma (Holzer *et al*., 2003). However, it is known that some individual asthmatics respond to one type of airway challenge but not another (Anderson *et al*., 2009; Barben *et al*., 2011; Porsbjerg *et al*., 2007).

Two important factors used in comparing the efficacy of different airway challenges are the sensitivity and specificity of being able to identify airway hyperresponsiveness and diagnose asthma. Ideally, a high sensitivity and a high specificity would be preferred characteristics of a bronchoprovocation test used in asthma diagnosis. However, a number of direct and indirect airway challenge techniques including mannitol have been reported to have widely varying levels of sensitivity (32% to 90%) (Anderson *et al*., 2009; Andregnette-Roscigno *et al*., 2010; Barban *et al*., 2011; Kersten *et al*., 2009; Koskela *et al*., 2003b; Porsbjerg *et al*., 2007; Sverrild *et al*., 2010) and specificity (73 to 98%)(Anderson *et al*., 2009; Barban *et al*., 2011; Sverrild *et al*., 2010). Interestingly, bronchoprovocation with mannitol compared favorably with other challenge tests with a sensitivity of 43% to 64% and a specificity of 73% to 98.4%.

Although, the accepted value for a positive response to inhaled mannitol is a fall in FEV_1 of ≥ 15%, lower cutoff values have been employed (Holzer *et al*., 2003; Sverrild *et al*., 2009). Given the comparatively low sensitivity of mannitol and other types of airway challenge in identifying asthmatics when more traditional higher

cutoff levels are used, a reduction in the cutoff value for positive response may be a useful consideration. In fact, Sverrild *et al* (2009) showed that the sensitivity for a positive response to inhaled mannitol challenge would increase if cut-off values less than a 15% fall in FEV_1 are used to define a positive bronchoprovocation response. In their study, the sensitivity of mannitol increased from 58.8% to 70.6% when the cutoff value for the fall in FEV_1 was reduced from ≥ 15% to ≥ 10%, with an acceptable fall in specificity from 98.4% to 93.0%.

1.3 Potential Pharmacological Significance and Therapeutic Role of Phospholipids

Various foods are a palatable cheap and rich source of phospholipids that can exert a range of biological actions with pharmacological potential. Bananas are one example of such foods (Hills and Kirkwood, 1989). A superior example is soy lecithin, becauseit contains markedly higher concentrations of surface active phospholipids than do bananas (Beare-Rogers *et al*., 1992; Helmerich and Koehler 2003; Hurst and Marttin, 1984; Nzai and Proctor, 1999; William and Aoyagi, 2007).

Phospholipids in endogenous lung surfactants (principally dipalmitoylphosphatidylcholine) are known to reduce the collapsing pressures generated by surface tension within the alveoli and small airways and thereby promote patency in these respiratory structures (Goerke and Clements, 1986; Hawgood, 1991; Hills, 1988; Macklem *et al*., 1970; Whitsett, 1991). These considerations raise an important clinical question as to whether phospholipids surfactants can be pharmacologically useful in the treatment of asthma.

Physical studies of banana suspensions have shown that the fruit contains substances which are highly surface active both at liquid and solid-liquid interfaces. Bananas contain lamellar-like bodies similar to those produced by alveolar type II cells, the principal source of surface active phosphatidylcholine in the lungs (Hills and Kirkwood, 1989). Ideally, the surface monolayer of lung surfactants needs to be compressed to achieve dramatic reductions in surface tension (Hills and Kirkwood

1989; Whitsett, 1991). In contrast, bananas vortexed in water display a dramatic reduction in surface tension from 72 to 10 dyne/cm without compression of the phospholipid monolayer (Hills and Kirkwood, 1989). Such high surface activity may potentially be of value clinically, and the same would be expected of the phospholipid component in soy lecithin.

Ueda and co-workers (1995) published a series of electromicrographic studies which clearly demonstrated an oligolamellar surfactant layer immediately adjacent to the epithelium of the alveoli and upper airways (Ueda *et al.*, 1995). Hills (2002a) states that this lamellar lining to the airways of the lung extends to the bronchi where, beneath the mucus layer, it is adsorbed to sites adjacent to chemoreceptors, if not directly adsorbed to the receptors themselves. In addition, Hills (2002a) hypothesized that surfactant would seem ideal for masking airway irritant receptors because surface active phospholipid in surfactant is capable of binding tenaciously to surfaces which prevents noxious inhaled agents from reaching the irritant receptors. Moreover, asthma has been associated with surfactant deficiency (Enhorning *et al.*, 1989), and this has led to speculation that agents that can uncover or otherwise compromise the adsorbed layer of surface active phospholipids by physical or biological means can potentiate asthmatic attacks (Hills, 1996a). Such unmasking or uncovering is a well-known concept in neurophysiology, and was introduced as a physical mechanism to explain the marked (up to 100 fold) sensitization of a reflex sometimes observed in the central nervous system (Hills, 1996a; Hills 1996b).

Hills (1996a) speculated that the unmasking of more irritant receptors by various sensitizing agents which remove the surface active phospholipids barrier could be the underlying mechanism for asthma. Citing the work done by Crawford and Young (1995), in which the administration of aerosolised exogenous surfactant in asthmatics resulted in improvement of the airway patency, Hills (1996a) argued that the known deficiency of surfactant in asthmatics compromise the barrier layer, leaving more receptors uncovered and so sensitize the lung to various triggers. To support this, Hills and Chen (2000) carried out a series of experiments to determine whether phospholipids applied to bronchial mucosa will reduce the sensitivity of irritants

receptors in rat airways to standard airway challenge with methacholine aerosol. Their results showed that surface active phospholipids have appreciable effects on irritant receptors, reducing neural response to a methacholine challenge by a comparable amount to that of salbutamol. As this supports the concept of surface active phospholipid masking bronchial irritant receptors, these workers suggested that placebo-controlled clinical trials, using exogenous surface active phospholipids as a means of controlling asthma should be considered (Hills and Chen, 2000).

Neural afferent pathways from the larynx and tracheobronchial tree have been shown to be extensively involved in reflex bronchoconstriction and the genesis of coughing (Nadel and Widdicombe, 1962; Widdicombe, 1963; Widdicombe, 1986). However, coughing can also be induced by pharyngeal stimulation (Irwin and Widdicombe, 2000). In addition, several studies have shown that local anaesthesia of the pharynx with lidocaine solution ameliorates the bronchoconstrictive response to exercise and inhaling cold air in asthmatics (McNally *et al.*, 1979; Strauss *et al.*, 1977). These findings suggest that neural receptors in the pharynx play a role in the development of bronchoconstriction and cough that can be induced by exercise and cold air. Contrary evidence has been reported regarding the effect of local anaesthesia on airway provocation sensitivity in asthmatics. Caire and associates (1989) reported that inhaled lidocaine aerosol did not block bronchoconstriction induced by hyperventilation of cold dry air in asthmatics. Therefore, in contrast to previously mentioned studies, Caire and associates (1989) concluded that it was unlikely that upper airway receptors could modulate bronchoconstriction. However, it is plausible that this disparity was due to inadequate receptor blockage following local anaesthetic inhalation.

It has been speculated that airway receptors can be masked by adhering phospholipids and that such an effect may de-sensitize these receptors and reduce the ability of inhaled trigger factors to initiate bronchoconstriction in asthmatics. There is also good experimental evidence which suggests that pharyngeal receptors play an important role in triggering bronchoconstriction in asthmatics and that such receptors can be pharmacologically modified by orally administered medications. This raises the

possibility that orally administered phospholipids may be of value in the pharmacological treatment of asthma. However, currently available clinical phospholipid surfactants are expensive. Soy lecithin is a cheap, readily available and palatable source of highly surface active phospholipids which bind to mucosal surfaces for extended periods, and provide protection at such surfaces. In view of these considerations, phospholipids show promise for the pharmacological treatment of asthma, and for studying how trigger factors induce bronchoconstriction and cough in asthma.

SECTION B

Experimental Investigation and Discussion

CHAPTER TWO

Airway Hyperresponsiveness Assessed By Inhaled Mannitol in Asthmatics

2.1 Introduction

Investigations into how exogenous phospholipids binding to the oropharyngeal mucosa may influence airway function in asthmatics could be achieved by comparing the effects of oropharyngeal phospholipid administration on airway responsiveness to inhaled bronchoprovocating agents. Airway hyperresponsiveness testing with inhaled bronchoprovocating agents is routinely done with asthmatic patients in lung function laboratories. However, a variety of different bronchoprovocating agents are used, which raises the question of which such agent is best suited for the purposes of this research.

Inhaled mannitol (Aridol™, Pharmaxis Ltd., Sydney, Australia) is a comparatively new product that serves a variety of uses in clinical research and in the diagnosis of asthma and other airway disorders (de Nijs *et al*., 2011), as well as a possible treatment option for patients with cystic fibrosis (Minasian, 2008; Spector, 2010).Its use is favored over other methods of bronchial hyperresponsiveness testing in many laboratories and clinics because it is convenient to use. As a standardized product, Aridol™ mannitol inhalers offer a means of delivering standardized known amounts of inhaled mannitol without the need for laboratory preparation of the bronchoprovocative agent (as required with the use of histamine and methacholine). In addition, good repeatability in the level of airway obstruction has been reported when mannitol airway challenges were performed on separate days (Anderson *et al*., 1997; Barben *et al*., 2003; Subbarao *et al*., 2000).

Inhaled mannitol is thought to indirectly produce bronchoconstriction by increasing the osmolarity of the periciliary fluid of the airway mucosa, thereby causing a release of mediators from airway cells such as mast cells and eosinophils which act on airway smooth muscle to cause bronchoconstriction (Anderson *et al*., 1997; Brannan *et al*., 2001; Brannan *et al*., 2003; Koskela *et al*., 2005; Leuppi *et al*., 2002). However, stimulation of C-fibre receptors which produces a central cough reflex as a result of the hyperosmolarity of the airway may also contribute (Koskela *et al*., 2005).

Histamine and methacholine are airway provoking stimuli which directly exert their effects by acting directly on airway receptors to elicit bronchoconstriction. In contrast, indirect acting stimuli include important examples of agents such as exercise and inhalation of cold dry air that commonly trigger asthmatic exacerbations and worsening asthma symptoms (Ballweg, 2012), and may therefore be superior to direct acting bronchoprovocative agents for revealing characteristics of airway inflammation in addition to the sensitivity of the airway smooth muscles. It has therefore been argued that testing of airway hyperresponsiveness with indirect provocation agents such as inhaled mannitol is more clinically relevant in airway assessment than testing with directly acting stimuli (Ballweg, 2012).

Asthmatics respond with greater bronchial responsiveness to inhaled mannitol than do healthy subjects (Anderson *et al*., 1997; Subbarao *et al*., 2000), and this is similar to the airway hyperresponsiveness elicited by other airway provocation agents. However, a significant number of asthmatics do not respond to inhaled mannitol (Anderson *et al*., 2009; Andregnette-Roscigno *et al*., 2010; Barban *et al*., 2011; Kersten *et al*., 2009; Koskela *et al*., 2003b; Porsbjerg *et al*., 2007; Sverrild *et al*., 2010). Also, for reasons which remain unclear, some asthmatics exhibit plateaus in their dose-response relationship during mannitol provocation such that no further decreases in FEV_1 are evident when more mannitol is inhaled (Anderson *et al*., 1997). Of interest, this pattern is similar to what is observed when methacholine is inhaled by normal subjects, who showed a plateau on the dose-response relationship (Moore *et al*., 1998).

A primary objective of this thesis will be to examine whether exogenous phospholipids binding to the oropharyngeal mucosa can influence airway function in asthmatics. This could be achieved by examining the effects of oropharyngeal phospholipid administration on the airway responsiveness to inhaled bronchoprovocating agents. As already discussed, inhaled mannitol may be a suitable and convenient means for inducing reproducible levels of airway provocation for this purpose. However, the available literature has revealed that a significant proportion of asthmatics do not respond to inhaled mannitol. This argues that it would be prudent to first carry out an initial study in order to better describe how asthmatics respond to inhaled mannitol in asthmatics, as well as identifying a group of asthmatic responders to inhaled mannitol who can later be recruited into the phospholipid – airway function study.

2.1.1 Aims of the Study

This study had the following aims:

1. To examine the convenience and suitability of a standardized inhaled mannitol product (Aridol™) in order to determine airway hyperresponsiveness in a group of physician diagnosed asthmatics.
2. To explore the sensitivity and dose-response relationship to inhaled mannitol in a group of physician diagnosed asthmatics.
3. To investigate the relationship between severity of asthma and the response to airway provocation with inhaled mannitol by determining whether the decrease in FEV_1 in response to mannitol airway provocation is dependent upon the baseline level of obstruction in a group of physician diagnosed asthmatics.
4. To identify a group of physician diagnosed asthmatics who respond to inhaled mannitol and can serve as participants for later phospholipids – airway function study.

2.2 Methods and Materials

2.2.1 Consent and Confidentiality of Information

This study was approved by the Human Research Ethics Committee of the University of New England (Approval No HE11/071). Each participant provided informed and signed consent prior to commencement of the study. Every participant was assigned an identification code to ensure that participants' data were only identifiable to the higher degree research candidate (HDR) candidate and his Principal Supervisor Dr Tom van der Touw.

2.2.2 Participant Selection

Men and women who had previously been diagnosed as having asthma by a medical practitioner were invited to participate in this study. After providing informed consent to participate in the study, adult (at least 18 years of age) asthmatics attended the laboratory to ascertain their suitability for the study. Potential participants were asked to refrain from taking substances that contain stimulants and lecithin (cola drinks, coffee, chocolate and confectionaries before coming to the laboratory on the day of each study. They were also to refrain from taking any short-acting bronchodilator medication such as Ventolin for at least 6 hours before coming to the laboratory. In addition, participants remained on prescribed preventer and long acting beta agonist medication throughout the study period. However, they were instructed to take their bronchodilator medication for symptomatic relief before the study if they believed this was necessary, and then inform the researchers that this has occurred and arrange for an alternate time to visit the laboratory. Participants were also asked to refrain from physical exercise during the entire day of their visit to the laboratory and to bring their personal Ventolin inhaler with them when they came to the laboratory. A Ventolin inhaler with a spacer device was always available in the laboratory.

After arrival at the laboratory, suitability of participants for this study was determined by means of a series of questions about their asthma history, and by spirometric

measurement of baseline forced expiratory lung function. The questions related to the participants' asthma history, current asthma status and known food allergies and were aimed at ensuring that the participants:

- Had been previously diagnosed with asthma by a medical practitioner.
- Had asthma that was well controlled with medication prescribed by their medical practitioner.
- Were not heavily reliant on short-acting bronchodilators such as Ventolin and did not take such medications more than 3 times a week
- Currently had a Ventolin inhaler which was been prescribed by their medical practitioner.
- Did not have significant worsening of asthma symptoms in the last 3 months or had a respiratory infection including colds and flues in the past 2 months.
- Had never been hospitalized for asthma.
- Were non-smokers.
- Were otherwise healthy without any significant illnesses other than asthma.
- Were not pregnant or lactating.
- Were 18 years of age or older.

To enter the study, participants were also required to have a forced expiratory volume of 1 second (FEV_1) of at least 60% of predicted. The best of three FEV_1measurements was used for this purpose.

2.2.3 Airway Provocation Protocol

Participants who met the asthma history and spirometric lung function criteria underwent progressive airway challenges with a dry powder preparation of mannitol from a halerimetric disposable Aridol inhaler (Aridol™, Pharmaxis Ltd., Sydney, Australia) (refer to Figure 2.1) using a previously described standardized protocol (Anderson *et al*., 1997). The progressive airway challenges were used to determine the cumulative dose of inhaled mannitol required to produce 5%, 10% and 15%

decreases in baseline FEV_1 (PD5, PD10 and PD15 respectively). Participants remained seated throughout the airway provocation protocol.

Figure 2.1 - Aridol™ capsules and disposable inhaler

A nose clip was positioned on the participant prior to airway provocation. Mannitol doses commenced at 0 mg (empty capsule served as the control), followed by 5, 10, 20, 40, 80, 160, 160 and 160 mg (the 80 mg and 160 mg doses were given as multiple 40mg doses because the maximum dose per capsule was 40 mg) to achieve a maximum total dose administered of 635 mg. Care was taken to ensure that all the powder within each capsule had been inhaled completely. After each mannitol inhalation, the subjects were instructed to hold their breath for 5 seconds and to exhale gently. Duplicate measurements of lung function were then made after 60 seconds post inhalation of each inhaled dose of mannitol, and the better of the two measurements was taken as the post inhalation FEV_1 at that time. Each serial best-of-two FEV_1 measurement was recorded and tabulated with its respective cumulative inhaled mannitol dose.

The FEV_1 measured after inhalation of the 0 mg capsule was taken as the pre-challenge FEV_1 baseline, and all subsequent measurements were referenced against this value to determine the percentage decrease (fall) in FEV_1 following each provocation with inhaled mannitol. A progressive provocation challenge test was deemed complete once the FEV_1 decreased by at least 15%, when there was a 10% decrease in lung function between successive provocations, or when a total dose of 635 mg of mannitol had been inhaled.

Care was taken to ensure that the FEV_1 had returned to at least the baseline pre-provocation level before participants left the laboratory. To ensure this, participants self-administered Ventolin from a metered dose inhaler through a spacer device if required. Participants who did not show at least a 10% decrease in their baseline FEV_1 after mannitol challenge were excluded from the later phospholipids – airway function study.

2.2.4 Spirometric Equipment

The experimental set up consisted of a spirometer (Super Spiro, SU6000, Micro Medical Ltd., Kent, England), a laptop computer with dedicated software (Spirometry Software, 36-SPC1000-STK, Micro Medical Ltd., Kent, England) which provides spirometric analysis of the recorded data on a laptop computer, a disposable microbial filter, disposable nose clip, a spacer device and a Ventolin metered dose inhaler (refer to Figure 2.2).

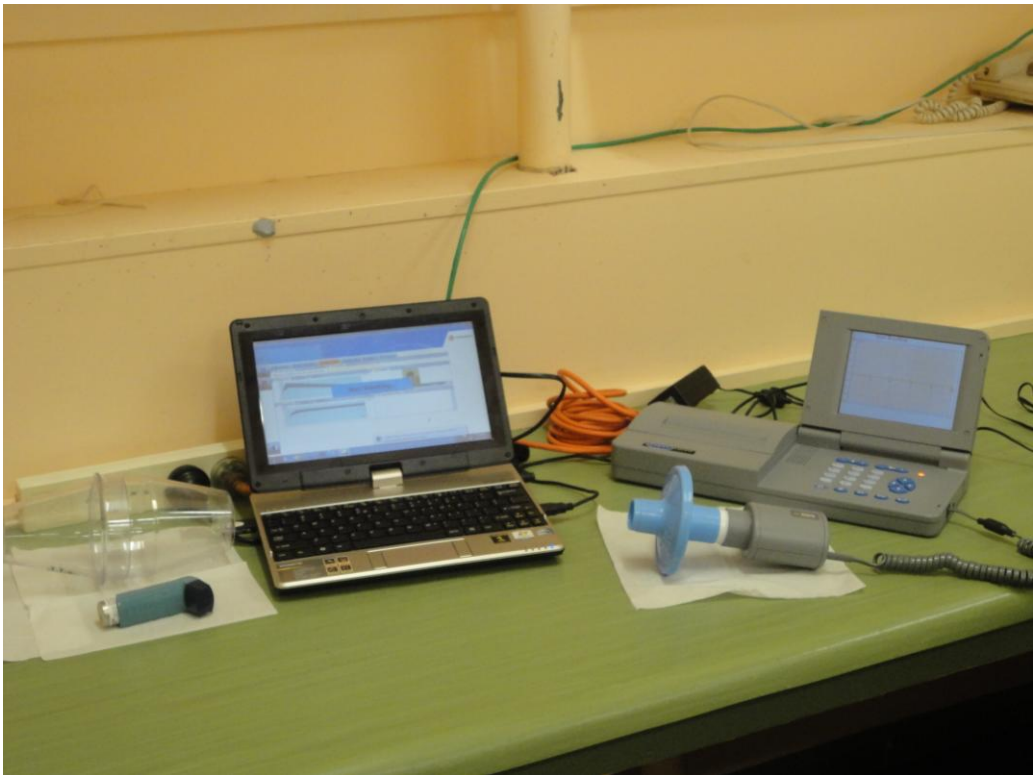

Figure 2.2 – Experimental set up showing the Super Spiro spirometer with microbial filter attached to the flow sensor, personal computer, Ventolin and a spacer device.

2.2.5 Classification of Asthma Severity

The severity of asthma of each participant recruited into the study was classified into one of 4 categories (Intermittent, Mild Persistent, Moderate Persistent, Severe Persistent) according to their daily medication regimen and response to treatment as described in the Global Initiative for Asthma guidelines (GINA, 2002).

2.2.6 Analysis

To ensure that spirometric data were only collected from satisfactory FVC manoeuvres, the suitability of each forced expiratory manoeuvre was automatically assessed by the Super Spiro spirometer and by visual inspection of each maximum expiratory flow-volume curve using the criteria of Johns and Pierce (2008). The asthma severity of each participant was determined according to their daily medication and response to treatment using GINA (2002) guidelines. Unless stated

otherwise, numeric data were reported as means ± 1 standard deviation. The response-to-dose ratio (RDR) was calculated as the cumulative % fall in FEV_1 per mg of cumulative inhaled mannitol. Statistical analyses of the data were carried out using Excel (2007 version, Microsoft) software, and consisted of paired and unpaired *t* tests (2 tailed) for comparisons between two groups, repeated measures one way analysis of variance and the least significant difference test for multiple comparisons, linear regression and linear correlation, with the null hypothesis being rejected at $p < 0.05$.

2.3 Results

2.3.1 Participants

A total of 24 participants met the inclusion criteria and participated in the study. Two participants did not show a decrease in baseline FEV_1 after the complete inhalation of the maximum allowable mannitol dose (635 mg), and another two participants showed a decrease in baseline FEV_1 of less than 5%. The remaining twenty participants consisted of 10 men and 10 women. Details of the 24 participants are shown in Table 2.1. Each participant had Ventolin prescribed by their medical practitioner. In addition, 16 participants were on daily inhaled corticosteroids (ICS) and 1 patient was on Montelukast (an oral leukotriene receptor antagonist). Each participant refrained from taking short-acting bronchodilators for at least 6 hours before coming to the laboratory but continued taking their preventer and long acting beta agonist medications if these medications had been prescribed. Before leaving the laboratory, the FEV_1 of each participant had returned to at least the level measured before airway challenge. No adverse effects were noted during the study and none of the participants showed or reported spirometric evidence of respiratory fatigue or exhaustion from the repeated FVC maneuvers in this study.

Table 2.1 - Anthropometric details showing the age, height, body mass index and baseline FEV_1 of 20 asthmatic participants.

	Men (n=10)	Women (n=10)	Total (n=20)
Age (yrs)	27 ± 11 (18 – 51)	27 ± 11 (19 – 51)	27 ± 11 (18 –51)
Height (cm)	178 ± 5 (169 – 185)	156 ± 8 (155 – 175)	170 ± 9 (155 – 185)
Body Mass Index (kg/M^2)	24.2 ± 2 (20.5 – 26.3)	25.6 ± 6 (20.9 – 40.8)	24.9 ± 4 (20.5 – 40.8)
Baseline FEV_1 (% Pred.)	83 ± 14 (61 – 107)	92 ± 12 (74 – 110)	88 ± 13 (61 – 110)

Note values are means ± 1 standard deviation. Minimum and maximum values are given in parenthesis.

2.3.2 Interindividual Variation in the Response to Mannitol Airway Challenge

As shown in Table 2.2 and Figure 2.3, substantial interindividual variation was observed in the FEV_1 responses to inhaled mannitol. The inhaled mannitol dose required to produce a maximum response varied substantially between different individuals, and no clear trend was evident in this variation. Most participants required a maximum dose of 635 mg of mannitol before a maximal decline in FEV_1 was observed, and this decline was mostly below 15%. Indeed, only 7 of the 24 participants (sensitivity of 29.1%) showed a maximum fall in FEV_1 of at least 15%, whereas 13 participants (sensitivity of 54.2%) showed a maximum fall in FEV_1 of at least 10%, and 20 participants (sensitivity of 83.3%) showed a maximum fall in FEV_1 of at least 5%. Most of the participants showed a maximum fall in FEV_1 of less than 15%, and twelve participants had the maximum dose of 635 mg administered before recording at least a 5% fall in FEV_1 from baseline. However, participants with a greater than 15% decrease in FEV_1 did so at varying maximal mannitol doses. None of the participants who showed a fall in FEV_1 of greater than 15% received the maximum mannitol dose of 635 mg. The greatest fall in FEV_1 observed was 23% and this was seen in two participants, but required markedly different mannitol doses (35 mg and 155 mg respectively). These features were evident in both men and women participants (refer to Figures 2.4 and 2.5).

Table 2.2 – Dose – response characteristics of inhaled mannitol in all 24 asthmatic participants.

Participant	Asthma Severity	Gender	Baseline FEV_1 (% pred)	Total mannitol dose (mg)	Max fall in FEV_1 (%)	Medication with ICS
1	Mild persist	Female	103	315	12.7	Yes
2	Mild persist	Female	110	635	0	Yes
3	Intermittent	Female	89	635	6.1	No
4	Mild persist	Female	96	635	0	Yes
5	Mild persist	Female	76	635	16.3	Yes
6	Mild persist	Female	110	635	11.6	Yes
7	Mod persist	Female	108	635	11.4	Yes
8	Mild persist	Female	77	635	2.7	Yes
9	Mild persist	Female	93	155	22.5	Yes
10	Mild persist	Female	74	475	17.5	No
11	Mild persist	Female	91	635	9.1	Yes
12	Mild persist	Female	83	315	17.7	Yes
13	Mild persist	Female	94	635	4.5	Yes
14	Intermittent	Male	107	235	17.5	No
15	Mild persist	Male	80	635	8.8	Yes
16	Mild persist	Male	85	635	13.5	Yes
17	Mild persist	Male	74	635	5.7	No
18	Intermittent	Male	95	635	10.1	No
19	Intermittent	Male	85	635	12.6	No
20	Mild persist	Male	72	35	23.1	No
21	Mod persist	Male	61	315	9.4	Yes
22	Mild persist	Male	97	635	5.2	Yes
23	Mild persist	Male	97	635	2.5	Yes
24	Mild persist	Male	76	155	16.7	No

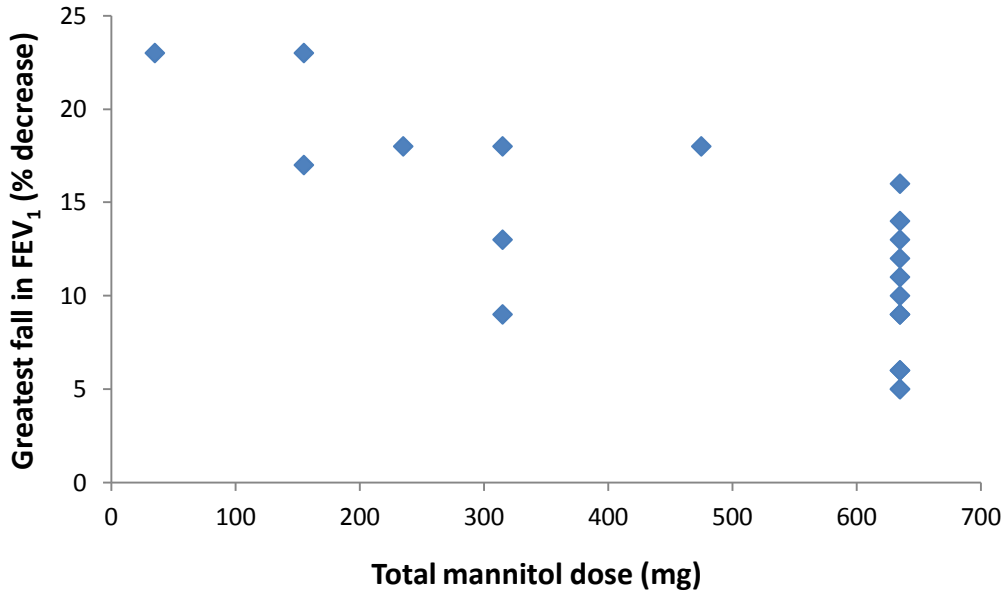

Figure 2.3 - Greatest fall in FEV_1 versus the total inhaled mannitol dose in the 10 men and 10 women asthmatics who displayed a fall in $FEV_1 \geq 5\%$.

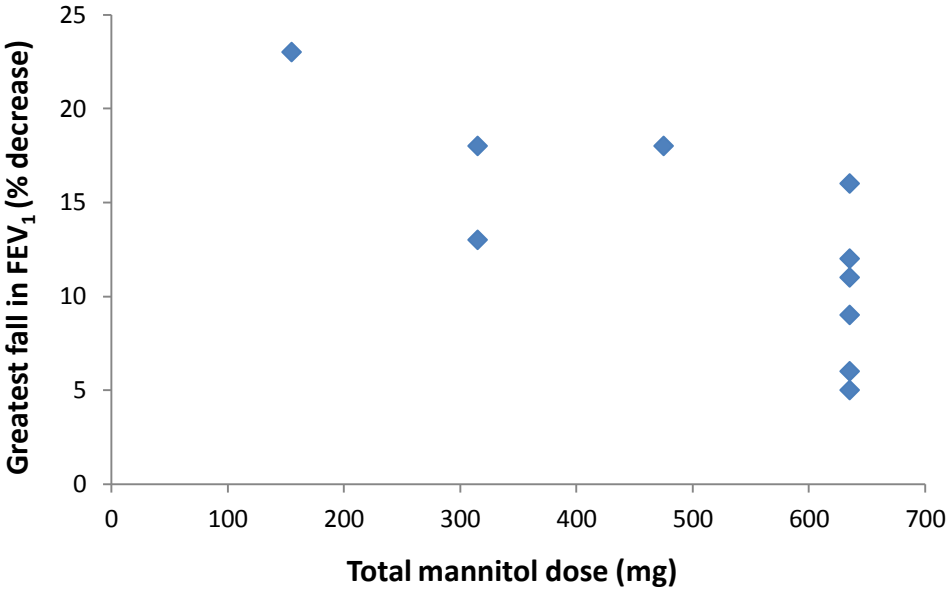

Figure 2.4 - Greatest fall in FEV_1 versus the total inhaled mannitol dose in the 10 women asthmatics who displayed a fall in $FEV_1 \geq 5\%$.

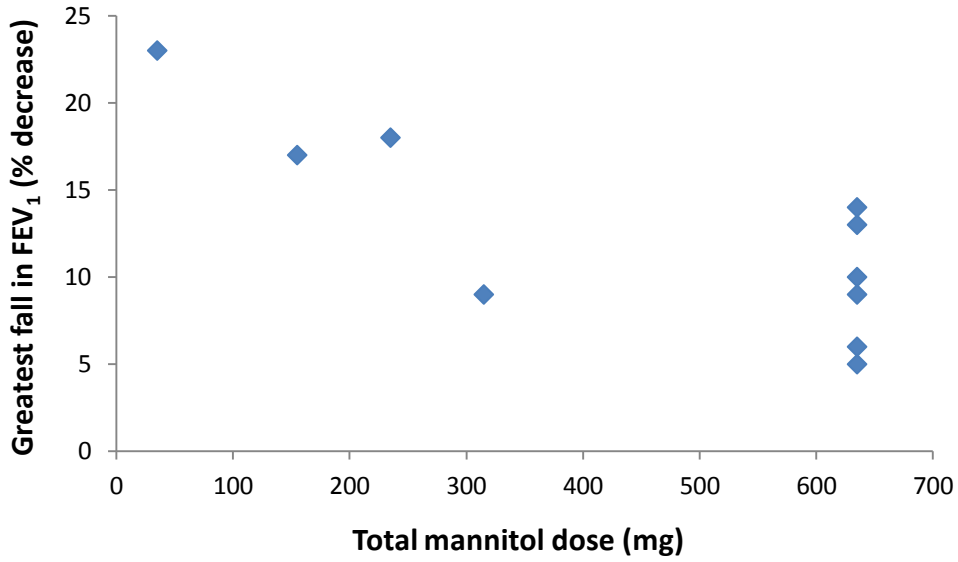

Figure 2.5 - Greatest fall in FEV_1 versus the total inhaled mannitol dose in the 10 men asthmatics who displayed a fall in $FEV_1 \geq 5\%$.

2.3.3 Role of Baseline FEV_1 and Asthma Severity on Airway Hyperresponsiveness

No relationship was evident between the baseline FEV_1 and the PD5 in the group as a whole (refer to Figure 2.6), the women participants (refer to Figure 2.7) or the men participants (refer to Figure 2.8). Similarly, no relationship was evident between the baseline FEV_1 and the PD10 (refer to Figures 2.9, 2.10 and 2.11), or between the baseline FEV_1 and the PD15 (refer to Figure 2.12). In addition, no association was apparent between the 24 participants' asthma severity and either their maximum fall in FEV_1 or their response-to-dose ratio for inhaled mannitol (refer to Table 2.3). Instead a wide overlap in these parameters was evident among the different grades of asthma severity. No association was also evident between the participants' age and the PD5, PD10 and PD15 (refer to Figures 2.13, 2.14 and 2.15).

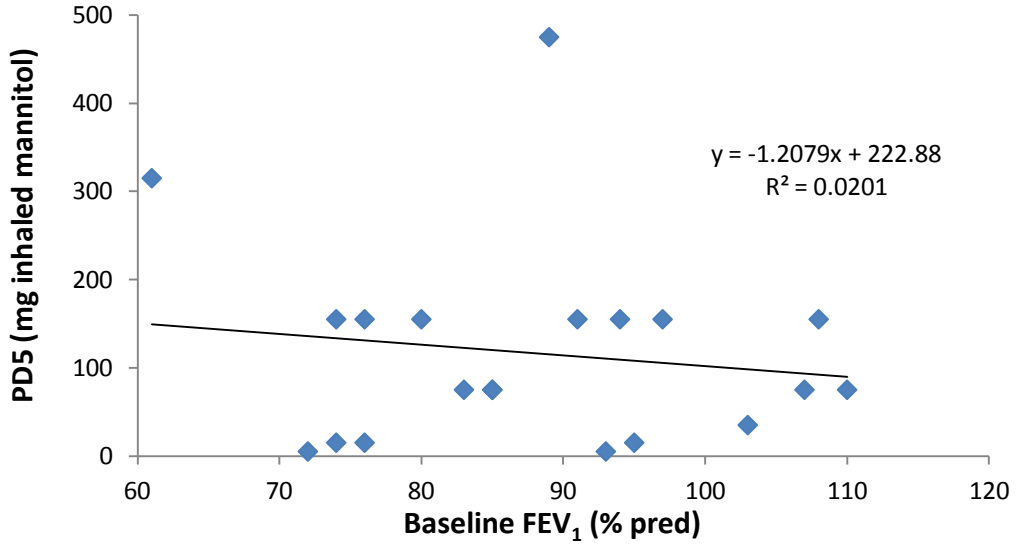

Figure 2.6 - Inhaled mannitol dose required to produce a 5% decrease in FEV_1 (PD5) versus the baseline FEV_1 (% predicted) in 10 men and 10 women asthmatics.

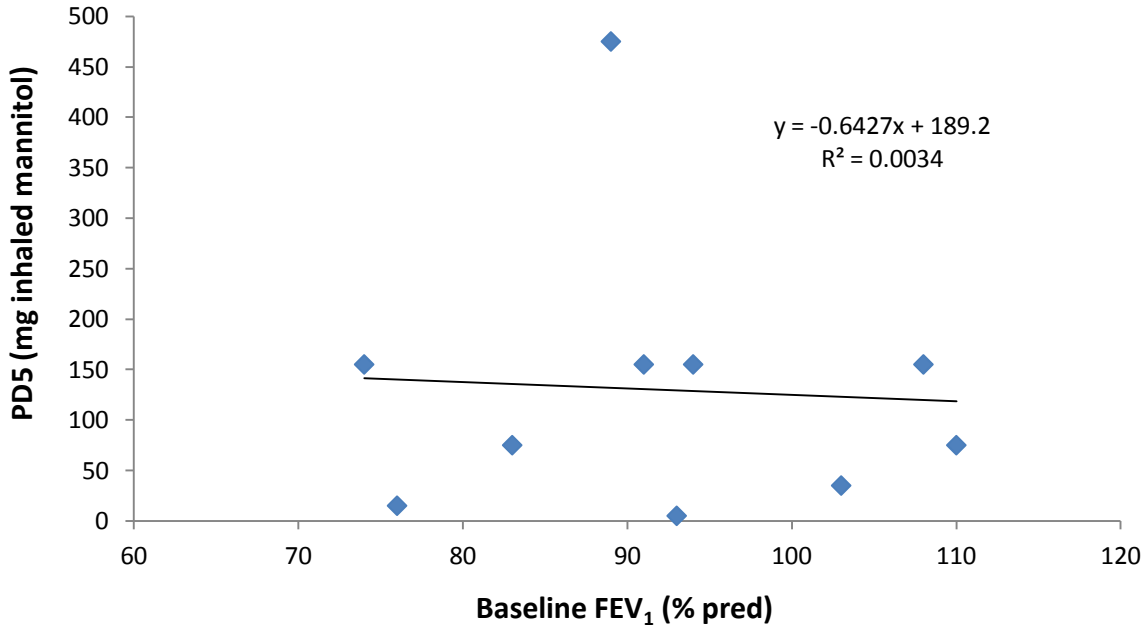

Figure 2.7 - Inhaled mannitol dose required to produce a 5% decrease in FEV_1 (PD5) versus the baseline FEV_1 (% predicted) in 10 women asthmatics.

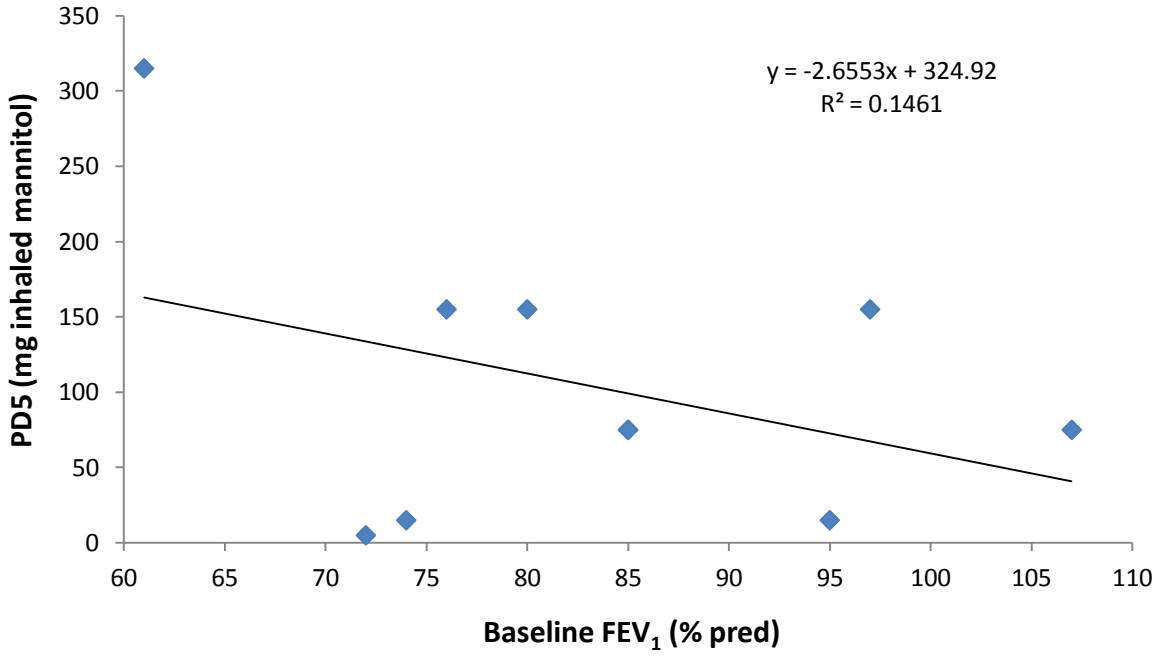

Figure 2.8 - Inhaled mannitol dose required to produce a 5% decrease in FEV_1 (PD5) versus the baseline FEV_1 (% predicted) in 10 men asthmatics

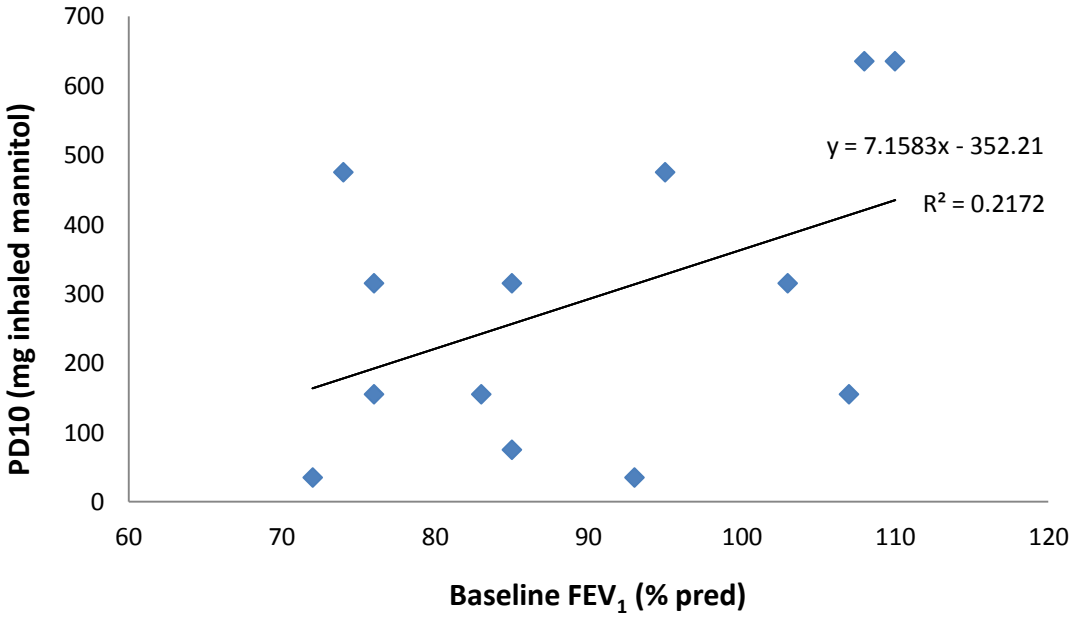

Figure 2.9 - Inhaled mannitol dose required to produce a 10% decrease in FEV_1 (PD10) versus the baseline FEV_1 (% predicted) in the 13 asthmatics who achieved a fall in FEV_1 of ≥ 10%.

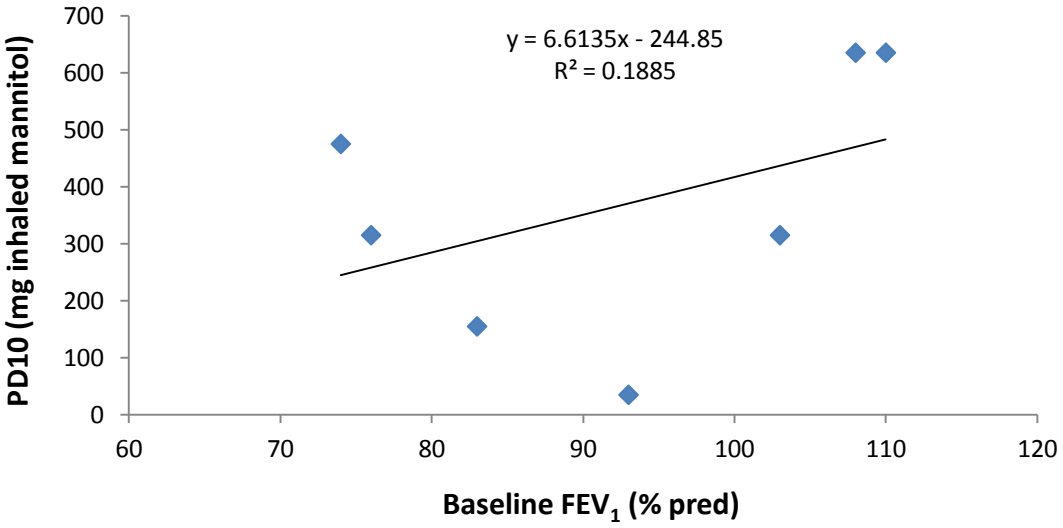

Figure 2.10 - Inhaled mannitol dose required to produce a 10% decrease in FEV_1 (PD10) versus the baseline FEV_1 (% predicted) in the 7 women asthmatics who achieved a fall in FEV_1 of ≥ 10%.

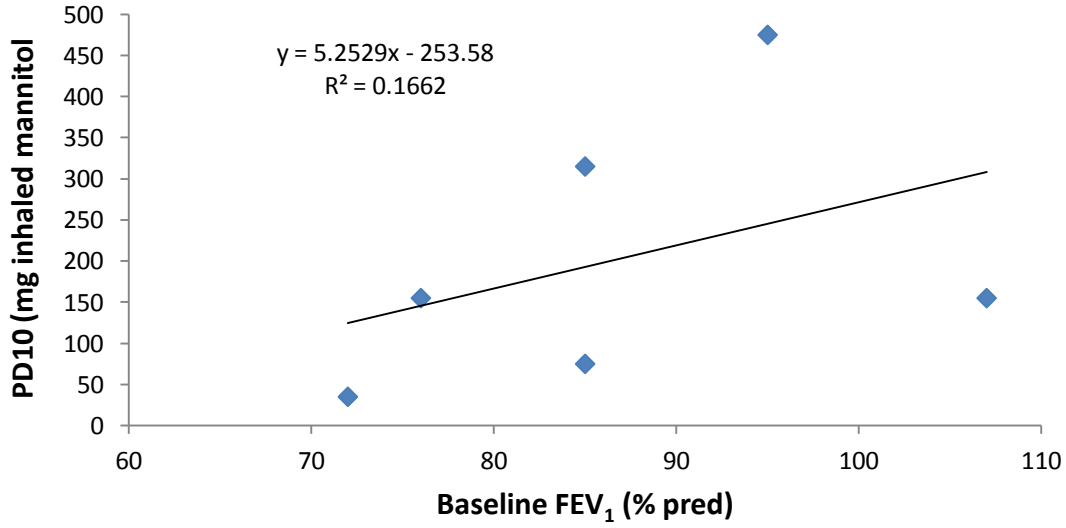

Figure 2.11 - Inhaled mannitol dose required to produce a 10% decrease in FEV_1 (PD10) versus the baseline FEV_1 (% predicted) in the 6 men asthmatics who achieved a fall in FEV_1 of ≥ 10%.

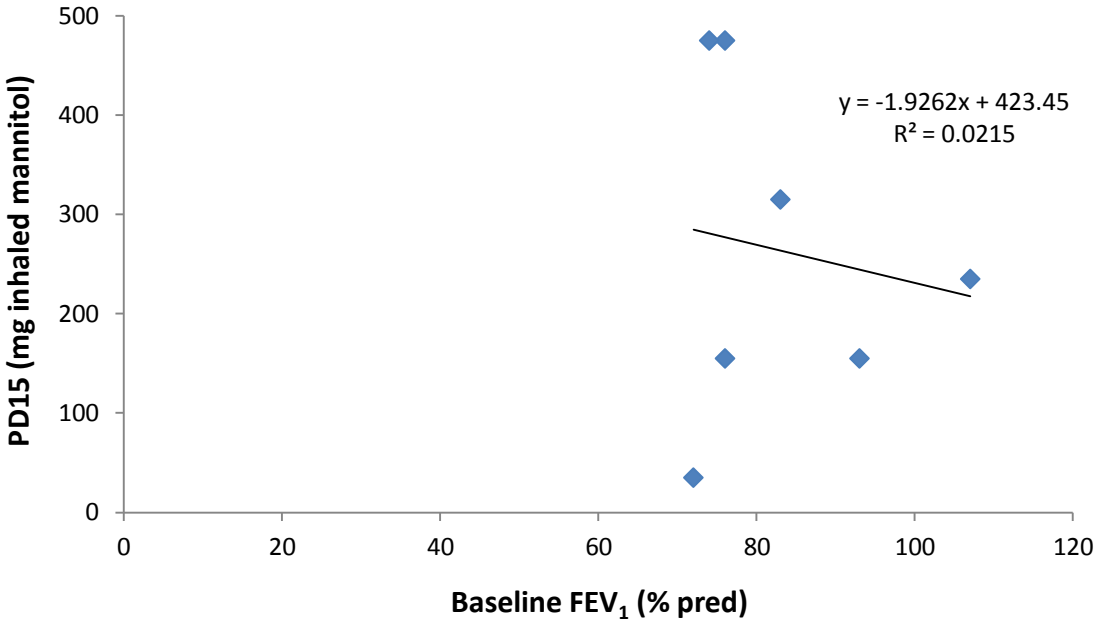

Figure 2.12 - Inhaled mannitol dose required to produce a 15% decrease in FEV_1 (PD15) versus the baseline FEV_1 (% predicted) in the 7 men and women asthmatics who achieved a fall in FEV_1 of ≥ 15%.

Table 2.3 – Comparison between asthma severity and response to inhaled mannitol in all 24 asthmatic participants.

Asthma severity (GINA 2002 classification)	No. of asthmatics	Maximum % fall in FEV_1 from baseline	RDR
Intermittent	4	6 - 18	0.009 - 0.077
Mild persistent	18	0 - 23	0 - 0.657
Moderate persistent	2	9 - 11	0.017 - 0.029
Severe persistent	0	_____	_____

Note: - the response-to-dose ratio (RDR) was calculated as the cumulative % fall in FEV_1 per mg of cumulative inhaled mannitol.

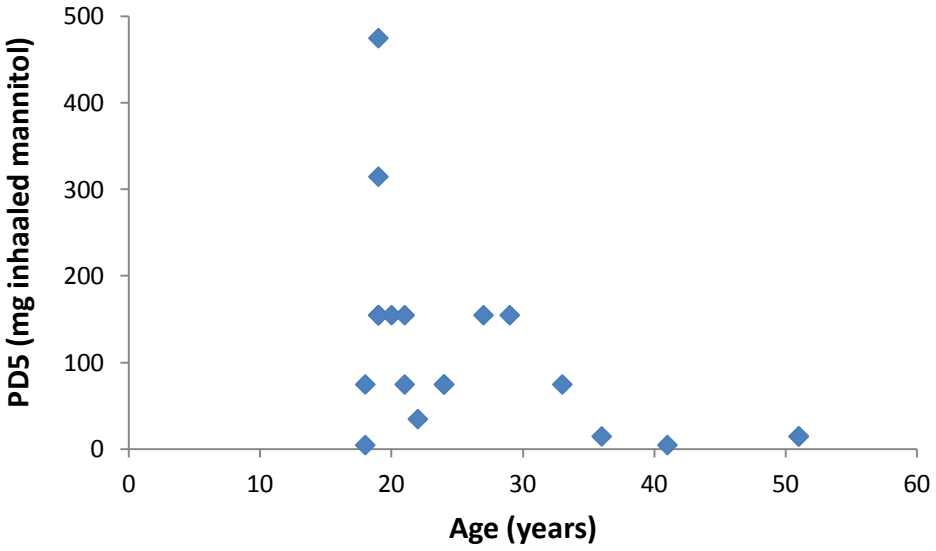

Figure 2.13 - Relationship between dose of inhaled mannitol required to produce a 5% decrease in baseline FEV_1 (PD5) and age in the 10 men and 10 women asthmatics who achieved a fall in $FEV_1 \geq 5\%$.

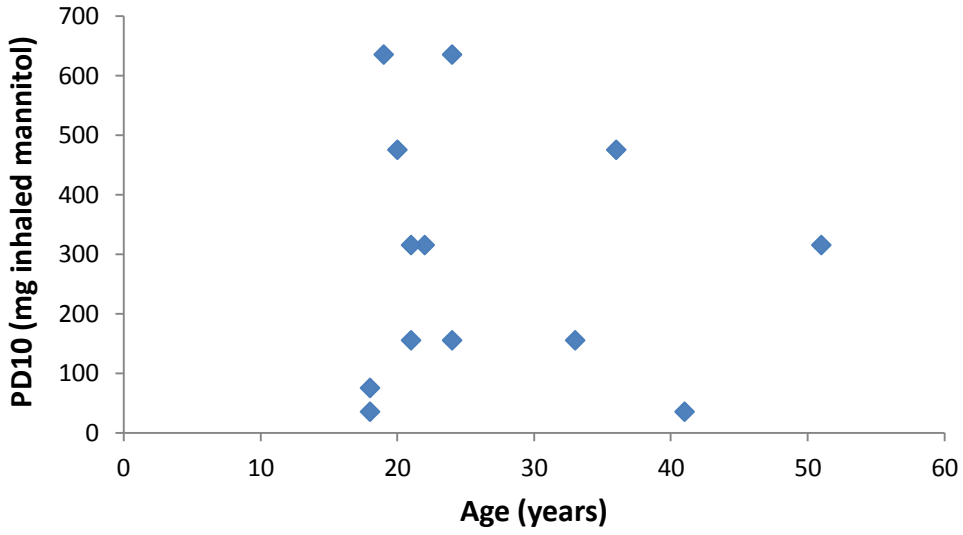

Figure 2.14 - Relationship between dose of inhaled mannitol required to produce a 10% decrease in baseline FEV_1 (PD 10) and age in the 10 men and 10 women asthmatics who achieved a fall in $FEV_1 \geq 10\%$.

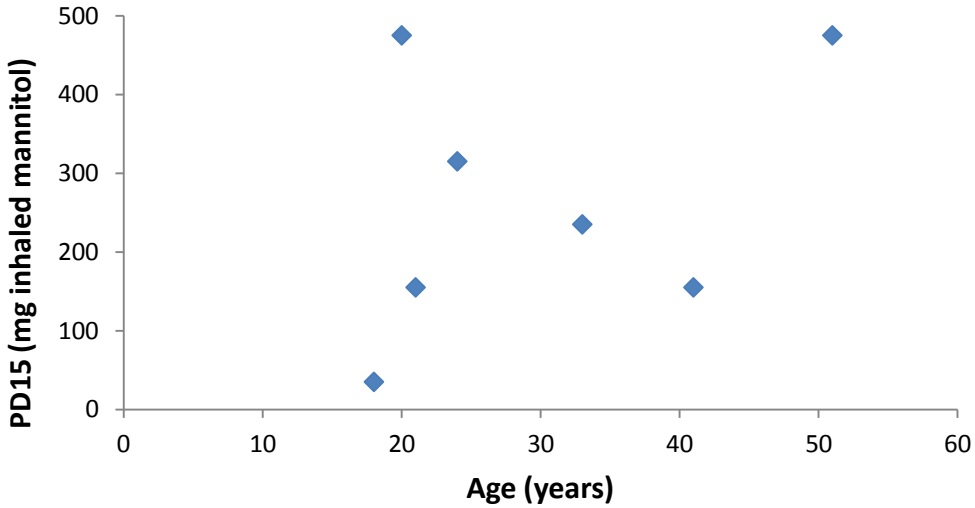

Figure 2.15 - Relationship between dose of inhaled mannitol required to produce a 15% decrease in baseline FEV_1 (PD15) and age in the 10 men and 10 women asthmatics who achieved a fall in $FEV_1 \geq 15\%$.

2.3.4 Dose - Response Relationships for Inhaled Mannitol

The group results revealed that increasing doses of inhaled mannitol resulted in progressively more severe airflow obstruction with a plateauing effect that became evident at inhaled mannitol doses greater than 300 mg (refer to Figures 2.16 and 2.17). Substantial differences in the dose – response relationships were also evident between different individuals. Some individuals showed progressively more severe airflow obstruction with increasing inhaled mannitol dose up to the maximum permissible dose of 635 mg, progressively more severe airflow obstruction with increasing inhaled mannitol dose and a plateau at the higher doses, and progressively more severe airflow obstruction followed by progressive resolution of airflow obstruction with increasing inhaled mannitol dose. Examples of each of these three types of curves are given in Figures 2.18, 2.19 and 2.20. In all, 3 asthmatics had a plateau in their dose-response curves (one with a fall in $FEV_1 > 15\%$ and two with a fall in FEV_1 between 5 and 15%), while 6 asthmatics with a fall in FEV_1 between 5 and 15% initially revealed progressively more severe airflow obstruction followed by progressive resolution of airflow obstruction with increasing inhaled mannitol dose. The individual dose – response relationships of each of the 20 participants are presented in Appendix 1.

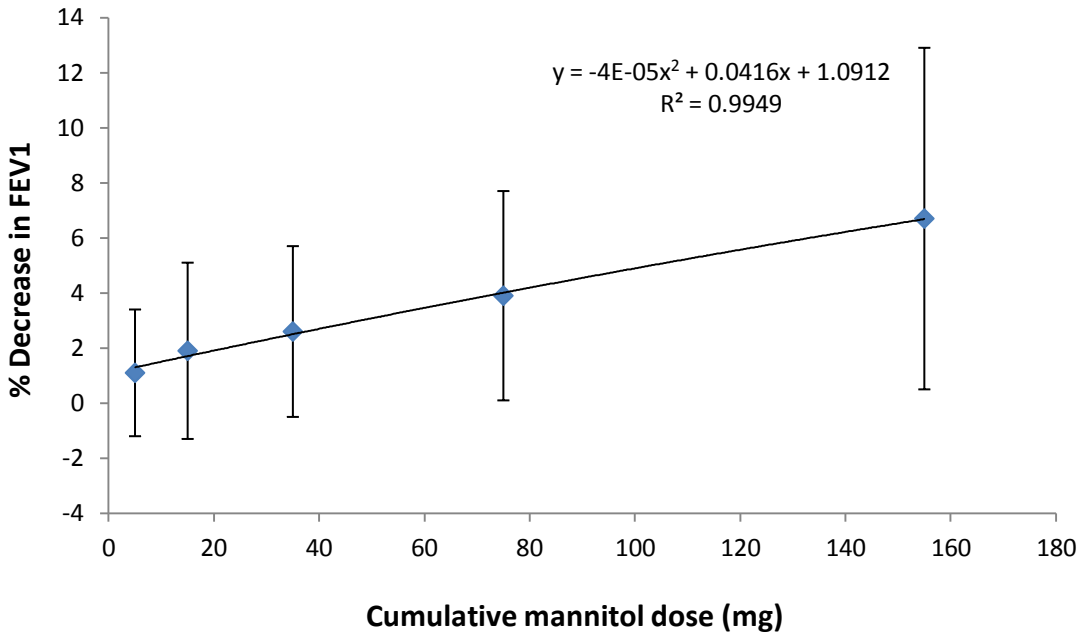

Figure 2.16 - Dose - response relationship between the mean % decrease in FEV_1 and the cumulative inhaled mannitol dose in the 19 men and women asthmatics who inhaled at least 155 mg of mannitol. Horizontal bars denote ± 1 SD from the mean values.

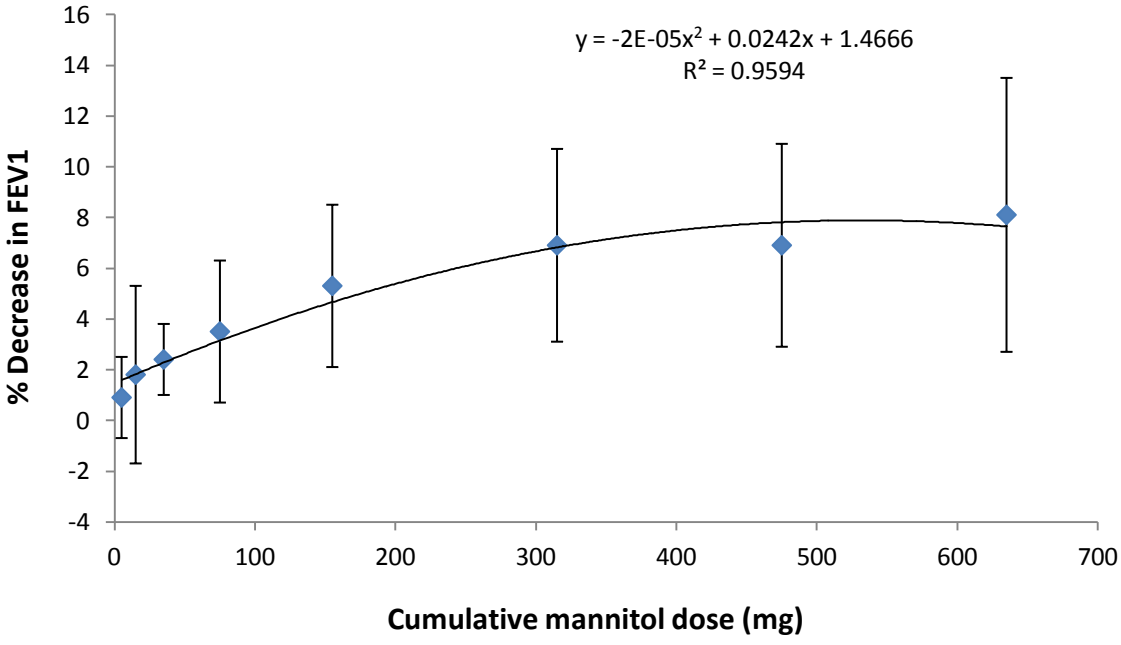

Figure 2.17 – Dose - response relationship between the mean % decrease in FEV_1 and the cumulative inhaled mannitol dose in the 12 asthmatic participants who received a total cummulative dose of 635 mg. Horizontal bars denote ± 1 SD from the mean values.

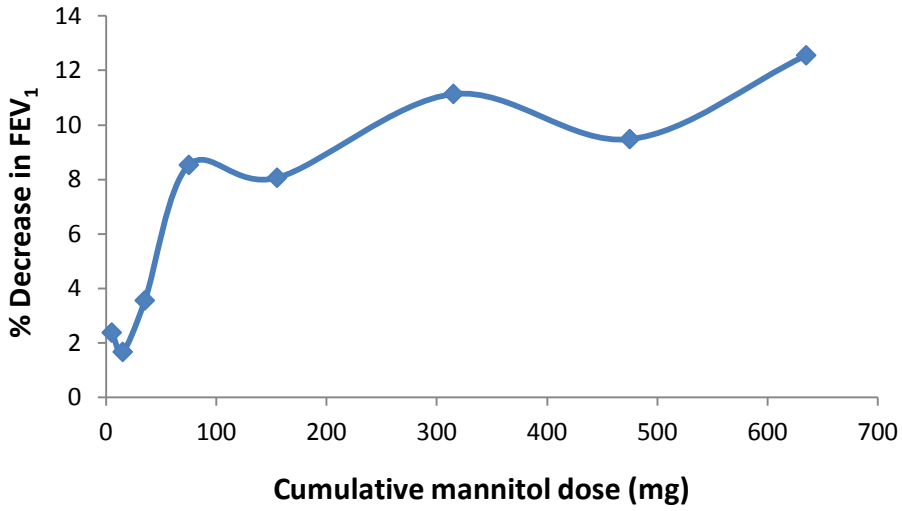

Figure 2.18 - Dose - response relationship between the % decrease in FEV_1 and the cumulative inhaled mannitol dose in an asthmatic man (participant no. 19) who continued to demonstrate progressively more severe airflow obstruction up to the maximum permissible dose (635mg) of inhaled mannitol.

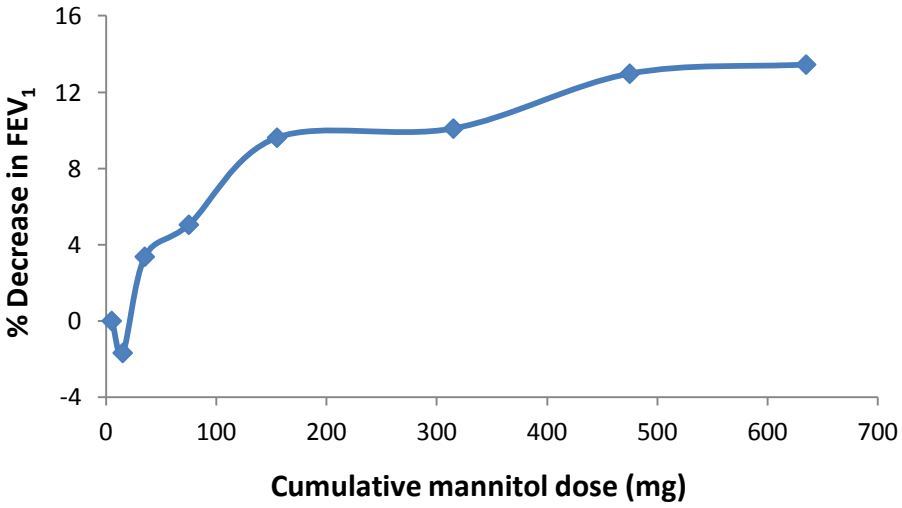

Figure 2.19 - Dose - response relationship between the % decrease in FEV_1 and the cumulative inhaled mannitol dose in an asthmatic man (participant no. 16) who demonstrated a plateau in the FEV_1 response.

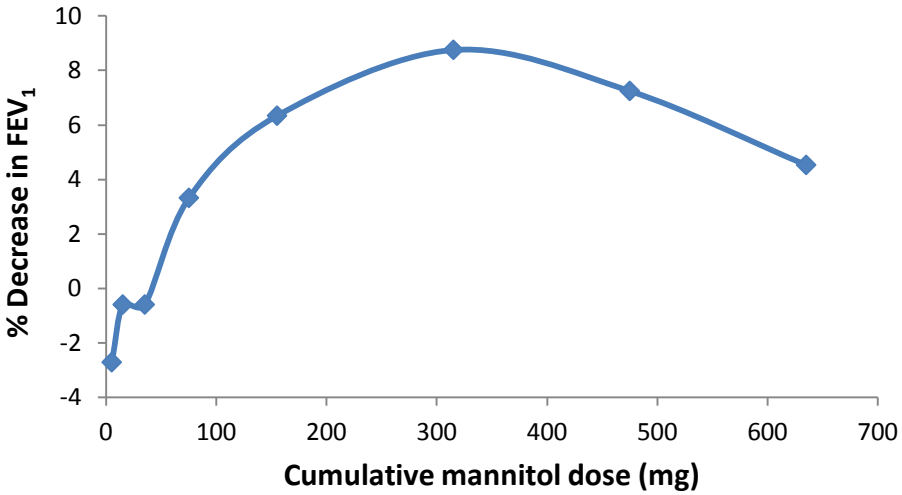

Figure 2.20 - Dose - response relationship between the % decrease in FEV_1 and the cumulative inhaled mannitol dose in an asthmatic man (participant no. 15) who displayed progressive progressively more severe airflow obstruction followed by resolution of airflow obstruction at the higher doses of inhaled mannitol.

The 7 asthmatics who demonstrated a fall in $FEV_1 \geq 15\%$ each showed progressively more severe airflow obstruction with increasing mannitol dose without evidence of a plateau (refer to Figure 2.21). However, the dose of inhaled mannitol required to elicit the maximum permissible fall in FEV_1 ranged from 35 mg to 635 mg. For comparison, the individual dose – response relationships for the other 6 participants who achieved a fall in $FEV_1 \geq 10\%$ and < 15% are shown in Figure 2.22.

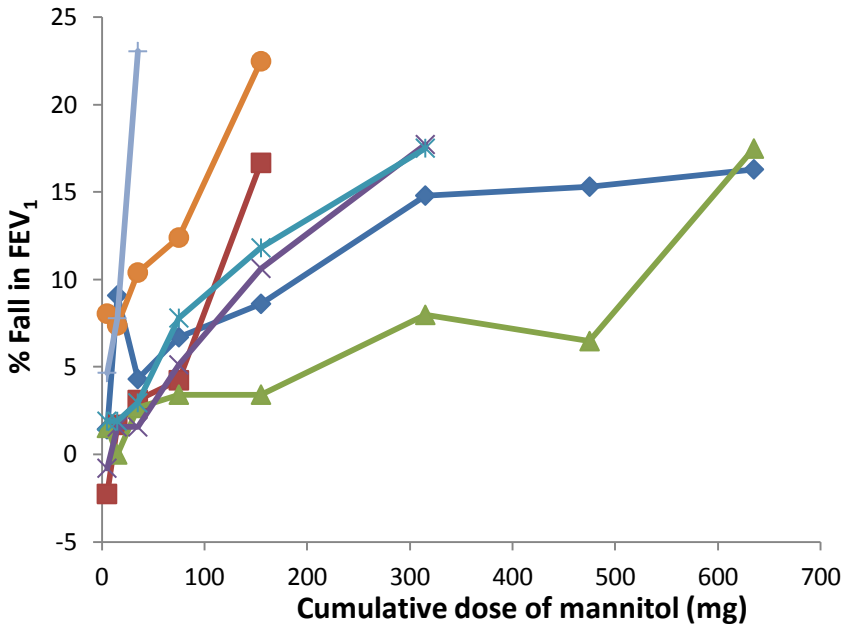

Figure 2.21 – Dose response relationship between the % decrease in FEV_1 and the cumulative inhaled mannitol dose in the 7 asthmatics who demonstrated a fall in fall in $FEV_1 \geq 15\%$.

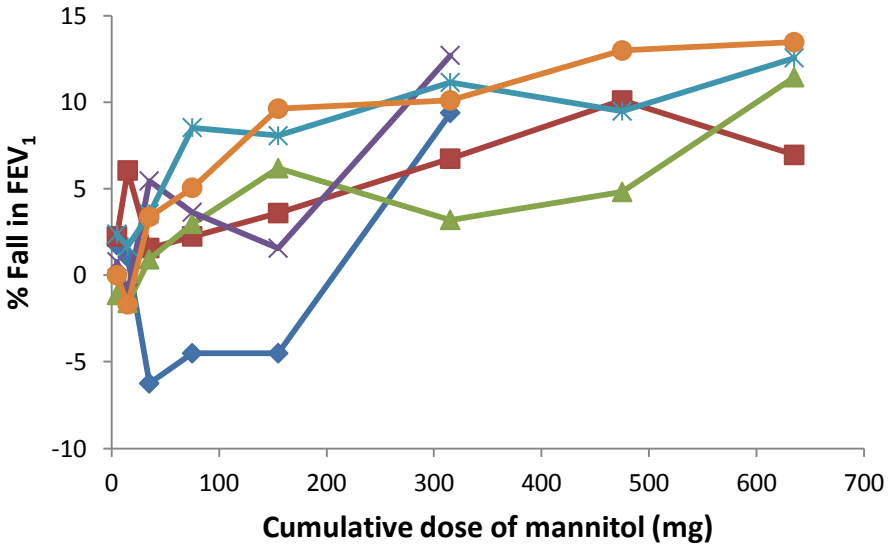

Figure 2.22 – Dose response relationship between the % decrease in FEV_1 and the cumulative inhaled mannitol dose in the 6 asthmatics who demonstrated a fall in fall in FEV_1 of $\geq 10\%$ and $< 15\%$.

2.3.5 Effects of Inhaled Corticosteroids

The baseline FEV_1 and responsiveness to inhaled mannitol (PD5, PD10 and RDR) of asthmatics on ICS did not differ significantly from values seen in the asthmatics who were not on ICS (all $p > 0.20$ with the 2-tailed unpaired Student's *t* test; refer to Table 2.4). However, the mean value for the RDR was numerically much lower in asthmatics on ICS but the standard deviations for the ICS RDR and non-ICS RDR were large. There were insufficient asthmatics who responded with a FEV_1 fall $\geq 15\%$ to carry a meaningful unpaired *t* test between the ICS and non-ICS PD15 data.

Table 2.4 – Effects of inhaled corticosteroids on baseline FEV_1 and responsiveness to inhaled mannitol

Parameter	Patients on ICS	Patients not on ICS	P value
Number of participants	12	8	---
FEV_1 (L)	90.1 ± 14.1	84.0 ± 12.4	0.34
PD5 (mg)	114.2 ± 86.0	121.3 ± 154.8	0.90
PD10 (mg)	309.3 ± 47.0	268.3 ± 183.2	0.74
RDR (% fall FEV_1/mg)	0.034 ± 0.039	0.117 ± 0.221	0.21

2.3.6 Effects of Mannitol Challenge on FVC and FEV_1/FVC

Table 2.5 compares the maximum falls in FEV_1, FVC and FEV_1/FVC in the 13 asthmatics who responded to inhaled mannitol challenge with a fall in FEV_1 of at least 10%. As noted, FVC fell substantially following mannitol challenge such that the fall in FEV_1/FVC was less than half that observed in the FEV_1.

Table 2.5 – Comparison of changes in FEV_1, FVC and FEV_1/FVC in the 13 asthmatics who demonstrated a ≥ 10% fall in FEV_1

Participant	Gender	Total mannitol dose (mg)	Max fall in FEV_1 (%)	Max Fall in FVC (%)	Max fall in FEV_1 /FVC (%)
1	Female	315	12.7	13.7	1
5	Female	635	16.3	12.3	6
6	Female	635	11.6	7.8	7
7	Female	635	11.4	10.7	2
9	Female	155	22.5	14.3	7
10	Female	475	17.5	8.8	7
12	Female	315	17.7	15.6	2
14	Male	235	17.5	9.6	7
16	Male	635	13.5	5.8	7
18	Male	635	10.1	3.7	3
19	Male	635	12.6	6.9	6
20	Male	35	23.1	4.6	16
24	Male	155	16.7	6	9
Mean			15.7	9.2	6.2
SD			4.1	3.9	3.9

2.4 Discussion

Although inhaled mannitol has become widely used in airway provocation in asthmatics in recent years, several unresolved features remain in the dose-response characteristics of inhaled mannitol in asthmatics. It was therefore considered prudent to first carry out a study to better describe the dose-response characteristics of inhaled mannitol in asthmatics before utilizing this form of airway provocation to examine the effects of oropharyngeal phospholipids administration on airway responsiveness in asthmatics. Airway challenges with inhaled mannitol in the 24 adult asthmatic participants in this study confirmed previous reports of ease of use, convenience and safety, with no ill effects noted in this study. However, this study also confirmed previous reports of a wide range of interindividual sensitivity to inhaled mannitol and atypical dose-response characteristics. Nevertheless, 12 of the asthmatic participants demonstrated sufficient sensitivity to inhaled mannitol to be suitable for recruitment into the subsequent study which will examine whether exogenous phospholipids binding to the oropharyngeal mucosa can influence airway function in asthmatics.

2.4.1 Suitability of Mannitol versus other Airway Challenge Techniques

2.4.1.1 Sensitivity and Specificity

Airway hyperresponsiveness has been regarded as a hallmark of asthma (Sverrild *et al*., 2009; Woolcock, 1988), and some earlier studies have concluded that that at least some forms of airway challenge such as exercise will induce significant airflow obstruction in almost all asthmatics (Jones *et al*., 1963). The development of inhaled dry mannitol powder as an airway challenge technique in recent years has stimulated considerable research into the comparative efficacy of different airway challenges for identifying airway hyperresponsiveness and diagnosing asthma. Ideally, a bronchoprovocation test should have both a high sensitivity and a high specificity for asthma. However, many recent studies have reported comparatively low levels of sensitivity and specificity for airway hyperresponsiveness as a diagnostic marker of asthma for a range of direct and indirect airway challenge techniques including mannitol inhalation. A review of the relevant literature revealed that reported values for sensitivity ranged from 32% to 90%

(Anderson *et al*., 2009; Andregnette-Roscigno *et al*., 2010; Barben *et al*., 2011; Kersten *et al*., 2009; Koskela *et al*., 2003b; Porsbjerg *et al*., 2007; Sverrild *et al*., 2010), whereas specificity ranged from 73 to 98% (Anderson *et al*., 2009; Barban *et al*., 2011; Sverrild *et al*., 2010). These studies further demonstrated that bronchoprovocation with mannitol compared favorably with other challenge tests with a sensitivity of 43% to 64% and a specificity of 73% to 98.4%.

It has been argued that unlike directly acting bronchoprovocation agents such as histamine and methacholine, indirect airway challenge tests are more closely related to natural stimuli that trigger bronchoconstriction in asthmatics (Brannan *et al*., 2009; Leuppi *et al*., 2002). The rationale for this is that the bronchoconstriction induced by indirect airway challenges is indicative of both airway hyperresponsiveness and airway inflammation, and that this may make indirect tests such as mannitol more specific for currently active asthma and for determining optimal treatment regimens for asthma (Brannan *et al*., 2005; Brannan *et al*., 2009; Koskela et al., 2003b; Leuppi *et al*., 2002). In support of this, several recent studies have shown reported that responsiveness to mannitol correlates better with markers of airway inflammation such as the percentage of sputum eosinophils (Porsbjerg *et al*., 2007) and exhaled nitric oxide levels than does responsiveness to methacholine (Porsbjerg *et al*., 2007; Sverrild *et al*., 2010).

Many studies have shown statistically significant correlations between airway hyperresponsiveness assessed by mannitol and other types of airway challenges (Anderson *et al*., 1997; Barben *et al*., 2011; Brannan *et al*., 2005; Koskela *et al*., 2003b). However, it is not uncommon for individual asthmatics to respond to one type of airway challenge but not another (Anderson *et al*., 2009; Barben *et al*., 2011; Porsbjerg *et al*., 2007).

2.4.1.2 Duration of Mannitol Airway Challenges

A number of studies have reported that airway challenges with inhaled mannitol take less time to perform than do other types of airway challenges (Anderson *et al*., 2009; Barben *et al*., 2011; Kersten *et al*., 2009). Mean times of 16 - 34 minutes have been reported to carry out airway challenges with mannitol (Anderson *et al*., 2009; Barben *et al*., 2011; Brannan *et al*., 2005; Kersten *et al*., 2009). These times compare favourably with the 14 - 42 minutes required in the present study. Shorter challenge times are more convenient and also have the advantage of less recovery occurring during inhalation of the total cumulative MDP dose.

2.4.1.3 Adverse Effects

Each of the 24 asthmatic participants in the present study tolerated the mannitol well, although coughing was noted in all 24 participants immediately after inhalation of mannitol powder. Such coughing was always of short duration and was never severe enough to warrant terminating further serial challenges with mannitol. These findings are in concordance with previous published studies which have reported an absence of severe adverse effects resulting from inhaled mannitol challenges, that inhaled mannitol is generally well tolerated, but that side effects such as coughing are not uncommon (Anderson *et al*., 2009; Barben *et al*., 2011; Brannan *et al*., 2005; Holzer *et al*., 2003; Kersten *et al*., 2009).

2.4.2 Interindividual Variation in the Response to Inhaled Mannitol

2.4.2.1 Interindividual Variation in Sensitivity

The sensitivity of inhaled mannitol to detect asthmatics is defined as the percentage of asthmatics tested who respond positively with a level bronchoconstriction which is routinely set at a fall in $FEV_1 \geq 15\%$ with a cumulative dose of inhaled mannitol of $\leq$ 635 mg. In the present study 7 of the 24 participants (29.1%) responded with a fall in FEV_1 of $\geq$ 15% to $\leq$ 635 mg of inhaled mannitol. This is marginally below the range of 32% to 90% previously reported (Anderson *et al*., 2009; Andregnette-Roscigno *et al*.,

2010; Barban *et al*., 2011; Kersten *et al*., 2009; Koskella *et al*., 2003b; Porsbjerg *et al*., 2007; Sverrild *et al*., 2010). A number of factors may potentially have contributed to this disparity. One important factor in this regard is asthma severity because sensitivity to airway challenge with mannitol in asthmatics can be increased in more severe cases of asthma (Brannan *et al*., 2005; Porsbjerg *etal*., 2007), although contrary findings have also been reported (Andregnette-Roscigno *et al*., 2010; Koskela *et al*., 2003b). The 7 asthmatics that showed a decrease in FEV_1 of $\geq$ 15% in response to inhaled mannitol could be classified into 3 groups according to their sensitivity to inhaled mannitol. The first group consisted of one asthmatic who required only 35 mg of mannitol, the second group consisted of 2 asthmatics who required between 75 mg and 155 mg, and the third group consisted of 4 asthmatics who required between 195 mg and the maximum recommended dose of 635 mg. This is in accord with the findings by Brannan *et al.* (2009) who similarly classified asthmatic responders into 3 groups according to the dose of inhaled mannitol required to elicit a fall in $FEV_1 \geq 15\%$. Nevertheless, no clear relationship was evident in the present study between asthma severity as measured by the baseline FEV_1 and the PD5, PD10 or PD15 (refer to Figures 14-20). In addition, each participant's asthma severity was graded according to GINA (2002) guidelines and no association was apparent between the participants' asthma severity and either their maximum fall in FEV_1 or their response-to-dose ratio for inhaled mannitol. Indeed, a wide overlap in these parameters was evident among the different grades of asthma severity. These findings suggest that asthma severity did not contribute to the low sensitivity to mannitol challenge in the present study.

Reduced sensitivity to airway challenge with inhaled mannitol in asthmatics by inhaled corticosteroids has been reported (Brannan *et al*., 2002; Koskela *et al*., 2003a; Leuppi *et al*., 2001). Therefore, it is plausible that inhaled corticosteroid medication may have contributed to the low sensitivity to mannitol in the present study because 16 of the 24 participants were on inhaled corticosteroid medication at the time of the study and they were instructed not to withhold this medication during the course of the study. No significant difference was noted between the ICS and non-ICS values for PD and RDR in the present study (refer to Table 9). However, the mean value for the RDR was numerically much lower in asthmatics who were taking ICS, which suggests that the

high prevalence of continuing ICS use may have contributed to the low sensitivity to mannitol in the present study. Only 5 of the 24 participants were on long-acting beta agonists and this number is too small for meaningful analysis. However, long acting beta agonist medication may have contributed somewhat to the reduced sensitivity to airway challenge with inhaled MDP in this study because 3 of these participants required 635 mg of MDP to achieve a 10% fall in FEV_1, and the other 2 participants achieved a fall in FEV_1 of less than 10% after inhaling 635 mg of MDP.

2.4.2.2 Dose-response Relationships

The PD15 for inhaled mannitol varies widely in asthmatics who respond to mannitol challenge. A review of the relevant literature revealed that reported values for the PD15 for inhaled mannitol in asthmatics varied widely between individuals, with PD15 values ranging from 2 mg to 617 mg (Anderson *et al*., 1997; Andregnette-Roscigno *et al*., 2010; Brannan *et al*., 2002; Kersten *et al*., 2009; Koskela *et al*., 2003b; Leuppi *et al*., 2002; Porsbjerg *et al*., 2007; Sverrild *et al*., 2009). This range compares favourably with the 35 mg to 635 mg PD15 range observed in the present study in the 7 asthmatics who achieved a fall in $FEV_1 \geq 15\%$. Studies by Brannan *et al* (2002) and Leuppi *et al* (2001) have reported mean response-to-dose ratios (RDR, calculated as the cumulative % fall in FEV_1 per mg of cumulative inhaled mannitol) of 0.18 and 0.012 % fall in FEV_1/mg of inhaled mannitol in their asthmatics participants who responded with a fall in $FEV_1 \geq 15\%$.This compares favorably with the mean (± SD) RDR of 0.16 ± 0.22 % fall in FEV_1/mg of inhaled mannitol in the 7 asthmatics in the present study who achieved a fall in $FEV_1 \geq 15\%$.

Few studies have reported detailed information on the dose-response curves of inhaled mannitol in asthmatics. Anderson *et al* (1997) and Kersten *et al* (2009) have published individual dose-response curves for each of their 56 asthmatic participants, and Brannan *et al* (2009) reported dose-response curves from 3 individuals with different levels of asthma severity. Each of these participants achieved a fall in FEV_1 of $\geq 15\%$ and each showed progressive falls in FEV_1 with increasing mannitol dosage, except for one participant from the study by Anderson *et al.* (1997) where FEV_1 failed

to fall much more than the 15% cutoff with cummulative mannitol inhalations at the higher dose range. The dose-response curves from the 7 asthmatics in the present study who achieved a fall in $FEV_1 \geq 15\%$ showed similar features to the published dose-response curves, including one participant where little further falls in FEV_1 were observed at the higher mannitol dosages once the fall in FEV_1 had reached the 15% cutoff (refer to Figure 29). The present study also provides dose-response curves for the 13 asthmatics who achieved a fall in FEV_1 of $\geq 5\%$ and $< 15\%$. Of interest, the dose-response curves of 2 of these participants showed a plateau at the higher doses (refer to Figure 27), and 6 of these participants initially revealed progressively more severe airflow obstruction followed by progressive resolution of airway obstruction with increasing inhaled mannitol dose (refer to Figure 28). The reasons for such atypical responses in asthmatics who respond weakly to inhale mannitol are unclear and there is a paucity of relevant asthmatic dose-response curve data in the literature.

2.4.3 Defining the Threshold Cut-off for a Positive Response

The sensitivity for a positive response to inhaled mannitol challenge would be expected to increase if cut-off values less than a 15% fall in FEV_1 are used to define a positive bronchoprovocation response. Most reported studies that have employed airway challenges with inhaled mannitol have used a decrease in FEV_1 of ≥ 15% to define a positive bronchoprovocation response. However, lower cutoff values have been employed to define a positive bronchoprovocation response (Holzer *et al*., 2003; Sverrild *et al*., 2009). Indeed, in the study by Sverrild *et al.* (2009), the sensitivity of mannitol for identifying asthmatics increased from 58.8% to 70.6% when the cutoff value for the fall in FEV_1 was reduced from ≥ 15% to ≥ 10%, and this was accompanied by only a modest fall in specificity from 98.4% to 93.0%. Such a reduction in the cutoff value can be useful given the comparatively low sensitivity of mannitol and other types of airway challenge in identifying asthmatics when more traditional higher cutoff levels are used. In the present study, reducing the cutoff value from the traditional ≥ 15% to ≥ 10% increased the number of positive responders to inhaled mannitol from 7 out of the 24 (29.2%) asthmatic participants tested to 13 (54.2%, 6 men and 7 women). This number of participants was considered sufficient

to conduct a subsequent study to examine whether exogenous phospholipids binding to the oropharyngeal mucosa can influence airway function in asthmatics, and to include gender specific analyses of the data.

2.4.4 Effects of Mannitol Challenge on FVC and FEV_1/FVC

During airway obstruction, the FVC is often reduced more than the slower and more relaxed measurements of vital capacity (VC) (ATS, 1995; BTS, 1994; Johns and Pierce, 2008; Pellegrino *et al*., 2005). Consequently, although the FVC is frequently measured instead of the VC (ATS, 1995), it is preferable to measure the VC rather than the FVC during airway obstruction (ATS, 1995; Johns and Pierce, 2008; Pellegrino *et al*., 2005). Mannitol airway challenges rely on repeated serial measurements of the FEV_1 which require FVC maneuvers, and these make it difficult to also carry out repeated slow VC maneuvers. Consequently, the FVC was measured instead of the slower VC in the present study. These issues are of interest because substantial falls in the FVC were observed after mannitol challenge in the present study, and consequently the fall in FEV_1/FVC was less than half that observed in the FEV_1. At first inspection, the small fall in FEV_1/FVC after airway challenge may seem atypical given that a disproportionately greater fall in maximal expiratory airflow relative to the maximal volume (*i.e.* the VC) is widely regarded as a defining feature of an obstructive ventilatory impairment, and can be determined by examining the FEV_1/VC (ATS, 1995; Pellegrino *et al*., 2005). This disparity may have occurred because the forced expiratory effort during the FVC maneuver resulted in more dynamic airway compression and earlier airway closure than would occur at equivalent lung volumes during the slower and more relaxed VC maneuvers (ATS, 1995; BTS, 1994; Johns and Pierce, 2008; Pellegrino *et al*., 2005).

2.4.5 Conclusion

The results of this study demonstrate that a commercially available inhaled mannitol product (Aridol^{TM}) is a convenient and safe means of evoking airway obstruction by airway challenge in asthmatics. However, a significant number of asthmatics did not develop significant airway obstruction after airway challenge with inhaled mannitol. This is consistent with previous published reports and emphasizes the need to screen the responses to mannitol airway challenges in a comparatively large number of asthmatics if one wishes to identify an adequate number of positive responders for subsequent research purposes. No relationship was evident between the asthma severity and the maximum fall in FEV_1, or the dose-to-response ratio for inhaled mannitol in either the men, women or the combined group of asthmatic participants. A relationship was also not evident between the participants' age and the PD5, PD10 or PD15. However, 16 of the 24 asthmatic participants were on current treatment with inhaled corticosteroids which, on theoretical grounds, may have accounted for the somewhat lower sensitivity (29.1%) to evoke a fall in FEV_1 ≥ 15% than has previously been reported in other studies. The present study also appears to be the first to document the dose-response curves to inhaled mannitol in asthmatics who responded to inhaled mannitol with a fall in FEV_1 between 5% and 15%. Eight of these 13 asthmatics demonstrated atypical dose-response curves, with 2 displaying a plateau at the higher doses of inhaled mannitol, and 6 displaying a progressive resolution of airway obstruction with cumulative higher doses of inhaled mannitol. Reducing the cutoff fall in the FEV_1 for a positive response to inhaled mannitol from the traditional ≥ 15% to ≥ 10% increased the number of positive responders from 7/24 (29.2%) participants to 13/24 (54.2%, 6 men and 7 women), but the ≥ 10% decrease in FEV_1 remained statistically significant compared to pre-provocation values. The 13 positive responders with the ≥ 10% decrease in FEV_1 cutoff were considered sufficient to conduct a subsequent study to examine whether exogenous phospholipids binding to the oropharyngeal mucosa can influence airway function in asthmatics and to provide sufficient male and female participants to include gender specific analyses of the results.

CHAPTER THREE

Effects of Oropharyngeal Administration of Exogenous Phospholipids on Airway Function in Asthmatics

3.1 Introduction

The factors responsible for regulating airway calibre in asthmatics are complex and not fully understood. Numerous trigger factors are known to exacerbate reversible bouts of bronchoconstriction, cough and wheeze in asthmatics (Kasper *et al*., 2005). Airway inflammatory immune processes play important roles in mediating these responses (Kasper *et al*., 2005). However, reflexes mediated via intrathoracic irritant, cough and afferent C fibre receptors also contribute (Pisarri *et al.*, 1992; Undem *et al.*, 2002). Laryngeal and pharyngeal receptors also appear to play a role in regulating airway calibre in asthmatics because stimulation of these receptors is known to evoke reflex bronchoconstriction in experimental animals **(**Widdicombe, 1988**),** and because local anaesthesia of the oropharynx attenuates exercise induced asthma (McNally *et al*., 1979).

Phospholipids bind to epithelial surfaces (Hills, 1996; Hills, 2002) and change the surface properties (Hills 1988, Hills 2002). Theoretically, phospholipids binding tenaciously to oropharyngeal receptors may attenuate airway provocation mediated via these receptors in asthmatics (Hills, 1996; Hills and Chen, 2000). Soy lecithin is a rich food source of phospholipids and could therefore be useful for topically applying high concentrations of phospholipids to the pharyngeal mucosa. This may be beneficial in protecting the asthmatic airways from provocation by natural stimuli such as exercise and mouth breathing. In addition, examination of the effects of oropharyngeal application of lecithin on airway challenges may provide insights into the neural regulation of airway caliber in asthmatics.

3.1.1 Purpose of the Study

The aim of this study was to determine whether topical application of soy lecithin to the oropharyngeal mucosa attenuates bronchoconstrictor and cough responses to airway challenge by inhaled mannitol in asthmatics.

3.2 Methods and Materials

3.2.1 Consent and Confidentiality of Information

This study was approved by the Human Research Ethics Committee of the University of New England (Approval No HE11/071). Informed and signed consent was obtained from each participant prior to their commencement in this study. Every participant was assigned an identification code to ensure that participants' data were only identifiable to the HDR candidate and his Principal Supervisor Dr Tom van der Touw.

3.2.2 Participant Selection

Patients with physician diagnosed asthma who had been successfully recruited into the airway hyperresponsiveness – mannitol challenge study (refer to Chapter 2) were invited to participate in the current study if they had achieved a fall in $FEV_1 \geq 10\%$ following progressive airway challenge with inhaled mannitol. Each potential participant was also carefully questioned to determine whether they had a history of food allergies, and especially allergy to soy or soy products. Any person with a history of food allergies was excluded from the study.

3.2.3 Study Protocol and Equipment

Participants attended the research laboratory on two separate days and were asked to refrain from taking beverages and foodstuffs that contain lecithin or caffeine and related xanthine alkaloids (cola drinks, coffee, chocolate and confectionaries) on both test days before coming to the laboratory. Participants remained on any prescribed preventer or long acting beta agonist medication throughout the study period. However, they were asked to refrain from taking any short-acting bronchodilator medication such as Ventolin for at least 6 hours before coming to the laboratory, but with the proviso that they take these medications if necessary for symptomatic relief. In such an event, the participant was advised that they inform the researchers that this has occurred so that arrangements for an alternate test date could be made. Participants were also advised to refrain from exercise during the entire day when they come to the laboratory.

Lung function was measured by forced expiratory spirometry prior to airway challenge (best of three baseline measurements) to ensure that the baseline FEV_1 was at least at 75% of predicted on each test day prior to airway challenge. On one test day, participants gargled 100 ml of water for 2 minutes and then expectorated the water. On the other test day, participants gargled an aqueous suspension of soy lecithin for 2 minutes and then expectorated the mixture. The aqueous lecithin suspension consisted of 8 g of soy lecithin (Puritan's Pride Ltd., NY, USA) which contained 1,680 mg of phosphatidylcholine and 960 mg of phosphatidylinositol), and made up to a volume of 100 ml with distilled water. The soy lecithin suspension was freshly vortexed for several minutes immediately prior to use. The order of water or lecithin treatment was randomised but was carried out at the same time of day (within 1 hour) on the two test days. For each subject, the two study days were conducted within a two week period with at least a 48 hour interval between successive study days.

Airway challenges were performed with inhaled mannitol and a disposable Aridol inhaler (Aridol™, Pharmaxis Ltd., Sydney, Australia) 15 minutes after gargling and expectorating on each of the two test days using the PD10 previously determined for each subject in the previous study (see Table 2.5). Because the PD10 had already been determined on the first test day, the minimum cumulative dose of inhaled mannitol that provoked ≥ 10% fall in FEV_1 was delivered as a cumulative dose in 40 mg increments on test days 2 and 3. Coughs which occurred within 60 seconds of each 40 mg dose were manually counted and recorded. The cough counts were carried out by the same person for each subject throughout the study period.

Lung function was measured by forced expiratory spirometry using a spirometer (Super Spiro, SU6000, Micro Medical Ltd., Kent, England), a laptop computer with dedicated software (Spirometry Software, 36-SPC1000-STK, Micro Medical Ltd., Kent, England) which provides spirometric analysis of the recorded data on a laptop computer, a disposable microbial filter and disposable nose clip. Forced expiratory maneuvers were performed 5, 10, 20, 30, 40, 50 and 60 minutes after airway challenge with inhaled mannitol. Two acceptable forced expiratory manoeuvres were recorded for each time period and the better of the two measurements was taken as the lung function values for each time period.

Each participant was asked to bring their personal Ventolin inhaler with them when they attended the laboratory, and a spacer device and a Ventolin metered dose inhaler was always immediately available in the laboratory. At the completion of each test period, spirometric measurements were made to ensure that the FEV_1 in each participant had returned to within 5% of their pre-challenge baseline level.

3.2.4 Data Analysis

Airway challenges with inhaled MDP have traditionally relied solely on FEV_1 measurements. However, for the purpose of this study, a more comprehensive spirometric assessment of lung function was warranted and this necessitated FVC

maneuvers. To ensure that spirometric data were only collected from satisfactory FVC manoeuvres, the suitability of each forced expiratory manoeuvre was automatically assessed by the Super Spiro spirometer and by visual inspection of each maximum expiratory flow-volume curve using the criteria of Johns and Pierce (2007).

The following forced expiratory parameters were determined and recorded using the Super Spiro Spirometry Software: forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC, peak expiratory flow rate (PEFR) and mid-maximal expiratory flow (MMEF). The following criteria were used to measure the sensitivity of subjects to mannitol airways provocation (Koskela *et al*., 2005).

1. Cough threshold (C2): This is the lowest cumulative dose (mg) of inhaled mannitol required to produce a minimum of 2 coughs during a single mannitol airway challenge test.

2. Cough-to-dose ratio (CDR): This is the ratio of the cumulative number of coughs to the cumulative dose of mannitol administered (mg) during a complete mannitol airway challenge. It was calculated by dividing the cumulative number of coughs to the cumulative dose of mannitol administered, expressed as coughs per 100 mg of mannitol.

3. Response-to-dose ratio (RDR): This describes the tendency of the subject's airways to obstruct in response to inhaled mannitol. It was calculated by dividing the final percentage fall in FEV_1 by the total cumulative dose (mg) of inhaled mannitol (Koskela *et al.*, 2005).

4. Total number of coughs: The total number of coughs produced during the total cumulative dose (mg) of inhaled mannitol.

Unless otherwise stated, all data are expressed as means ± 1 standard deviation. The Student's paired *t*- test (2 tailed) was used to compare between post-lecithin and post-water values for each lung function parameter. The Student's paired *t* test (2 tailed) was also used to compare between post-lecithin and post-water values of C2, CDR, and total cough data. The null hypothesis was rejected at $p < 0.05$ (2-tailed). Statistical analyses of the data were carried out using Excel (2007 version, Microsoft). The null hypothesis was rejected at $p < 0.05$.

3.3 Results

3.3.1 Participants

Thirteen previously identified adult asthmatics (refer to Tables 2.2 and 2.5 in Chapter 2) who achieved a fall in $FEV_1 \geq 10\%$ following progressive airway challenge with inhaled mannitol were recruited into this study. However, one female participant (participant number 25) discontinued on the basis of unavailability for the study. The remaining 12 participants (6 men, 6 women) successfully completed the study. All data presented in this thesis chapter relates only to these 12 participants.

The anthropometric characteristics and the dose of inhaled MDP delivered to the 12 participants in this study are given in Table 3.1. Mean ± 1 SD values for the group were as follows: age 28.5 ± 10 years, height 170 ± 9 cm, body weight 76 ± 18 kg. The baseline FEV_1 when the patients arrived at the laboratory was 93 ± 12 % predicted. Each participant had Ventolin prescribed by their medical practitioner. In addition, 9 participants were on daily inhaled corticosteroids and 1 patient was on Montelukast (an oral leukotriene receptor antagonist). Each participant refrained from taking short-acting bronchodilators for at least 6 hours before coming to the laboratory but continued taking any other prescribed medications including preventer and long acting beta agonists. Before leaving the laboratory, the FEV_1 of each participant had returned to at least the level measured before airway challenge. No adverse effects were noted during the study and none of the participants showed or reported

spirometric evidence of respiratory fatigue or exhaustion from the repeated FVC maneuvers in this study.

Table 3.1 – Characteristics of asthmatic participants

Participant	Gender	Age (yrs)	Height (cm)	Body weight (kg)	Dose of Mannitol given (mg)
1	M	33	178	83	235
2	M	21	180	69.5	635
3	M	29	169	75	635
4	M	18	178	65	635
5	M	35	182	83	35
6	M	19	180	85	155
7	F	22	170	63.4	315
8	F	51	164	73.5	635
9	F	24	157	62.1	635
10	F	19	175	125	635
11	F	41	168	65	155
12	F	24	155	61.5	475
Mean	------	28	171.3	75.9	432
SD	------	10.2	9.1	17.7	236

3.3.2 Reproducibility of Baseline Lung Function on the Two Study Days

Baseline spirometric lung function measurements (FEV_1, FVC, FEV_1/FVC, PEFR and MMEF) made before the commencement of airway challenges did not in general differ significantly between the two test days (refer to Table 3.2). The exception to this was the baseline PEFR which was significantly higher on the lecithin test day compared to the water test day.

Table 3.2 – Baseline lung function on the two test days

Parameter	Water test day	Lecithin test day	*P* (paired *t* test)
FEV_1 (L)	3.7 ± 0.9	3.7 ±0.9	0.66
FVC (L)	4.5 ± 1.1	4.5 ± 1.1	0.74
FEV_1/FVC	82.9 ± 8.1	83.2 ± 8.9	0.75
PEFR (L/Min)	491.2 ± 112.2	506.7 ± 115.1	0.02
MMEF (L/sec)	3.9 ± 1.2	3.9 ± 1.2	0.84

3.3.3 Magnitude of Changes in Lung Function

Each spirometric lung function index (FEV_1, FVC, FEV_1/FVC, PEFR and MMEF) decreased significantly after airway challenge on the water test day. However, the FVC and PEFR failed to decrease significantly (both $p > 0.05$) when the asthmatic participants had gargled with lecithin (refer to Table 3.3). In addition, gargling with lecithin significantly attenuated the responses to airway challenge for FEV_1, FVC and PEFR when compared with respective values obtained after gargling with water.

Table 3.3 – Maximum changes in lung function during airway challenge

	Water			Lecithin			% of Placebo		
Parameter	**After Placebo**	**Minimum after challenge**	***p***	**After Placebo**	**Minimum after challenge**	***p***	**Water**	**Lecithin**	***p***
FEV_1 (L)	3.7 ± 0.9	3.4 ± 1.0	0.0003	3.7 ± 0.9	3.6 ± 0.9	0.0082	91.1± 6.9	96.5 ± 3.5	0.011
FVC (L)	4.5 ± 1.1	4.3 ± 1.2	0.0337	4.5 ± 1.1	4.5 ± 1.1	0.1842	95.3± 6.5	99.0 ± 2.4	0.047
FEV_1/FVC (%)	82.9 ± 7.7	78 ± 8.6	0.0007	82.3 ± 8.3	79.5 ± 8.7	0.0003	94.8± 3.8	96.5 ± 2.2	0.111
PEFR (L/Min)	484.3± 108.5	432.8 ± 107.8	0.0006	485.1 ± 116.7	468.8 ± 105.7	0.0915	89.1± 8.1	97.0 ± 5.9	0.001
MMEF (L/sec)	3.8 ± 1.2	3.2 ± 1.2	0.0001	3.7 ± 1.2	3.5 ± 1.2	0.0109	81.2± 10.8	91.9 ± 7.5	0.004

3.3.4 Effects of Lecithin on Lung Function

3.3.4.1 FEV_1

Figure 3.1 shows FEV_1 changes for 1 hour after airway challenge with inhaled mannitol for the 12 participants. For each time period over the 60 minutes post-challenge period, the FEV_1 was significantly higher after gargling with lecithin compared to after gargling with water (all $p < 0.05$, paired t tests). As seen in Figure 3.2, the FEV_1 changes in the 6 male participants were numerically higher after lecithin compared to after water for each time period over the 60 minutes post-challenge. However, none of these numeric differences reached statistical significance (all $p < 0.05$, paired t tests). Figure 3.3 shows FEV_1 changes in the 6 female participants for 1 hour after airway challenge with inhaled mannitol. The post-challenge decrease in FEV_1 for the women was significantly attenuated after garling with lecithin compared to after gargling with water for each of the time periods over the 60 minutes post-challenge period (all $p < 0.05$, paired t tests).

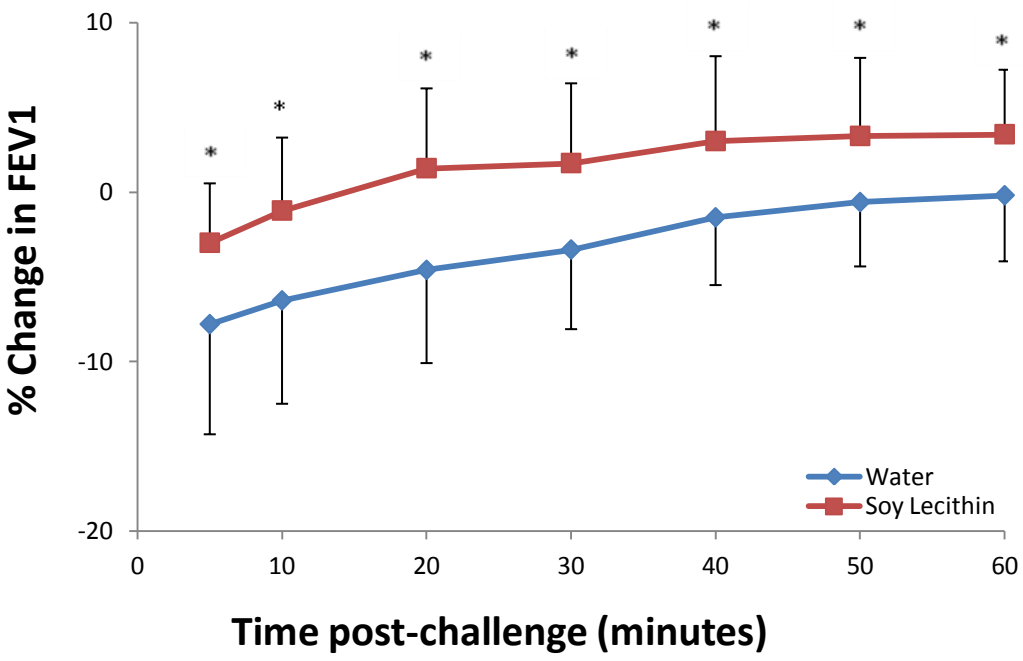

Figure 3.1 – Time course of mean FEV_1 changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 12 adult asthmatics. * denotes $p < 0.05$

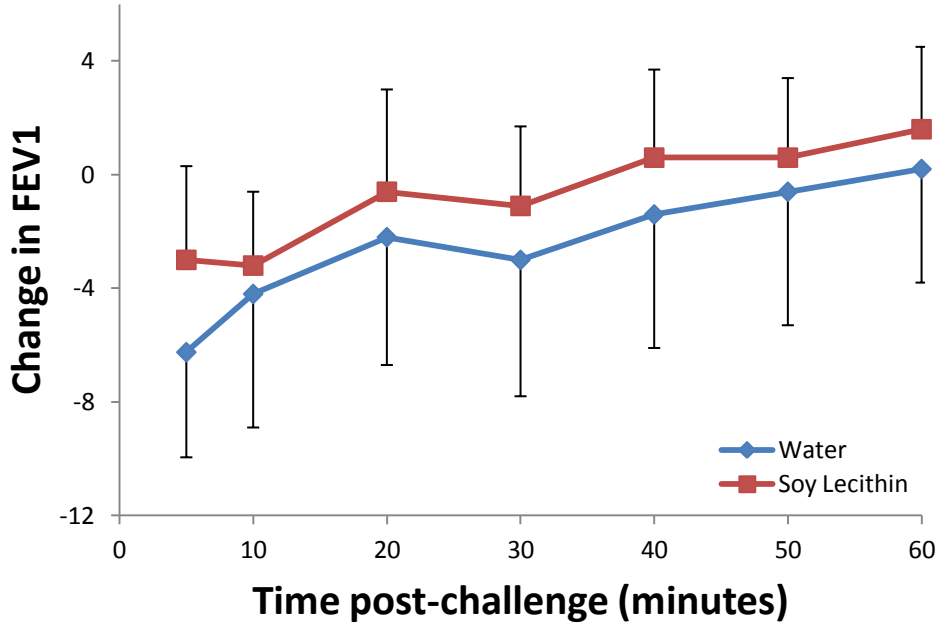

Figure 3.2 – Time course of mean FEV_1 changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic men. None of the lecithin values differed significantly (all $p > 0.05$) from their respective water values.

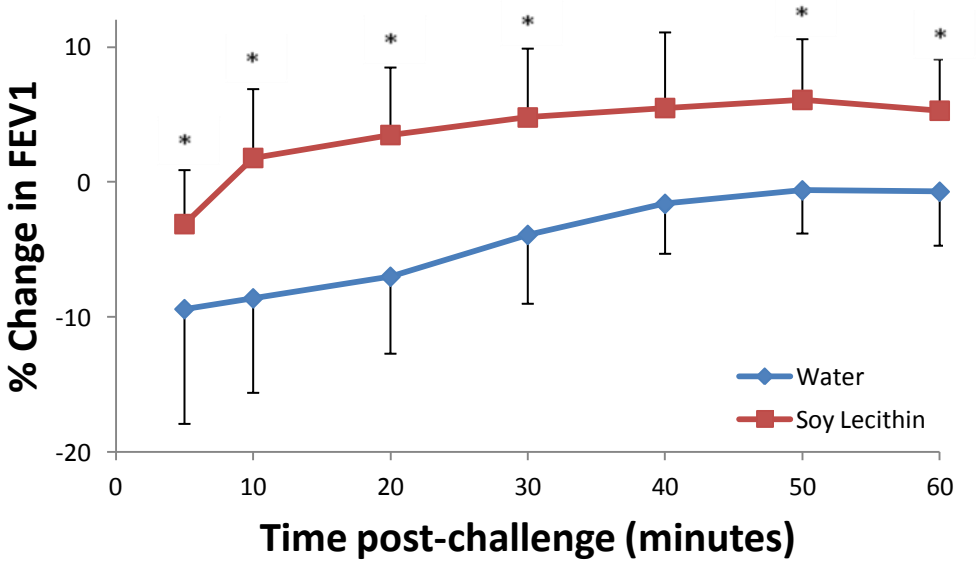

Figure 3.3 – Time course of mean FEV_1 changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic women. * denotes $p < 0.05$

3.3.4.2 FVC

Figure 3.4 shows the FVC changes for 1 hour after airway challenge in all 12 participants. The FVC was significantly higher after lecithin compared to after water for each time period over the 60 minutes post-challenge period (all $p < 0.05$, paired t tests). For the 6 men, the FVC changes were marginally numerically higher after gargling with lecithin than after gargling with water for each time period over the 60 minutes post-challenge period (refer to Figure 3.5). However, these numeric differences in the men did not reach statistical significance (all $p > 0.05$, paired t tests). Figure 3.6 shows the FVC changes in the 6 asthmatic women during the 1 hour after airway challenge. The women's FVC was significantly higher after gargling with lecithin compared to after gargling with water for each time period during the 60 minutes post-challenge period (all $p < 0.05$, paired t tests).

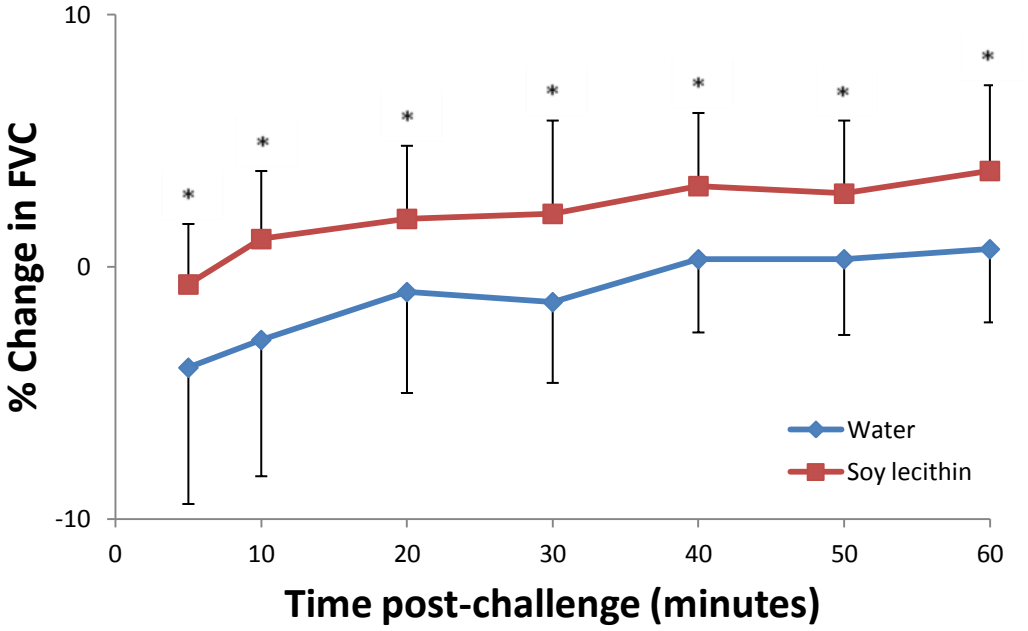

Figure 3.4 – Time course of mean FVC changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 12 adult asthmatics. * denotes p < 0.05

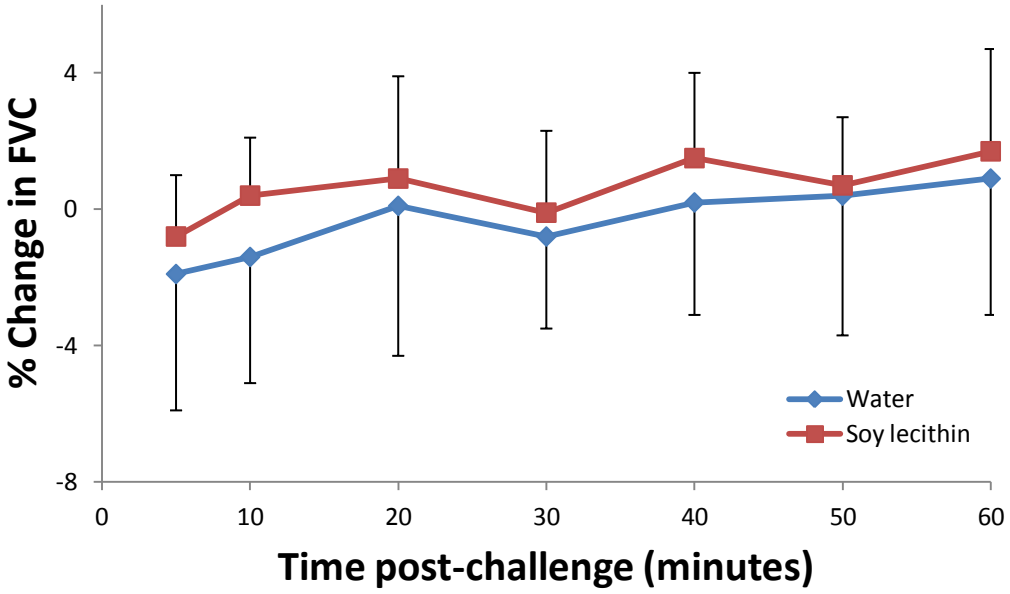

Figure 3.5 – Time course of mean FVC changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic men. None of the lecithin values differed significantly (all $p > 0.05$) from their respective water values.

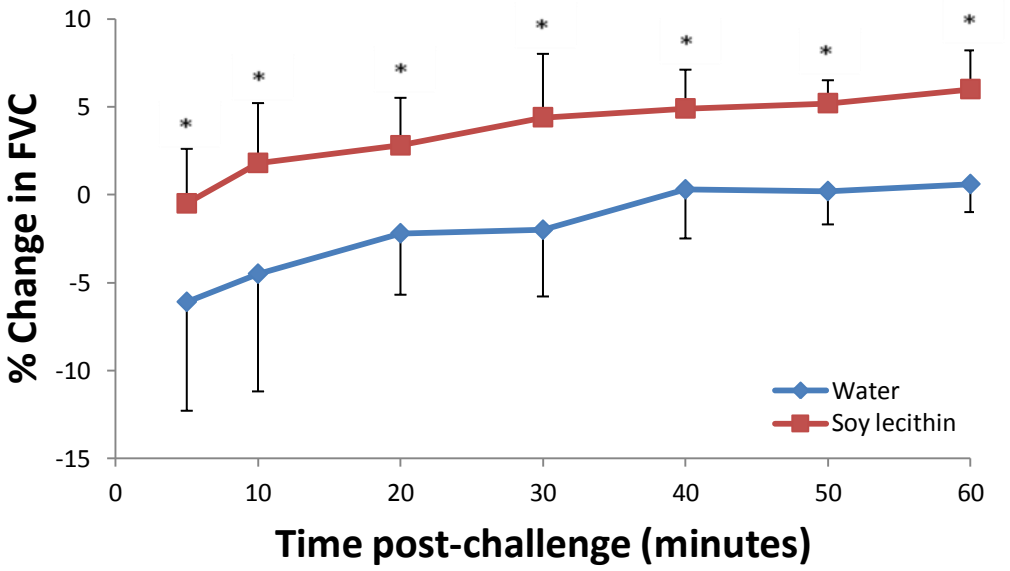

Figure 3.6 - Time course of mean FVC changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic women. * denotes $p < 0.05$

3.3.4.3 FEV_1/FVC

The FEV_1/FVC was numerically higher after gargling with lecithin compared to after gargling with water for each time period for the 60 minutes post-challenge period in the 12 asthmatics, the 6 men asthmatics and the 6 women asthmatics although none of these numeric differences reached statistical significance (all $p > 0.05$, paired t tests).

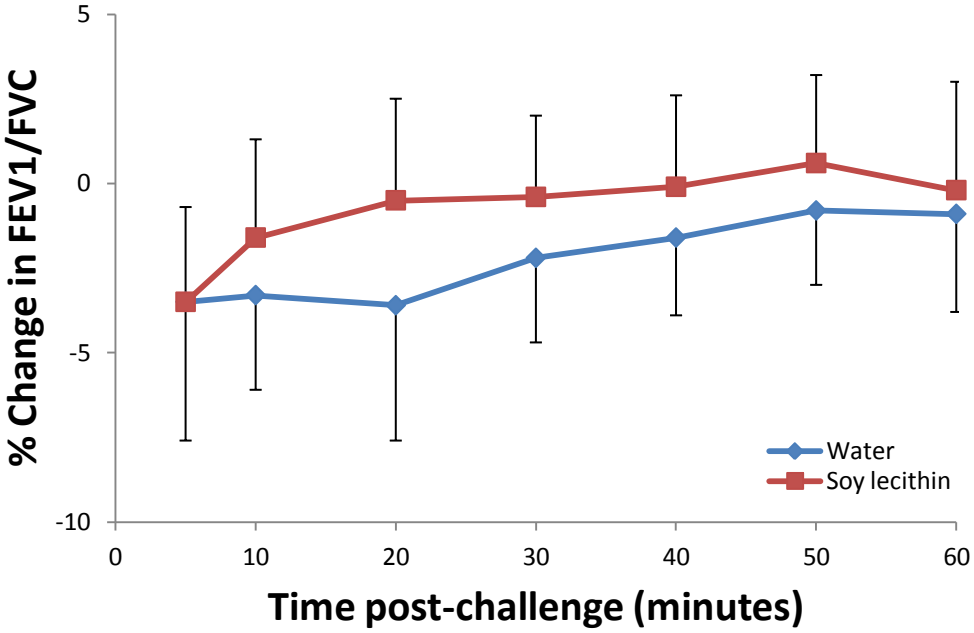

Figure 3.7 - Time course of mean FEV_1/FVC changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 12 adult asthmatics. None of the lecithin values differed significantly (all $p > 0.05$) from their respective water values.

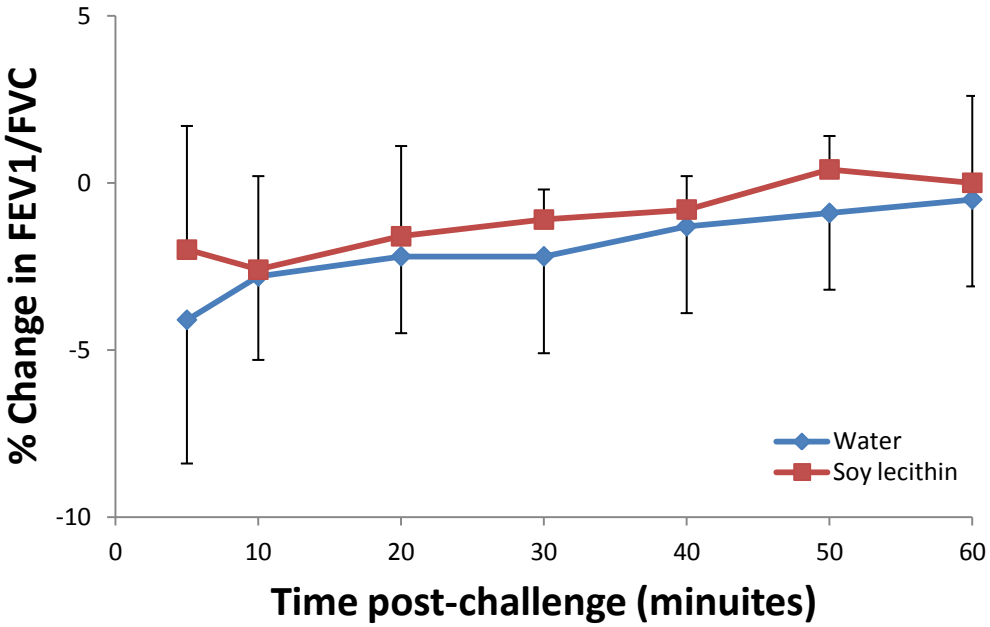

Figure 3.8 – Time course of mean FEV_1/FVC changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic men. None of the lecithin values differed significantly (all $p > 0.05$) from their respective water values.

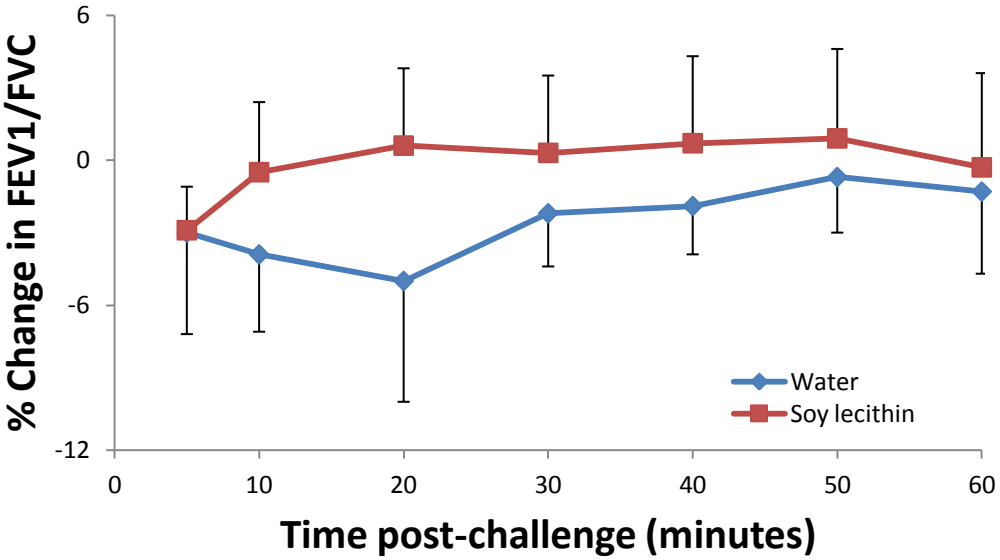

Figure 3.9 – Time course of mean FEV_1/FVC changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic women. None of the lecithin values differed significantly (all $p > 0.05$) from their respective water values.

3.3.4.4 PEFR

PEFR changes in the 12 participants were significantly higher after gargling with lecithin compared to after gargling with water throughout the 60 minutes post-challenge period (all $p < 0.05$, paired t tests; refer to Figure 3.10). For the 6 men, PEFR changes throughout the 60 minutes post-challenge period were numerically higher after gargling with lecithin compared to after gargling with water (refer to Figure 3.11). However, the numeric differences only reached statistical significance for the 5 minutes post-provocation time ($p < 0.05$, paired t tests). As shown in Figure 3.12, PEFR in the 6 women asthmatics was numerically higher throughout the 60 minutes post-provocation period after gargling with lecithin compared to after gargling with water. However, these numeric differences were only statistically significant ($p < 0.05$, paired t tests) at 40 and 60 minutes post-provocation.

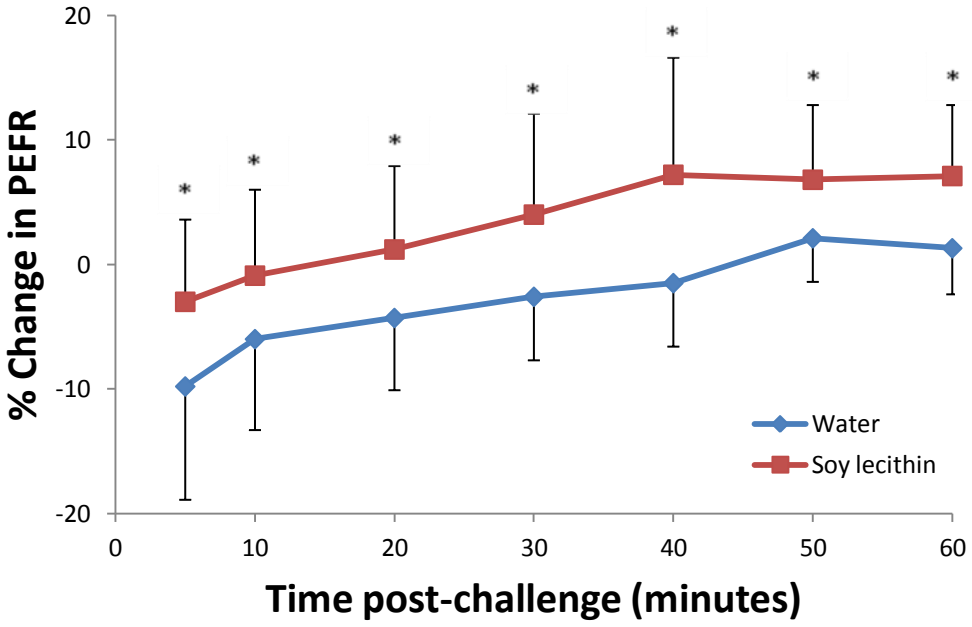

Figure 3.10 – Time course of mean PEFR changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 12 adult asthmatics. * denotes p < 0.05

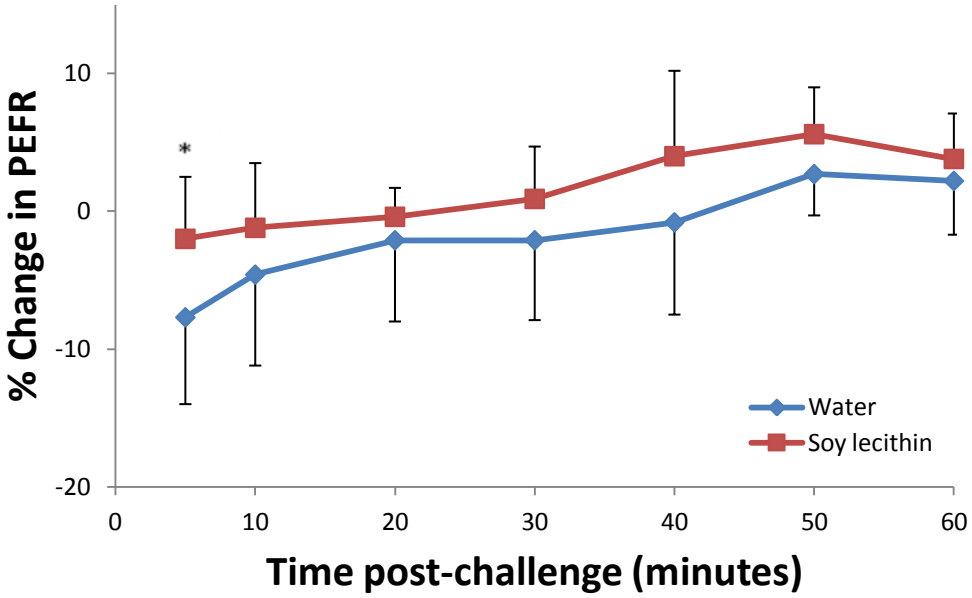

Figure 3.11 – Time course of mean PEFR changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic men. * denotes p < 0.05

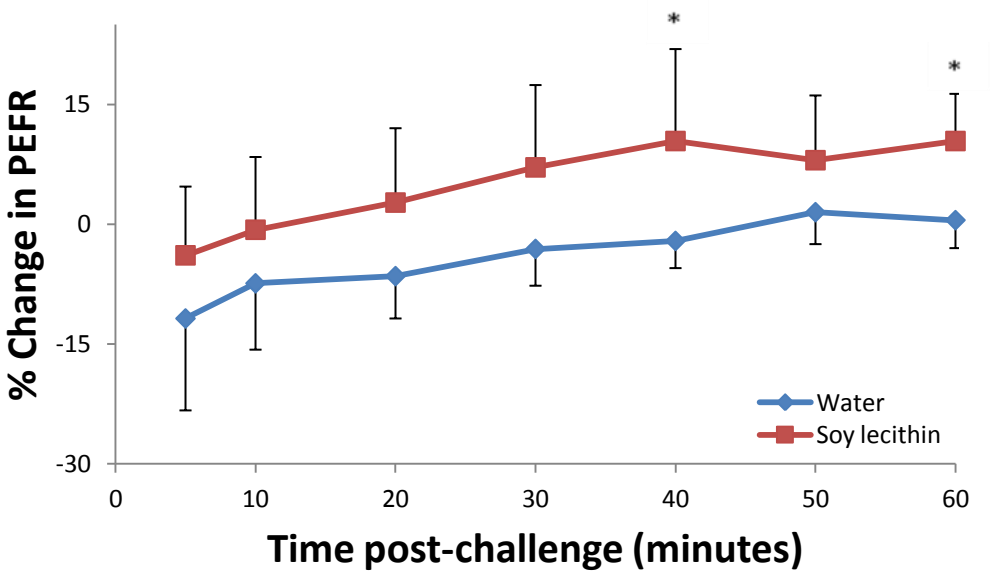

Figure 3.12 – Time course of mean PEFR changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic women. * denotes p < 0.05

3.3.4.5 MMEF

For the 12 asthmatics the MMEF was numerically higher throughout the 60 minute post-provocation period after gargling with lecithin compared to after gargling with water (refer to Figure 3.13). However, these numeric differences only reached statistical significance ($p < 0.05$, paired t test) at 5, 10 and 30 minutes post-provocation. The MMEF in the 6 men was numerically higher throughout the 60 minutes post-challenge period after gargling with lecithin compared to after gargling with water (refer to Figure 3.14), but none of these differences reached statistical significance (all $p > 0.05$, paired t tests). In the 6 women, the MMEF was numerically higher throughout the 60 minute post-provocation period after gargling with lecithin compared to after gargling with water (refer to Figure 3.15), but these difference only reached statistical significance ($p < 0.05$, paired t tests) at 5 and 10minutes post-provocation.

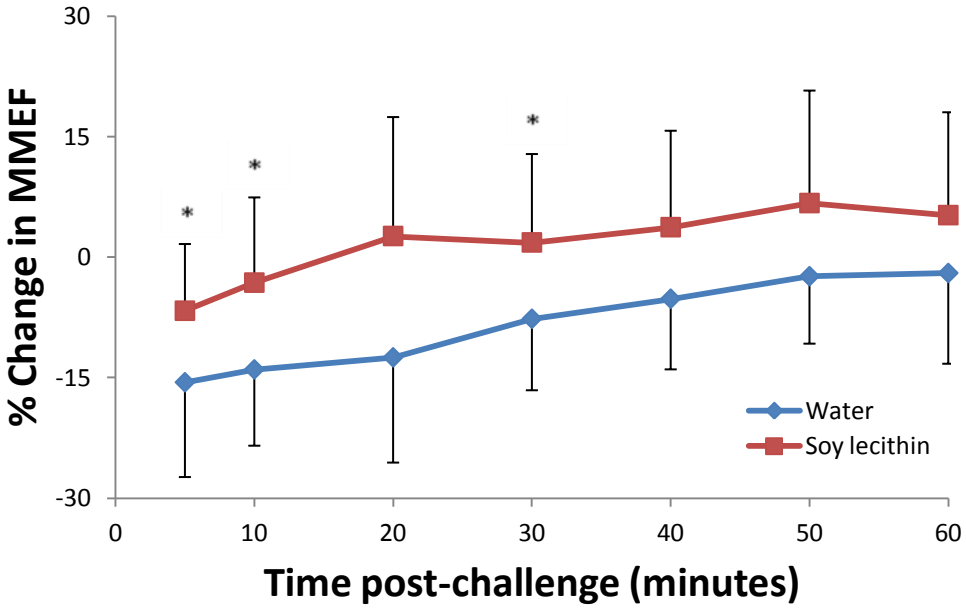

Figure 3.13 – Time course of mean MMEF changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 12 adult asthmatics. * denotes p < 0.05

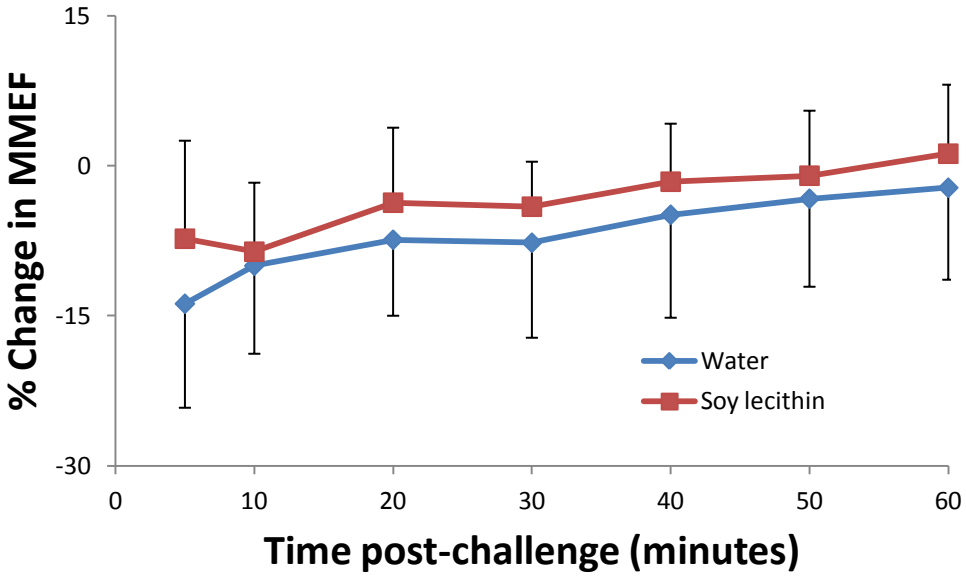

Figure 3.14 – Time course of mean MMEF changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic men. None of the lecithin values differed significantly (all $p > 0.05$) from their respective water values.

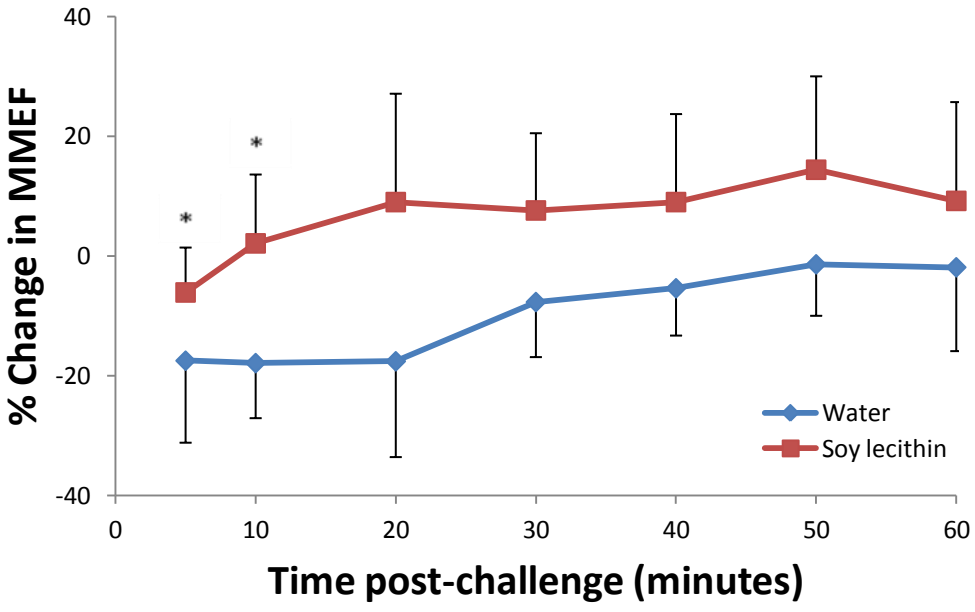

Figure 3.15 – Time course of mean MMEF changes (+ or - 1 SD) from airway challenge with inhaled mannitol following oral gargling of lecithin and water in 6 asthmatic women. * denotes $p < 0.05$

3.3.4.6 Spirometric Recovery Relative to Pre-provocation Baseline

In the 12 asthmatic participants three of the forced expiratory measurements of lung function (FEV_1, FVC and PEFR) recovered to above pre-provocation baseline levels during the 60 minute post-provocation period on the lecithin test day (refer to Table 3.4). Although such increases in lung function to above pre-provocation baseline levels on the lecithin test day were evident to a minor extent in the 6 men) PEFR only), they were clearly evident in the 6 women (refer to Tables 3.5 and 3.6). The increases in lung function to above pre-provocation baseline levels on the lecithin test day were small but were statistically significant (all $p < 0.05$) and were not observed when the participants had gargled with water (refer to Tables 3.7 to 3.9). In addition, for the 12 asthmatics the increases in lung function to above pre-provocation baseline levels on the lecithin test day first became evident 40 minutes after airway provocation with inhaled mannitol and persisted throughout the remainder of the 60 minute post-provocation period.

Table 3.4 – Spirometric indices in 12 asthmatics before and after gargling with lecithin and airway challenge with inhaled mannitol

	Time after airway challenge with inhaled mannitol					
Parameter	**baseline**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
FEV_1 (L)	3.7 ± 0.9	3.7 ± 0.9	3.7 ± 0.9	3.8 ± 0.9*	3.8 ± 0.9*	3.8 ± 0.9*
FVC (L)	4.5 ± 1.1	4.6 ± 1.1	4.6 ± 1.0	4.6 ± 1.1*	4.6 ± 1.1*	4.6 ± 1.1*
FEV_1/FVC	83.2 ± 8.9	81.9 ± 8.7	82.0 ± 8.2	82.3 ± 8.3	82.8 ± 8.2	82.2 ± 8.2
PEFR (L/min)	507 ± 115	489 ± 110	501 ± 108	514 ±109*	516 ±116*	516 ±111*
MMEF (L/sec)	3.9 ± 1.2	3.8 ± 1.3	3.8 ± 1.2	3.8 ± 1.2	4.0 ± 1.3	3.9 ± 1.2

* Denotes $p < 0.05$ relative to baseline levels (paired *t* tests)

Table 3.5 – Spirometric indices in 6 men asthmatics before and after gargling with lecithin and airway challenge with inhaled mannitol

	Time after airway challenge with inhaled mannitol					
Parameter	**baseline**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
FEV_1 (L)	4.3 ± 0.6	4.3 ± 0.6	4.2 ± 0.6	4.3 ± 0.6	4.3 ± 0.6	4.4 ± 0.6
FVC (L)	5.3 ± 0.7	5.4 ± 0.9	5.3 ± 0.8	5.4 ± 0.7	5.4 ± 0.7	5.4 ± 0.7
FEV_1/FVC	80.5 ± 5.0	79.3 ± 6.8	79.7 ± 5.3*	79.8 ± 4.9	80.8 ± 5.2	80.5 ± 5.6
PEFR (L/min)	559 ± 113	556 ± 107	563 ± 107	576 ± 110	589 ± 112	580 ± 114*
MMEF (L/sec)	3.9 ± 0.7	3.8 ± 0.9	3.8 ± 0.8	3.9 ± 0.8	3.9 ± 0.9	4.0 ± 0.9

* Denotes $p < 0.05$ relative to baseline levels (paired *t* tests)

Table 3.6 – Spirometric indices in 6 women asthmatics before and after gargling with lecithin and airway challenge with inhaled mannitol

	Time after airway challenge with inhaled mannitol					
Parameter	**baseline**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
FEV_1 (L)	3.1 ± 0.8	3.2 ± 0.9	3.2 ± 0.9 *	3.3 ± 0.8 *	3.3 ± 0.9 *	3.2 ± 0.8 *
FVC (L)	3.7 ± 0.7	3.8 ± 0.8 *	3.8 ± 0.8*	3.8 ± 0.7*	3.9 ± 0.8*	3.9 ± 0.7 *
FEV_1/FVC	84.2 ± 10.9	84.5 ± 10.1	84.3 ± 10.3	84.7 ± 10.6	84.8 ± 10.6	83.8 ± 10.7
PEFR (L/min)	411 ± 65	422 ± 68	440 ± 73	452 ± 69	443 ± 67	453 ± 66*
MMEF (L/sec)	3.5 ± 1.5	3.8 ± 1.8	3.8 ± 1.7	3.8 ± 1.6	4.0 ± 1.7	3.7 ± 1.6

* Denotes $p < 0.05$ relative to baseline levels (paired *t* tests)

Table 3.7 – Spirometric indices in 12 asthmatics before and after gargling with water and airway challenge with inhaled mannitol

	Time after airway challenge with inhaled mannitol					
Parameter	**baseline**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
FEV_1 (L)	3.7 ± 0.9	3.6 ± 0.9*	3.6 ± 0.9*	3.7 ± 0.9	3.7 ± 0.9	3.7 ± 0.9
FVC (L)	4.5 ± 1.1	4.4 ± 1.2	4.4 ± 1.1	4.5 ± 1.1	4.5 ± 1.1	4.5 ± 1.1
FEV_1/FVC	82.9 ± 8.1	80.0 ± 8.8*	81.3 ± 9.2*	81.7 ± 8.7*	82.3 ± 8.7	82.3 ± 9.4
PEFR (L/min)	491 ± 112	466 ± 114*	473 ± 112	478 ± 111	495 ± 115*	492 ± 115
MMEF (L/sec)	3.9 ± 1.2	3.4 ± 1.2*	3.6 ± 1.3*	3.7 ± 1.3*	3.8 ± 1.3	3.9 ± 1.4

* Denotes $p < 0.05$ relative to baseline levels (paired *t* tests)

Table 3.8 – Spirometric indices in 6 men asthmatics before and after gargling with water and airway challenge with inhaled mannitol

	Time after airway challenge with inhaled mannitol					
Parameter	**baseline**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
FEV_1 (L)	4.2 ± 0.6	4.2 ± 0.6	4.1 ± 0.7	4.2 ± 0.7	4.2 ± 0.7	4.3 ± 0.6
FVC (L)	5.2 ± 0.7	5.3 ± 0.8	5.2 ± 0.8	5.3 ± 0.7	5.3 ± 0.8	5.3 ± 0.8
FEV_1/FVC	81.0 ± 5.2	79.3 ± 6.8	79.3 ± 7.2	80.0 ± 6.5	80.3 ± 6.3	80.7 ± 6.2
PEFR (L/min)	548 ± 106	535± 101	535 ± 106	542 ± 104	562 ± 108	560 ± 109
MMEF (L/sec)	4.0 ± 0.8	3.7 ± 1.0	3.7 ± 1.1	3.8 ± 1.0	3.9 ± 1.0	3.9 ± 0.9

All $p > 0.05$ relative to baseline levels (paired *t* tests)

Table 3.9 – Spirometric indices in 6 women asthmatics before and after gargling with water and airway challenge with inhaled mannitol

	Time after airway challenge with inhaled mannitol					
Parameter	**baseline**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
FEV_1 (L)	3.1 ± 0.8	2.9 ± 0.9*	3.0 ± 0.9	3.1 ± 0.9	3.1 ± 0.9	3.1 ± 0.9
FVC (L)	3.7 ± 0.7	3.6 ± 0.8	3.6 ± 0.8	3.7 ± 0.8	3.7 ± 0.8	3.7 ± 0.7
FEV_1/FVC	84.8 ± 9.8	80.7 ± 11.0	83.2 ± 11.2	83.3 ± 10.8	84.3 ± 10.8	84.0 ± 12.2
PEFR (L/min)	421 ± 71	396 ± 83*	410 ± 82	414 ± 80	429 ± 82	425 ± 80
MMEF (L/sec)	3.7 ± 1.5	3.1 ± 1.5	3.5 ± 1.6	3.6 ± 1.6	3.7 ± 1.7	3.7 ± 1.9

* Denotes $p < 0.05$ relative to baseline levels (paired *t* tests)

3.3.5 Changes in Cough Indices

As shown in Table 3.10, gargling with lecithin reduced two of the cough indices' (CDR and Total number of coughs) responses to airway challenge with inhaled mannitol compared to the levels seen when gargling was done using water. These numeric differences reached statistical significance ($p < 0.050$ with the paired Students' *t* test. However the cough threshold did not change ($p = 0.90$). Although the RDR was numerically lower on the lecithin test day in 11 of the 12 participants, the numeric differences between the two test days was small compared to the interindividual variation, and a significant difference was therefore not detected using the paired Student's *t* test. However, the RDR was found to be significantly lower on the lecithin test day when these data were reanalyzed *post priori* with the nonparametric Wilcoxon sign test for paired data (null hypothesis rejected at $p < 0.05$) (Alder and Roessler, 1972).

Table 3.10 - Cough indices and response-to-dose ratio for inhaled mannitol after gargling with water and lecithin

Indices	**Water**	**Lecithin**	***p* value**
C2	90 ± 150	93 ± 103	0.90†
CDR	3.6 ± 2.5	2.4 ± 1.6	0.02†
RDR	0.04 ± 0.08	0.02 ± 0.03	0.01*
Total number of coughs	15 ± 10.7	10 ± 7.8	0.01†

† Determined by the paired Students *t* test.

* Determined by the nonparametric Wilcoxon sign test for paired data.

3.4 Discussion

This is the first study to examine whether topical application of exogenous phospholipids to the oropharynx and adjacent areas can significantly attenuate the bronchoconstrictor and cough responses to indirect airway challenges with inhaled mannitol in asthmatic men and women. Soy lecithin is a rich food source of phospholipids (Helmerich and Koehler, 2003; Hurst and Martin, 1984**;** Press *et al*., 1981; Weihrauch and Son, 1983; Zeisel *et al*., 2003) and phospholipids were therefore topically applied to the oropharyngeal mucosa by having the asthmatic subjects gargle an aqueous suspension of soy lecithin. The present study demonstrated that gargling with soy lecithin attenuated the airway obstruction and cough induced by inhaled mannitol challenge and these findings are consistent with a protective effect when phospholipids are topically applied to the oropharynx in asthmatics. The findings of this study provide new insights into extrathoracic control of airway function in asthmatics and raise a number of potentially significant therapeutic implications which warrant further investigation.

3.4.1 Mechanism of Action

Gargling with lecithin significantly attenuated the airway obstruction and cough evoked by mannitol challenge in the present study. It is significant to note that, unlike the total number of coughs and the cough-to-dose ratio, the cough threshold to inhaled mannitol was not higher on the lecithin test day. This may mean that the total number of coughs and the cough-to-dose ratio were more sensitive cough indices than the cough threshold. Neural afferent pathways from the larynx and tracheobronchial tree have been shown to be extensively involved in reflex bronchoconstriction and the genesis of coughing (Nadel and Widdicombe, 1962; Widdicombe, 1963; Widdicombe, 1986). However, coughing can also be induced by pharyngeal stimulation (Irwin and Widdicombe, 2000). In addition, McNally *et al*. (1979) and Rodriguez-Martinez *et al.* (1973) demonstrated that local anaesthesia of the pharyngeal mucosa attenuated the airway obstruction induced by indirect airway

challenges with exercise and cold air breathing in asthmatics, suggesting that pharyngeal neural receptors may also be involved in inducing airway obstruction in asthmatics. Thus, although little explored, these studies suggest that pharyngeal neural receptors can play a significant role in the regulation of airway caliber and the genesis of cough in asthmatics.

Although definitive evidence is lacking, it has been hypothesized that mast cell degranulation resulting from transient changes in the osmolarity of the periciliary airway fluid is responsible for the induction of airway obstruction in asthmatics during indirect airway challenges with exercise and inhalation of cold dry air (Anderson, 1985; McFadden and Gilbert, 1994**)**. A similar mechanism has been proposed for airway challenges with inhaled mannitol powder (Brannan *et al*., 2009; Leuppi *et al*., 2002). However, local anaesthesia of the pharyngeal and adjacent upper airway mucosa has been shown to attenuate and even completely prevent airway obstruction induced by indirect airway challenges with exercise and cold air breathing in asthmatics (McNally *et al*., 1979; Rodriguez-Martinez *et al*., 1973). Other studies have reported that inhalation of nebulized local anaesthetic solutions can benefit asthmatics and reduce reliance on inhaled corticosteroids and bronchodilators (Hunt *et al*., 1996; Hunt et al., 2004). These findings have been used as supportive evidence for a role of upper airway neural receptors in the airway responses to indirect airway challenges (McNally *et al*., 1979; Rodriguez-Martinez *et al*., 1973). However, contrary findings showing an absence of beneficial effects have also been reported (Caire *et al*., 1989; Fanta *et al*., 1980). These disparities may reflect differences in the degree of local anaesthetic block achieved, or may reflect interindividual variation in the importance of upper airway neural receptor involvement in regulating airway caliber.

The protective effects from lecithin on lung function and cough observed in the present study have not previously been reported and the responsible mechanisms have not been determined. Phospholipid molecules are electrostatically adsorbed onto mucosal surfaces (Hills, 1988; Hills, 2002; Hills and Kirkwood, 1989; Van der Touw and Fewings, 2009). This occurs because of the electrostatic attraction between negatively charged radicals (e.g. carbonyl and sulphonyl groups) on the mucosal surfaces and the positive groups on the bipolar head of the phospholipids molecules (Hills, 1988; Hills, 2002). Hills (2002) hypothesized that lung surfactant would be well suited for masking airway irritant receptors because tenacious binding of surface active phospholipids to the airway mucosa would form a barrier which prevents noxious inhaled agents from reaching the irritant receptors. Moreover, asthma has been associated with surfactant deficiency (Enhorning *et al.*, 1989), and this has led to speculation that agents that can uncover or otherwise compromise the adsorbed layer of surface active phospholipids by physical or biological means can sensitize the lung to various triggers and potentiate asthmatic attacks (Hills, 1996). Such "unmasking" or "uncovering" is a well-known concept in neurophysiology, and was introduced as a physical mechanism to explain the up to 100 fold sensitization of a reflex sometimes observed in the central nervous system (Hills, 1996). In support of this postulated mechanism, Hills and Chen (2002) demonstrated that surface active phospholipids applied to bronchial mucosa reduced the sensitivity of irritant receptors in rat airways to airway challenge with methacholine aerosol. These workers suggested that placebo-controlled clinical trials, using exogenous surface active phospholipids as a means of controlling asthma should be considered (Hills and Chen, 2002). However, these reports have been confined to the lower respiratory tract and there do not appear to be any studies that have examined whether surface active phospholipids can mask upper airway neural receptors, or what the airway mechanics consequences of such an action would be if such an effect occurred.

In view of the above mentioned considerations, there is evidence in the literature that pharyngeal neural receptors play a role in the control of airway caliber and the genesis of coughing, and that phospholipids can mask neural airway receptors and impair their sensitivity. The results of the present study are consistent with these findings and suggest that phospholipids applied topically to the oropharynx in asthmatics can mask localized mucosal neural receptors and thereby attenuate airway obstruction and cough that have been reflexly mediated when these receptors have been stimulated by airway challenge with inhaled mannitol powder. These findings provide additional evidence that pharyngeal mucosal neural receptors can play a significant role in eliciting airway obstruction and cough during indirect airway challenge in asthmatics.

3.4.2 Critique of Methodology

The pre-challenge PEFR was significantly higher on the lecithin test day compared to the water test day. In contrast, prechallenge levels of the four other forced expiratory lung function indices were reproducible over the two test days. Measurements of PEFR show greater variability than other expiratory spirometric indices of lung function (American Thoracic Society, 1995). It is therefore tempting to speculate whether this greater variability may have spuriously led to the higher prechallenge PEFR levels seen on the lecithin test day.

Reproducibility of baseline lung function was deemed to be a necessary condition to enable meaningful comparisons between post-airway challenge data on the water and lecithin test days. The experimental protocol was therefore designed to minimize any variations in baseline lung function over the experimental period. A number of steps were incorporated into the experimental protocol in order to optimize reproducibility between the two test days. Firstly, the asthmatic participants were carefully screened to ensure that their asthma was currently well controlled, that there had been no significant worsening of asthma symptoms in the last three months, and that there had not been a respiratory infection in the past two months (refer to section 2.2.2). Secondly, care was taken to ensure that the intervening period between the two test

days did not exceed one week. In addition, lung function fluctuates periodically during each 24 hour cycle (Hetzel, 1988**)**, and to minimize the influence of this periodic fluctuation on the experimental data experiments for each participant on the two test days were carried out at the same time of day (within one hour). Participants were also asked to refrain from taking any short-acting bronchodilator medications and to refrain from physical exercise before attending the laboratory on each of the two test days. In addition, caffeine is known to exert a bronchodilator action (Kivity *et al*., 1990), and participants were therefore asked to refrain from taking any caffeine containing foodstuffs or beverages before attending the laboratory on each of the two test days. Lastly, many raw foods contain significant quantities of phospholipids (Hills and Kirkwood, 1989; Lundberg, 1959; Weihrauch and Son, 1983; Zeisel *et al*., 2003), and lecithin is widely used in the processing of many foods (Helmerich and Koehler, 2003; Hurst and Martin, 1984). This study was designed to examine whether phospholipids can influence airway function in asthmatics (an effect which was confirmed by the results of this study), and participants were therefore asked to refrain from ingesting any foodstuffs before attending the laboratory on each of the two test days.

McNally *et al* (1979) and Rodriguez-Martinez *et al* (1973) demonstrated that local anaesthesia of the pharyngeal mucosa attenuated the airway obstruction induced by indirect airway challenges with exercise and cold air breathing in asthmatics. Exercise and cold air inhalation are believed to initiate airway obstruction in asthmatics by transiently changes the osmolarity of the periciliary fluid (Anderson *et al*., 1985; McFadden and Gilbert, 1994), and a similar mechanism is believed to initiate airway obstruction in asthmatics during airway challenges with inhaled mannitol powder (Brannan *et al*., 2009; Leuppi *et al*., 2002). Indeed, airway challenges with inhaled mannitol were originally devised as a simpler and more convenient alternative which required only minimal equipment and provided an indirect airway challenge comparable to that obtained by other indirect challenges such as exercise and cold air breathing (Brannan *et al*., 2009; Leuppi *et al*., 2002). In view of these considerations, inhaled mannitol was considered to be the most appropriate form of indirect airway challenge for the present study.

Soy lecithin is a rich food source of cheap and readily available phospholipids (Helmerich and Koehler, 2003; Hurst and Martin, 1984**;** Press *et al*., 1981; Weihrauch and Son, 1983; Zeisel *et al*., 2003). Gargling with a concentrated aqueous suspension of soy lecithin was therefore chosen as the means of topically applying phospholipids to the pharyngeal mucosa in the present study. Based on the manufacturer's reported phospholipid content of the soy lecithin used in this study, each participant gargled a 100 ml suspension containing 1,680 mg phosphatidylcholine and 960 mg phosphatidylinositol. However, the quantity of phospholipids that adhered to the pharyngeal mucosa could not be determined. In addition, the distribution of the adhering phospholipids remains unknown. Subjects were carefully instructed not to swallow any of the lecithin suspension to minimize any possible systemic effects. Most of the adhering phospholipids would have likely been located on the oral and oropharyngeal mucosa following gargling, but adhesion onto adjacent mucosal surfaces (e.g. laryngopharyngeal and nasopharyngeal) cannot be discounted. Researchers have commented that inhaled mannitol powder during mannitol airway challenge is likely to preferentially deposit on the mucosa of the larger airways such as the oropharynx (Anderson *et al*., 1997; Kersten *et al*., 2009; Leuppi *et al*., 2002). This could potentially have augmented the protective effect of phospholipids in the present study, given that the phospholipids were topically applied to the same region before airway challenge. Further studies will be required to determine whether a comparable protective effect from phospholipids can be achieved against direct types of airway challenge or other types of indirect airway challenge.

The protective effects on lung function in this study have been attributed to lecithin's high phospholipid content. However, the soy lecithin used in this study also contains significant amounts of other components, including the essential fatty acids linoleic acid and linolenic acid. It is therefore possible that such non phospholipid components were responsible for, or contributed to, the protective effect on lung function. To counter this possibility, phospholipids are known to bind tenaciously onto mucosal surfaces (Hills, 1988; Hills, 2002; Hills and Kirkwood, 1989; Van der Touw and Fewings, 2009). In addition, phospholipids delivered to the intrathoracic airways have previously been shown to reduce airflow obstruction and improve heterogeneity of

ventilation in asthmatics (Crawford and Young, 1995), as well as reduce irritant receptor stimulation from airway challenge with methacholine in rat intrathoracic airways (Hills and Chen, 2002).Thus, while a definitive answer to this issue will require further studies with pure phospholipids, there is supportive evidence in the literature suggesting that the phospholipid component of soy lecithin was responsible for the protective effect on lung function observed in the present study.

To take into account any placebo responses, the effects of phospholipids on airway challenge responses were compared with the effects of water on airway challenge responses. Participants were informed that the study aimed to determine whether water or an aqueous suspension of soy lecithin was best for protecting the airways during airway challenge. Despite this, the bronchoconstriction and cough evoked by mannitol airway challenge were significantly lower when the asthmatic participants had gargled with lecithin compared to when they had gargled with water.

3.4.3 Clinical Implications

In addition to providing further insights into the control of airway function in asthmatics, the findings of this study have a number of implications for the diagnostic testing and clinical management of asthma and other respiratory conditions. Previous studies have reported that local anaesthesia of the oropharyngeal and adjacent mucosal surfaces in asthmatics attenuates the airway obstruction induced by exercise challenge (McNally *et al*., 1979) and cold air breathing (Rodriguez-Martinez *et al*., 1973). These findings compliment the findings from the present study that the airway obstruction and cough induced by inhaled mannitol challenge is attenuated by pretreatment with topical application of phospholipids to the mucosa of the oropharynx and adjacent airways. This raises the possibility that topical application of phospholipids to the mucosa of the oropharynx and adjacent airways of asthmatics may attenuate bronchoconstriction induced by exercise or inhalation of cold air. Exercise induced asthma is a common complaint in many physically active asthmatics (Anderson, 1985; Jones *et al*., 1963; McFadden and Gilbert, 1994). In addition, enforced mouth breathing in asthmatics has been shown to result in a progressive

deterioration in FEV_1 under resting conditions (Hallani *et al*., 2008), and to increase the severity of exercise induced bronchoconstriction (Shturman-Ellstein *et al*., 1978). Moreover, a high proportion of asthmatics suffer from hay fever (Corren, 1997) and many asthmatics breathe predominantly via their mouths (Stensson *et al*. 2010). Thus, enhanced airway cooling and drying such as occurs during mouth breathing may be a common cause of exacerbations of airway obstruction in asthmatics, and a protective effect by topical application of phospholipids could be of clinical relevance to many asthmatics.

This study was not designed to examine whether gargling with lecithin improved baseline lung function in asthmatics. Nevertheless, three of the forced expiratory measurements of lung function (FEV_1, FVC and PEFR) recovered to above pre-provocation baseline levels during the 60 minute post-provocation period on the lecithin test day. Although these improvements were small, they were statistically significant (all $p < 0.05$) and were not observed when the participants had gargled with water. Moreover, these increases in lung function to above pre-provocation baseline levels first became evident 40 minutes after airway provocation with inhaled mannitol and persisted throughout the remainder of the 60 minute post-provocation period, suggesting that pharyngeal application of phospholipids can improve lung function in asthmatics for a considerable period of time. These findings raise the possibility that pharyngeal application of phospholipids may have offered some protection against airway cooling and drying. Such a protective effect could have therapeutic potential, and this possibility warrants further study and pharmacological evaluation. Further research is also warranted into whether pharyngeal application of phospholipids can offer a protective effect against other common factors that can exacerbate asthma such as air pollution, smog and upper airway irritation from viral respiratory tract infections.

Gargling with phospholipids offered significant protection against coughing during airway challenge in the present study. This raises the possibility that application of phospholipids to the oropharynx and adjacent regions (*e.g*. by lozenges) may potentially be beneficial in alleviating upper airway irritation and cough in a variety

of disorders. Such a protective action against cough and throat irritation warrants further study, especially when one considers the high annual incidence of these symptoms in the population as a result of colds and influenza infections. It is also worth considering that endogenous surface active phospholipids in the plasma membrane of cells making up the upper respiratory tract mucosa play a vital role in protecting the upper respiratory tract from viral infection (Parsons *et al*, 1998). This suggests that application of exogenous phospholipids to the mucosa of the upper respiratory tract may offer protection against viral respiratory tract infections such as the common cold and flu. Such common viral respiratory tract infections are also important exacerbating factors in asthma (Folkerts *et al*., 1998; Germ and Lemanske, 2003; Lemanske and Busse, 2006), which raises the possibility that application of exogenous phospholipids to the upper respiratory tract mucosa may offer protection against such exacerbations.

The FEV_1 in the present study was significantly higher after gargling with lecithin compared to after gargling with water over the entire 60 minutes post-challenge period. However, the design of the present study did not directly address the duration of the protective action offered by phospholipids, and it is not known if the protective effect occurred solely at the time of the inhaled mannitol challenge, or if it persisted throughout the entire 60 minute post-challenge period. Although little is known about the pharmacokinetics of topically applied phospholipids, data from a various studies suggest that exogenous phospholipids can be retained for at least several hours after being topically administered to an oral or respiratory mucosal surface (Jokic*et al*., 1998; Kirkness *et al*., 2003; Miki *et al*., 1992; Van der Touw and Fewings, 2008). Such a sustained effect could be of sufficient duration to be pharmacologically useful. However, sustaining the protective oropharyngeal phospholipid lining may require that no food or liquids be ingested for sustained periods after phospholipid administration, and this may prove to be impractical. In addition, a sustained phospholipid coating adhering to dental and other oral or pharyngeal surfaces may potentially predispose towards the development of dental caries and poor oral hygiene. These issues warrant further study.

Hills (1996; 2002) hypothesized that molecular adhesion of phospholipids to mucosal surfaces can protect the airways by providing a barrier that masks airway receptors (Hills, 1996; Hills, 2002). Supportive evidence for this concept comes from studies that have delivered phospholipids to the intrathoracic airways and noted reduced airflow obstruction and improved heterogeneity of ventilation in asthmatics (Crawford and Young, 1995), and reduced irritant receptor stimulation from airway challenge with methacholine in rat intrathoracic airways (Hills and Chen, 2000). However, this raises the possibility that masking of neural receptors and other types of receptors at the intrathoracic airway mucosa following inhalation of nebulized phospholipids could also impede the action of medications such as inhaled bronchodilators and inhaled corticosteroids that act at these receptor sites. Nevertheless, it seems unlikely that application of phospholipids to the upper airway mucosa, as was done in the present study, would interfere with the action of medications such as inhaled bronchodilators and inhaled corticosteroids because such inhaled medications for airway obstruction act within the intrathoracic airways.

One of the most common types of food allergy in children is soy allergy (Savage *et al.*, 2010). The risk of evoking an allergic response to soy was mitigated in the present study by carefully interviewing each potential asthmatic participant and excluding any person who stated that they had a history of food allergy. However, soy allergy could adversely impact on the potential value of soy lecithin derived phospholipids as pharmaceuticals, and this may necessitate using either utilizing phospholipids derived from other sources or highly purified phospholipids.

3.4.4 Conclusion

This is the first study to examine whether topical application of exogenous phospholipids to the oropharynx and adjacent areas can significantly attenuate the bronchoconstrictor and cough responses to indirect airway challenges with inhaled mannitol in asthmatic men and women. Soy lecithin is a rich food source of cheap and readily available phospholipids, and gargling an aqueous suspension of soy lecithin was chosen to apply phospholipids to the oropharyngeal mucosa. Both the FEV_1 and cough responses to mannitol challenge were significantly attenuated after prechallenge gargling with soy lecithin compared to prechallenge gargling with water. Effects on the FEV_1 were mirrored by effects on the FVC, FEV_1/FVC, PEFR and MMEF. In addition, the degree of attenuation appeared to be greater in women than in the men. Supportive evidence from the literature suggests that the high phospholipid component of soy lecithin was responsible for the protective effects on lung function observed in the present study. However, further studies with pure phospholipids will be required to definitively test this.

This study did not address how phospholipids applied to the oropharynx can protect asthmatics against mannitol airway challenge, and further studies will be required to elucidate the mechanisms involved. However, the literature suggests that masking of oropharyngeal neural receptors as a result of phospholipids binding onto the oropharyngeal mucosa results in diminished reflex bronchoconstriction and cough during airway challenge. The findings of this study therefore provide new evidence suggesting that extrathoracic afferent pathways are involved in the regulation of airway function and cough during asthmatic exacerbations.

The present study was not designed to examine whether gargling with lecithin improved baseline pre-provocation lung function and it is not known whether oropharyngeal application of phospholipids can protect asthmatics from common naturally occurring types of indirect airway challenges such as exercise and the inhalation of cold dry air. Nevertheless, evidence from this study suggests that pharyngeal application of lecithin can improve baseline lung function in asthmatics,

with small but significant and sustained increases in FEV_1, FVC and PEFR to above baseline levels seen 40 minutes after airway provocation. Specific studies will need to be conducted to confirm and expand on these findings. However, a protective effect by oropharyngeal phospholipids against inhalation of cold dry air or exercise is plausible because these challenges, like inhaled mannitol powder, are believed to evoke airway provocation in asthmatics by an osmotic effect in the airway periciliary fluid.

The findings of this study have a number of potentially significant pharmacological implications which warrant further investigation. Oropharyngeal application of phospholipids may have therapeutic potential in the management of asthma by protecting the airways against factors that commonly provoke worsening symptoms such as inhaling cold dry air and exercise. In addition, the attenuation of cough observed in the present study suggests that oropharyngeal application of phospholipids (*e.g.* by lozenges) may potentially be beneficial in alleviating upper airway irritation and cough in other common disorders such as viral respiratory tract infections.

CHAPTER FOUR

Conclusions

Stimulation of oropharyngeal neural receptors is known to contribute to the bronchoconstriction induced by airway challenge with exercise and cold air in asthmatics, and phospholipids can mask and desensitize similar neural receptors in the intrathoracic airways. These findings raise the possibility that application of phospholipids to the oropharyngeal mucosa can attenuate the bronchoconstriction induced by airway challenge in asthmatics. This intriguing possibility has not previously been studied but could provide new insights into the regulation of airway caliber in asthmatics, as well as having potential therapeutic implications for the clinical management of asthma. These considerations prompted the author to conduct the investigations presented in this thesis.

4.1 Assessment of Airway Hyperresponsiveness with Inhaled Mannitol in Asthmatics

A range of different types of airway challenges have been used in lung function laboratories to induce experimental bronchoconstriction in asthmatics and careful consideration was given to the type of airway challenge that would be best suited for the purposes of the studies presented in this thesis. Airway challenge with inhaled mannitol has proven popular in lung function laboratories in recent years because of its convenience and ease of use, and an inhaled mannitol product with a disposable inhaler (Aridol^{TM}) has recently become commercially available. In addition, mannitol airway challenge is believed to evoke bronchoconstriction by similar mechanisms as other types of indirect airway challenges such as exercise and hyperventilation of cold dry air. For these reasons, mannitol airway challenges with Aridol^{TM} appeared suitable for the purposes of the studies presented in this thesis. However, mannitol challenge is a comparatively new type of airway challenge and some asthmatics do not respond to inhaled mannitol. An initial study was therefore carried out to examine

the suitability and safety of mannitol airway challenge in asthmatics. The other chief purpose of this initial study was to identify an adequately sized cohort of asthmatics who had sufficient sensitivity to inhaled mannitol to be suitable for recruitment into the primary study of this thesis which examined whether exogenous phospholipids binding to the oropharyngeal mucosa influence airway function in asthmatics.

A total of 24 adult asthmatic participants were recruited into the first study. Airway challenge with Aridol^{TM} was found to be a convenient and safe means of evoking airway obstruction by airway challenge in asthmatics, which confirms reports from previous studies. No adverse effects were noted apart from some transient coughing during the airway challenges. As previously reported in other studies, there was marked interindividual variation in the degree of bronchoconstriction evoked by airway challenge with inhaled mannitol, with the FEV_1 responses ranging from no change to a 23% fall. This is consistent with previous published reports and emphasizes the need to screen the responses to mannitol airway challenges in a comparatively large number of asthmatics if one wishes to identify an adequate number of positive responders for subsequent research purposes.

The data from 4 of the participants were excluded from detailed analysis because the FEV_1 in these participants fell less than 5% after airway challenge with inhaled mannitol. A review of the literature indicated that a fall in FEV_1 of at least 10% was the minimum acceptable level of bronchoconstriction from airway challenges with inhaled mannitol. Thirteen of the asthmatics met this criteria and this number was considered sufficient to conduct the subsequent study to examine whether exogenous phospholipids binding to the oropharyngeal mucosa can influence airway function in asthmatics. The number of asthmatic positive responders to mannitol airway challenge was marginally below the lower range reported in previous studies and this may have reflected the high proprtion of participants (16 out of 24) who were on current treatment with inhaled corticosteroids.

No relationship was evident between asthma severity and the maximum fall in FEV_1 or the dose-to-response ratio for inhaled mannitol. These findings are consistent with reports from previous studies. The present study also appears to be the first to document the dose-response curves to inhaled mannitol in asthmatics who responded to inhaled mannitol with a fall in FEV_1 between 5% and 15%. This is perhaps not surprising given that most studies dealing with mannitol airway challenge in asthmatics have used a $\geq$ 15% decrease in FEV_1 cutoff for a positive bronchoconstrictor response. Of interest, 8 of the 13 asthmatics who responded to inhaled mannitol with a fall in FEV_1 between 5% and 15% demonstrated atypical dose-response curves, with 2 participants displaying a plateau at the higher doses of inhaled mannitol, and 6 participants displaying a progressive resolution of airway obstruction with cumulative higher doses of inhaled mannitol. The reasons for this atypical dose-response behavior are unresolved at this time, but this warrants further investigation given the widespread use of airway challenge with inhaled mannitol in lung function laboratories.

4.2 Effects of Oropharyngeal Administration of Exogenous Phospholipids on Airway Function in Asthmatics

This is the first study that has examined whether topical application of exogenous phospholipids to the oropharynx and adjacent areas significantly attenuated the bronchoconstrictor and cough responses to indirect airway challenges with inhaled mannitol in asthmatic men and women. Twelve of the 13 adult asthmatics (6 men and 6 women) who demonstrated a fall in $FEV_1 \geq 10\%$ after airway challenge with inhaled mannitol in the first study were successfully recruited into the second study. For the purposes of the second study, the twelve asthmatics were airway challenged with equivalent doses of inhaled mannitol on two separate days after they had either gargled with water or an aqueous suspension of soy lecithin in random order. Soy lecithin is a processed food with a high phospholipid content, and because the oropharynx serves as both a respiratory and digestive pathway, gargling of an aqueous suspension of soy lecithin was chosen as a suitable means of topically applying a high concentration of phospholipids to the oropharyngeal mucosa.

Prechallenge lung function values were reproducible over the two experimental test days. However, both the FEV_1 and cough responses to mannitol challenge were significantly attenuated after prechallenge gargling with soy lecithin compared to prechallenge gargling with water. A similar protective effect by lecithin was also seen with the FVC, FEV_1/FVC, PEFR and MMEF. Although the present study was not designed to examine whether gargling with lecithin improved baseline pre-provocation lung function, small but significant and sustained increases in FEV_1, FVC and PEFR to above baseline pre-provocation levels were observed 40 minutes after airway challenge. Such a protective effect was not observed when the participants had gargled with water, suggesting that pharyngeal application of lecithin can improve baseline lung function in asthmatics. In addition, the increases in lung function to above pre-provocation baseline levels on the lecithin test day first became evident 40 minutes after airway provocation with inhaled mannitol and persisted throughout the remainder of the 60 minute post-provocation period. These findings suggest that pharyngeal application of phospholipids can improve lung function in asthmatics for a considerable period of time, and raise the possibility that pharyngeal application of phospholipids may have offered some sustained protection against airway cooling and drying. Of interest, the degree of protection by lecithin appeared to be greater in women than in the men. The reason for this remains unknown and it is possible that such an apparent gender effect may reflect the small sample size studied for each gender (6 participants per gender).

The findings of this study provide new evidence suggesting that oropharyngeal afferent pathways are involved in the reflex regulation of airway function and cough during asthmatic exacerbations. Supportive evidence from the literature suggests that the high phospholipid component of soy lecithin was responsible for the protective effects on lung function observed in the present study. However, further studies with pure phospholipids will be required to definitively test this. Additional studies will also be required to elucidate the mechanisms involved in the protective effects of lecithin. However, based on the published literature it is plausible that attenuated reflex bronchoconstriction and cough resulting from the masking of oropharyngeal neural receptors by adhering phospholipids was responsible.

Specific studies will need to be conducted to confirm and expand the findings of the present study to other types of airway challenges, and to specifically determine the duration of the protective action offered by phospholipids. However, a protective effect by oropharyngeal phospholipids against natural asthma trigger factors such as inhalation of cold dry air or exercise is plausible because these factors, like inhaled mannitol powder, are believed to evoke airway provocation in asthmatics by an osmotic effect in the airway periciliary fluid.

If confirmed, sustained protective effects from orpharyngeal phospholipids could potentially have significant pharmacological implications that warrant further investigation. In particular, oropharyngeal application of phospholipids may have therapeutic potential in the management of asthma by providing the airways with sustained protection against factors that commonly provoke worsening asthmatic symptoms such as inhaling cold dry air and exercise. Furthermore, the attenuation of cough observed in the present study suggests that oropharyngeal application of phospholipids (*e.g.* by lozenges) may potentially be beneficial in alleviating upper airway irritation and cough in other common disorders such as viral respiratory tract infections.

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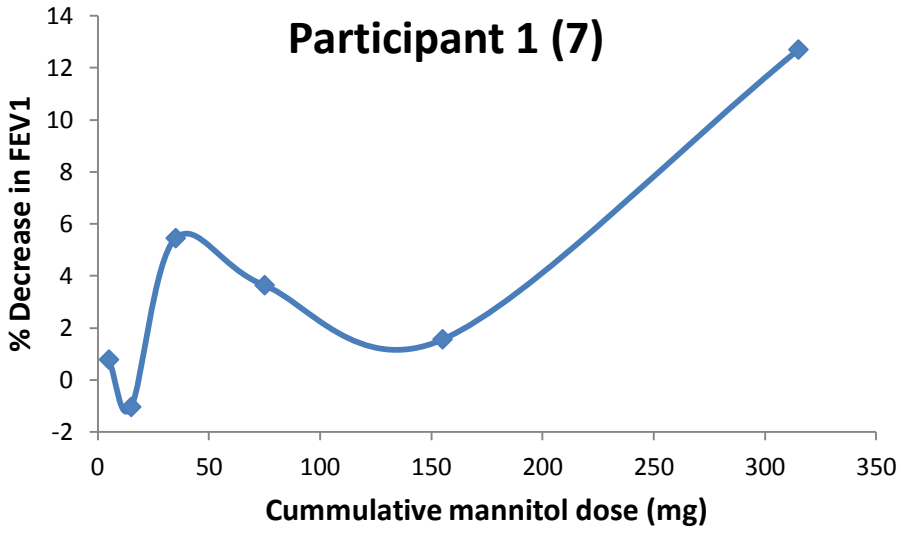
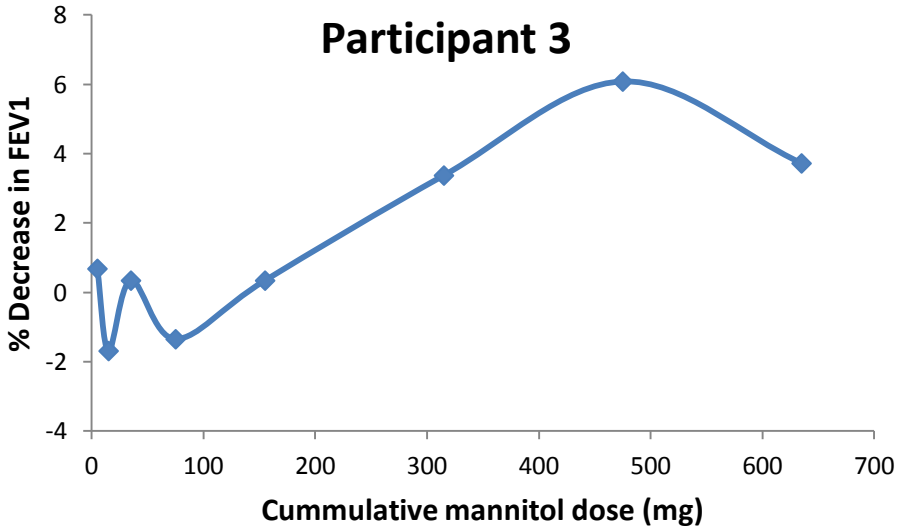
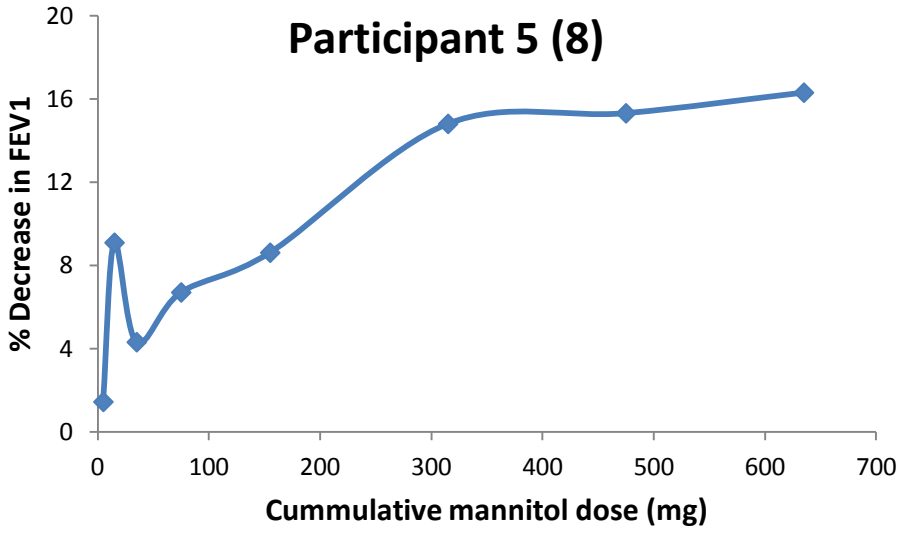
Appendix 1

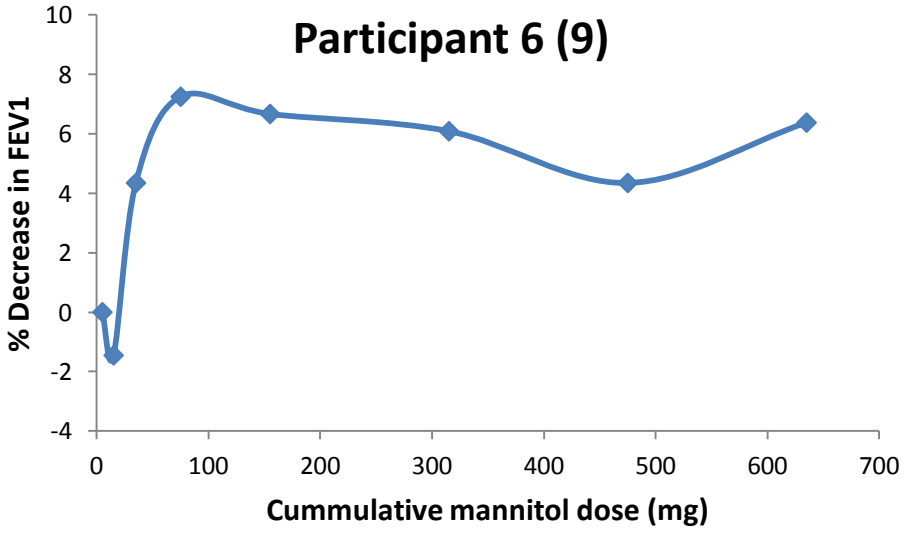
Individual dose-response curves for inhaled mannitol in 20 adult asthmatics

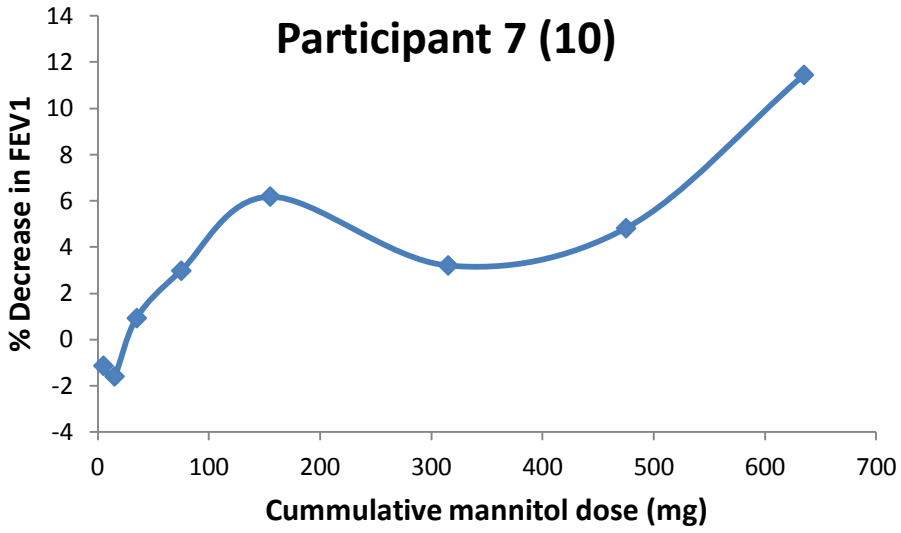
Note: Participant numbers at the top of each curve refer to the 20 asthmatics who demonstrated a fall in $FEV_1 \geq 5\%$ in chapter 2, and the numbers in parenthesis refer to the participant numbers of the 12 asthmatics in chapter 3 of the thesis.

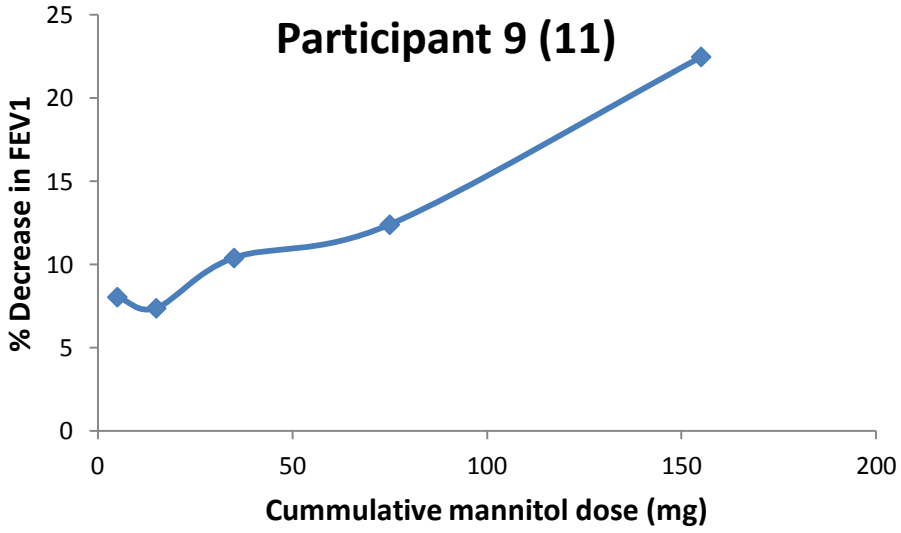

Participant 1 (7)
% Decrease in FEV1
14
12
10
8
6
4
2
0
-2
0
50
100
150
200
250
300
350
Cummulative mannitol dose (mg)

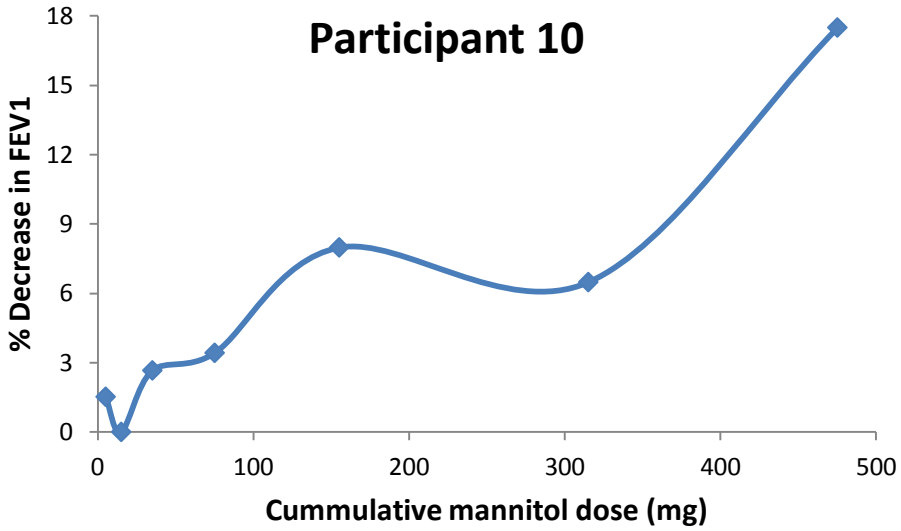

Participant 3
% Decrease in FEV1
8
6
4
2
0
-2
-4
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

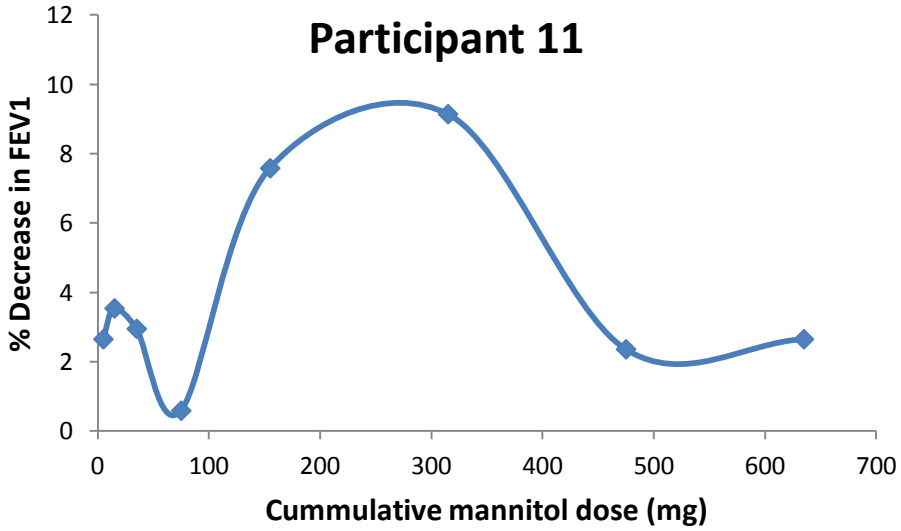

Participant 5 (8)
% Decrease in FEV1
20
16
12
8
4
0
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

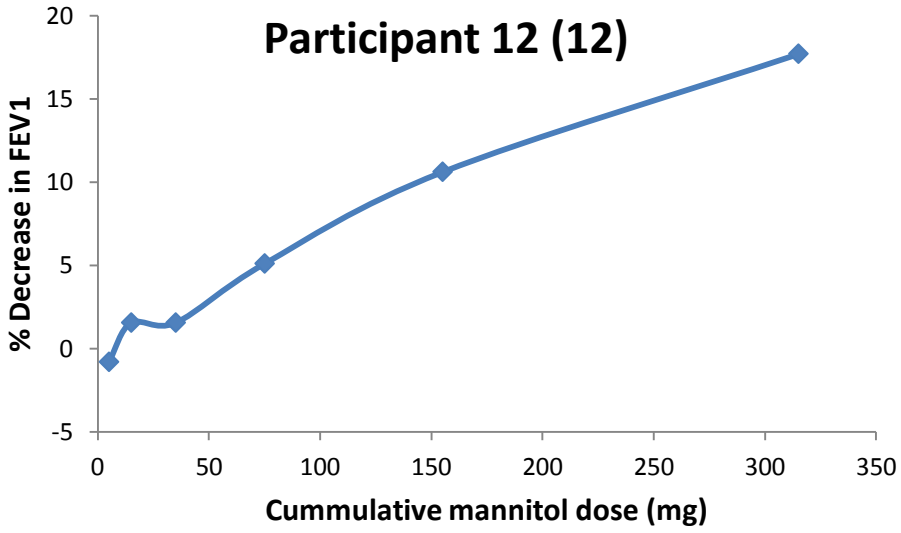

Participant 6 (9)
% Decrease in FEV1
10
8
6
4
2
0
-2
-4
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

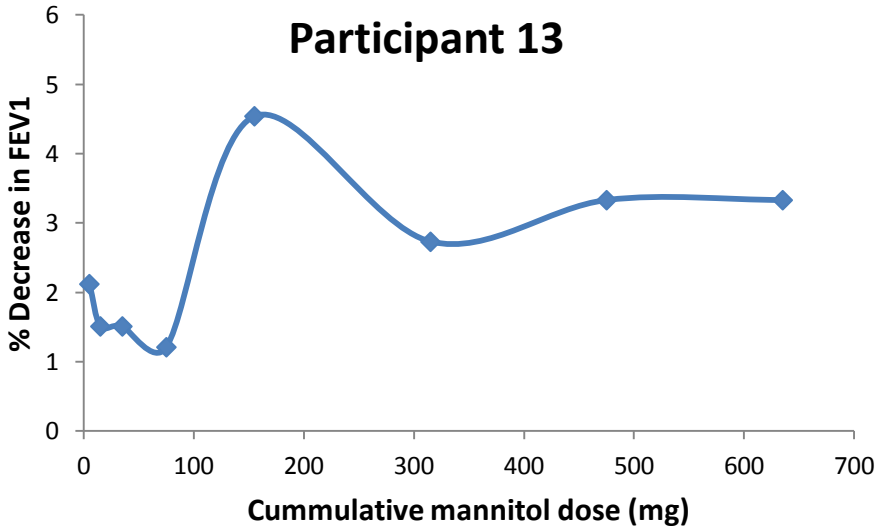

Participant 7 (10)
% Decrease in FEV1
14
12
10
8
6
4
2
0
-2
-4
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

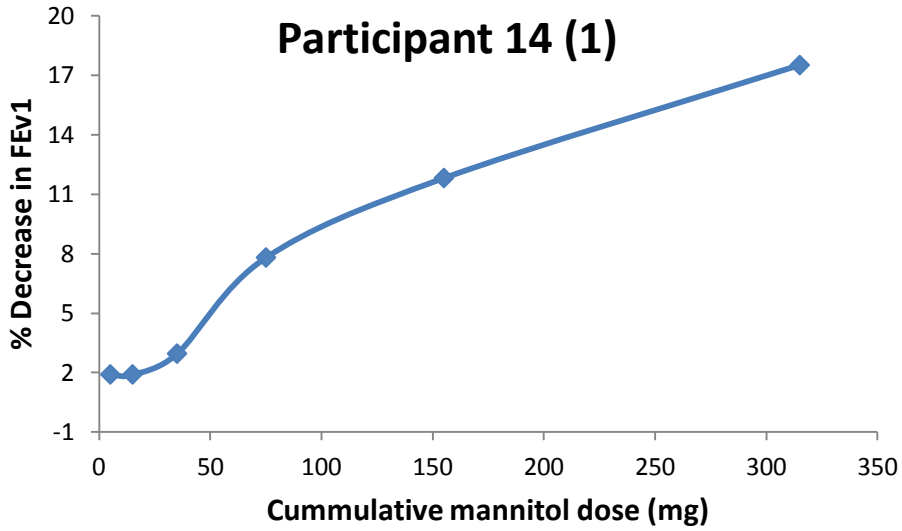

Participant 9 (11)
% Decrease in FEV1
25
20
15
10
5
0
0
50
100
150
200
Cummulative mannitol dose (mg)

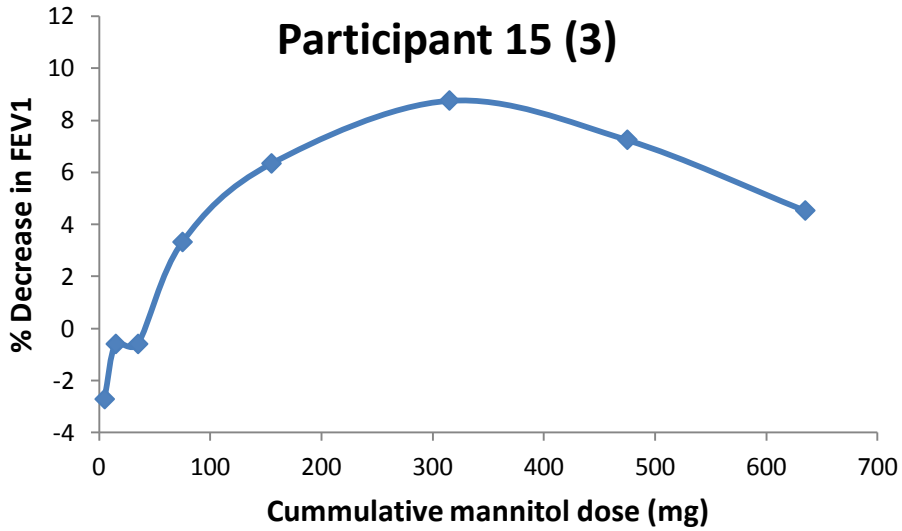

Participant 10
% Decrease in FEV1
18
15
12
9
6
3
0
0
100
200
300
400
500
Cummulative mannitol dose (mg)

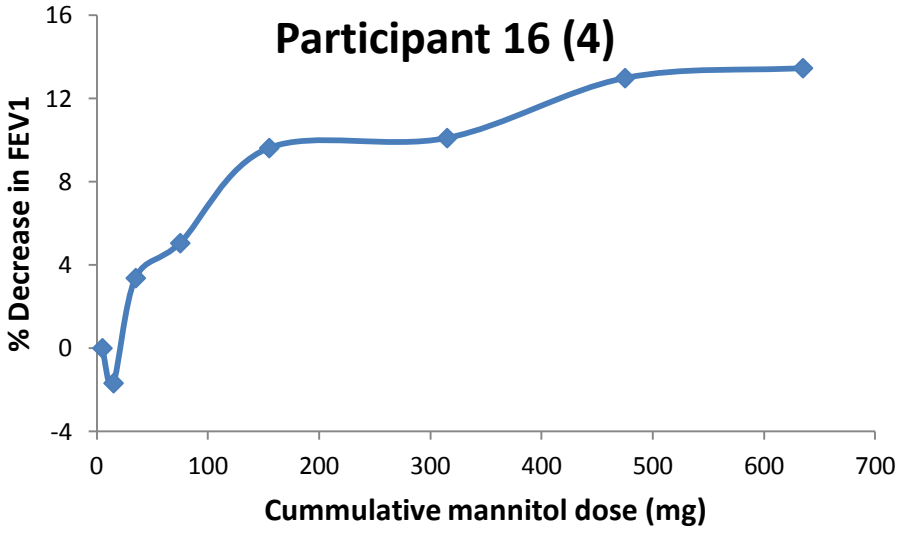

Participant 11
% Decrease in FEV1
12
10
8
6
4
2
0
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

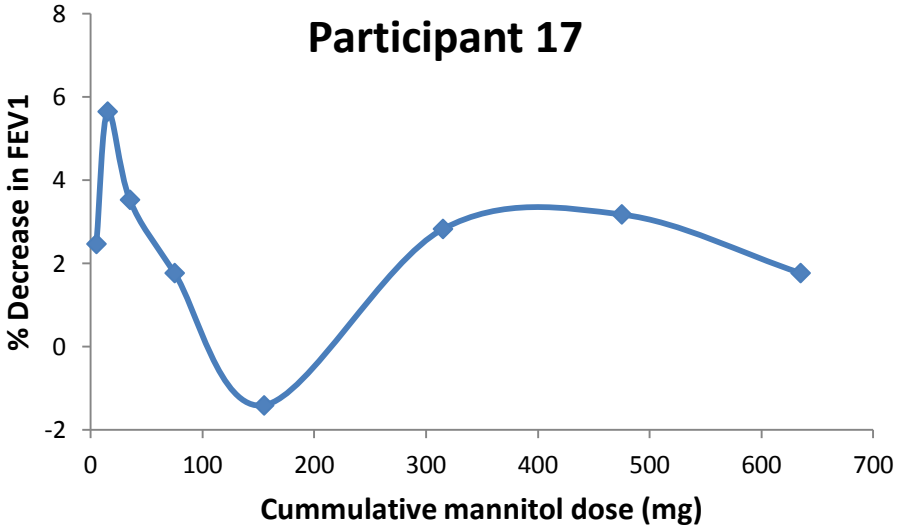

Participant 12 (12)
% Decrease in FEV1
20
15
10
5
0
-5
0
50
100
150
200
250
300
350
Cummulative mannitol dose (mg)

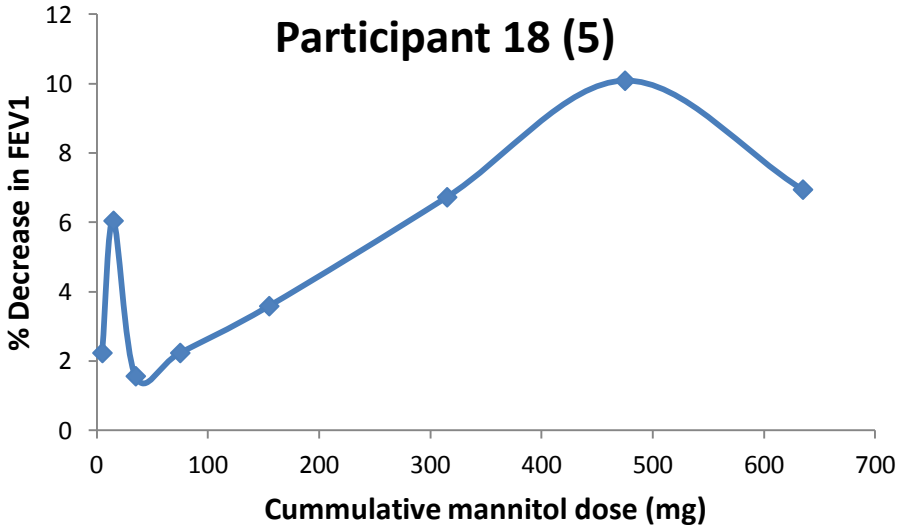

Participant 13
% Decrease in FEV1
6
5
4
3
2
1
0
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

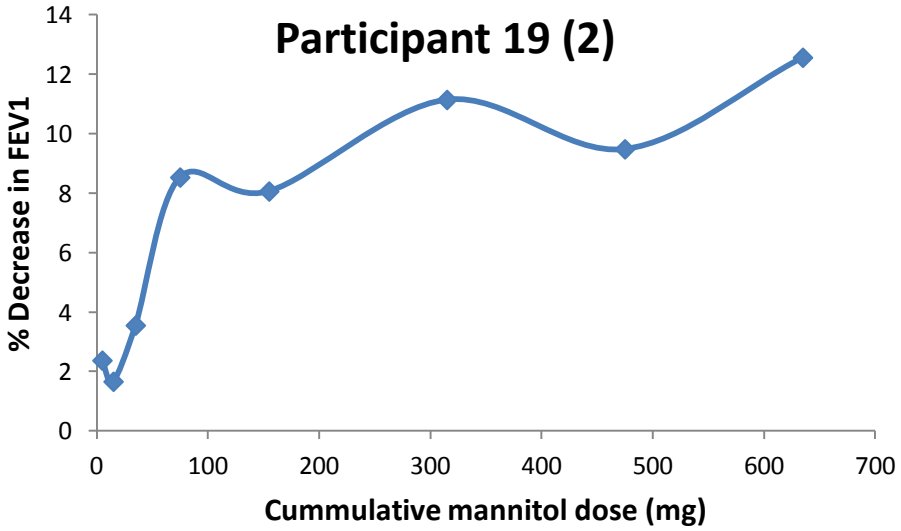

Participant 14 (1)
% Decrease in FEv1
20
17
14
11
8
5
2
-1
0
50
100
150
200
250
300
350
Cummulative mannitol dose (mg)

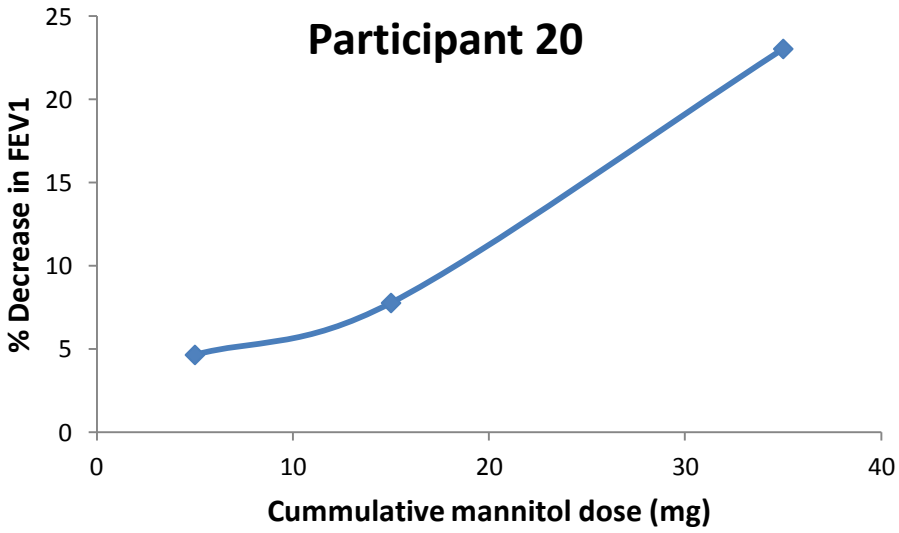

Participant 15 (3)
% Decrease in FEV1
12
10
8
6
4
2
0
-2
-4
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

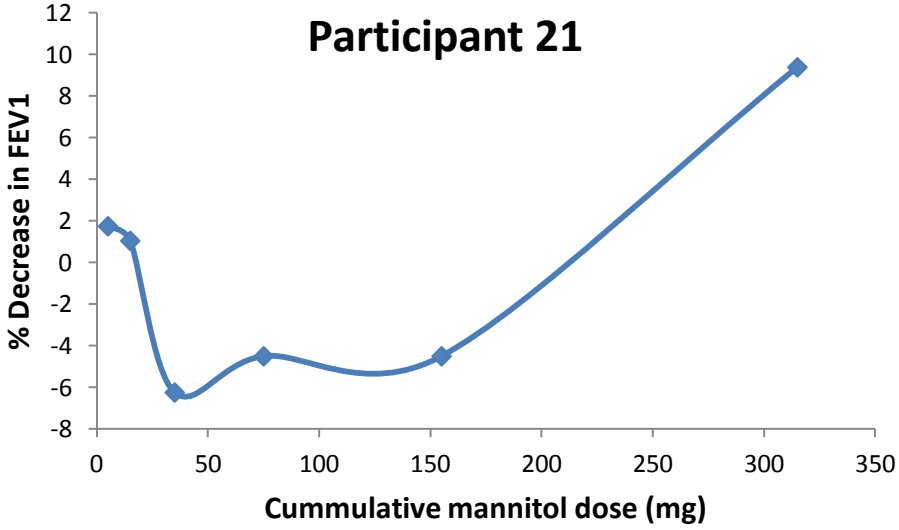

Participant 16 (4)
% Decrease in FEV1
16
12
8
4
0
-4
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

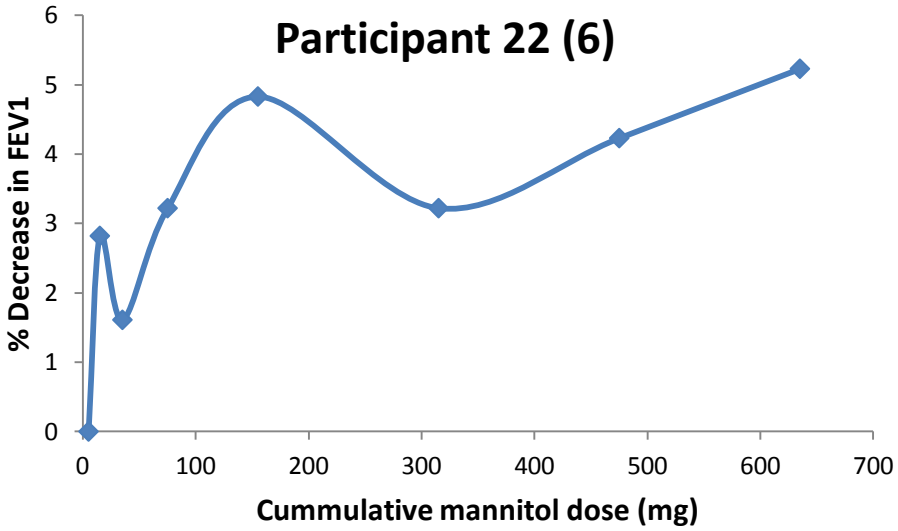

Participant 17
% Decrease in FEV1
8
6
4
2
0
-2
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)

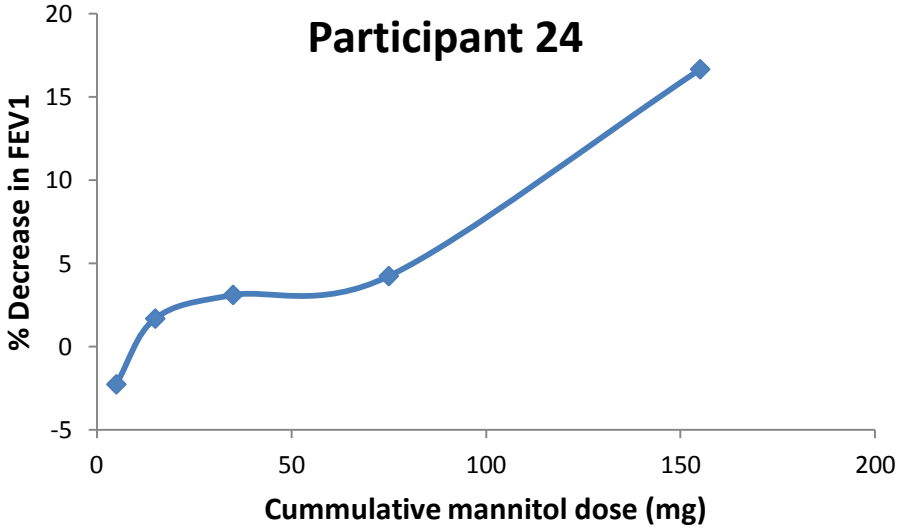

Participant 18 (5)
% Decrease in FEV1
0
2
4
6
8
10
12
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)


Participant 19 (2)
% Decrease in FEV1
0
2
4
6
8
10
12
14
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)


Participant 20
% Decrease in FEV1
25
20
15
10
5
0
0
10
20
30
40
Cummulative mannitol dose (mg)


Participant 21
% Decrease in FEV1
12
10
8
6
4
2
0
-2
-4
-6
-8
0
50
100
150
200
250
300
350
Cummulative mannitol dose (mg)


Participant 22 (6)
% Decrease in FEV1
0
1
2
3
4
5
6
0
100
200
300
400
500
600
700
Cummulative mannitol dose (mg)


Participant 24
% Decrease in FEV1
-5
0
5
10
15
20
0
50
100
150
200
Cummulative mannitol dose (mg)

Appendix 2

Abstract presented at the Annual Scientific Meeting of the Thoracic Society of Australia and New Zealand, Canberra, April, 2012.

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