Effect of atorvastatin on asthma control and airway inflammation: a randomised controlled trial

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Dedication

This thesis is dedicated to the memory of Dr Stuart Wood, Senior Lecturer in General Practice at the University of Glasgow, who died in March 2006.

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Abbreviations

4S	Scandinavian Simvastatin Survival Study
AHR	Airway Hyperresponsiveness
APC	Antigen Presenting Cell
ATS	American Thoracic Society
BAL	Bronchoalveolar Lavage
BTS	British Thoracic Society
CCL2	Chemokine (C-C motif) Ligand 2
CD40L	CD40 Ligand
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-Reactive Protein
DTT	Dithiothreitol
FE_{NO}	Exhaled Nitric Oxide
FEF_{25-75}	Forced Expiratory Flow rate from 25% to 75% of exhalation
FEV_1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GM-CSF	Granulocyte Macrophage – Colony Stimulating Factor
GP	General Practice or General Practitioner
GPASS	General Practice Administration system for Scotland
GTP	Guanosine Triphosphate
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HMG CoA	3-HydroxyMethyl-3-Glutaryl Co Enzyme A
HUVEC	Human Umbilical Vein Endothelial Cells
ICAM	Intercellular Adhesion Molecule
IFN-γ	Interferon γ
IgE	Immunoglobin type E
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthetase
IPF	Idiopathic Pulmonary Fibrosis
LABA	Long Acting ß agonist
LDL	Low Density Lipoprotein
LFA-1	Leucocyte Function-associated Antigen-1
LPS	Lipopolysaccharide
LTB_4	Leukotriene B_4
MCP-1	Monocyte Chemoattractant Protein-1 (also known as Chemokine (C-C motif) Ligand 2, *CCL2*)
MDI	Metered Dose Inhaler
MHC	Major Histocompatibility Complex
MIP1α	Macrophage Inflammatory Protein 1α
MMP	Matrix Metalloproteinase
MPO	Myeloperoxidase
NF-κB	Nuclear Factor-κB
NO	Nitric Oxide

OVA	Ovalbumin
PBS	Phosphate Buffered Saline
PEF	Peak Expiratory Flow
RANTES	Regulated on Activation, Normal T cell-Expressed and Secreted protein
RPMI	Roswell Park Memorial Institute culture medium
sICAM	Soluble Intercellular Adhesion Molecule
TCR	T Cell Receptor
TG	Triglycerides
TGF-β1	Transforming Growth Factor-β1
T_H1	T Helper cell type 1
T_H2	T Helper cell type 2
T_H0	T Helper cell type 0
TNF-α	Tumour Necrosis Factor-α
VCAM	Vascular Cell Adhesion Molecule
VLDL	Very Low Density Lipoprotein

SUMMARY

Background

Statins are inhibitors of the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, in cholesterol biosynthesis. As such, they have been widely used in clinical practice as cholesterol lowering agents to reduce morbidity and mortality from coronary artery disease. There is evidence from clinical studies and in vitro experiments that statins have additional anti-inflammatory properties in atherosclerotic disease, which are unrelated to their lipid lowering activity.

Clinical studies have previously suggested that statins might show a beneficial clinical effect in inflammatory diseases, such as rheumatoid arthritis and multiple sclerosis. Furthermore, preliminary data obtained in models of pulmonary inflammation suggest that the effects manifest in rheumatoid patients can be achieved also in asthma. A proof of concept study was designed to test the hypothesis that atorvastatin improves asthma control and airway inflammation in adults with asthma.

Methods

Fifty four adults with allergic asthma were recruited to a 22-week crossover randomised controlled trial comparing the effect on asthma control and airway inflammation of oral atorvastatin 40 mg daily with that of a matched placebo. Each treatment was administered for 8 weeks separated by a 6-week washout period. The primary outcome was morning peak expiratory flow. Secondary outcomes included spirometry, asthma control questionnaire (ACQ) score, asthma quality of life questionnaire (AQLQ), provocation

concentration to methacholine (PC_{20}) and inflammatory markers: exhaled nitric oxide, sputum differential cell count, sputum supernatant and serum inflammatory markers such as interleukin-6 (IL-6), IL-5, IL-8, sICAM-1, TNF-α, leukotriene B_4 (LTB_4) and high sensitivity C-reactive protein (hsCRP), and blood lymphocyte proliferation.

Results

At 8 weeks, the change in mean morning PEF, as compared with baseline, did not differ between the atorvastatin and placebo treatment periods [mean difference -0.5 L/min, 95% CI -10.6 to 9.6, p=0.921]. No statistically significant effect of atorvastatin was seen in evening PEF, or methacholine responsiveness (PC_{20}). Out of all spirometry results, only post-salbutamol FVC showed a statistically significant result, which was slightly lower in the atorvastatin group [treatment difference -0.1L, 95% CI -0.2 to 0.0, p=0.037]. There was also no change in ACQ or AQLQ.

No change was seen in exhaled nitric oxide. The total cell counts recovered from sputum were similar after atorvastatin compared to after placebo treatment. After 8 weeks, the mean absolute and relative sputum macrophage count was significantly reduced after atorvastatin compared to placebo [mean absolute difference -44.9×10^4 cells, 95% CI -80.1 to -9.7, p=0.029]. There was a reciprocal increase in the relative proportion of sputum neutrophils [mean proportion difference 13.1%, 95% CI 1.8 to 24.4, p=0.025], but there were no significant changes in the absolute count of these cells or the counts and proportions of the other sputum cell phenotypes under atorvastatin treatment.

The sputum concentrations of inflammatory cytokines and mediators were similar after atorvastatin compared to after placebo treatment other than LTB_4 which was significantly reduced [mean difference -88.1 pg/mL, 95% CI -156.4 to -19.9, p=0.014].

No significant difference was seen in the concentration of any serum marker of inflammation between atorvastatin and placebo treatment periods. The change in hsCRP was of borderline significance [mean difference -0.65 mg/L, 95% CI -1.38 to 0.09, p=0.082], but there were no changes in sICAM-1, TNF-α, IL-5, IL-6 and IL-8. There was no significant difference in lymphocyte proliferation.

The biochemical effects of atorvastatin therapy were reflected in significant reduction in concentration of serum lipids; cholesterol (mean difference -1.71 mmol/l, 95% CI -1.94 to -1.48 p<0.0001), and HDL-cholesterol (mean difference -0.14 mmol/l, 95% CI -0.26 to -0.02 p=0.026), but not triglycerides. There were significant, albeit modest, increases in mean bilirubin, AST and ALT. There was no difference in compliance, assessed by number of tablets returned and by biochemical results.

There was no correlation between changes in LTB_4 or IL-8 and sputum macrophage count, sputum neutrophil count, or PEF. The only correlation observed between the variables that were compared was between sputum macrophages and neutrophils.

Adverse event rates were similar in patients taking atorvastatin compared with placebo. Equal numbers of patients were lost to follow-up in both arms of the study. One patient died of unrelated causes while taking the placebo medication.

Conclusions

There were no clinically important improvements in a range of clinical indices of asthma control after eight weeks of treatment with atorvastatin despite expected changes in serum lipids. There were however changes in airway inflammation and in particular, a reduction in the absolute sputum macrophage count after atorvastatin compared to placebo and an associated reduction in sputum LTB_4 and a trend towards lower CRP.

The lack of any evidence of clinical benefit of atorvastatin in allergic asthma confirms and extends the findings of a smaller randomised placebo controlled crossover trial of simvastatin in 16 subjects with asthma, which showed no change in clinical outcomes or inflammatory markers.

It is unlikely that altering duration of treatment, washout period or type of statin used would have changed the outcome of the study. However, as all patients were receiving inhaled corticosteroid as part of their asthma therapy, it is possible that this may have masked any modest anti-inflammatory effects of the statin. Baseline asthma inflammation may also have been too low to show any significant improvement.

Despite the postulated anti-inflammatory actions of statins, it seems that they may not be appropriate for the inflammatory phenotype associated with atopic asthma. The reduction in alveolar macrophage count found in patients with allergic asthma may however have relevance to the treatment of chronic lung diseases such as COPD in which alveolar macrophage function has been implicated in the pathogenesis.

1. INTRODUCTION

1.1 Asthma

1.1.1 Definition

Asthma is a clinical syndrome characterised by increased responsiveness of the tracheo-bronchial tree to a variety of stimuli. The major symptoms of asthma are paroxysms of dyspnoea, wheezing and cough, which may vary from mild and almost undetectable to severe and unremitting. This is caused by airway smooth muscle hyperresponsiveness, which is the excessive narrowing of the airway lumen caused by stimuli that would cause little or no narrowing in the normal individual. The primary physiological manifestation of this hyperresponsiveness is variable airways obstruction.

Asthma is defined by the Global Initiative for Asthma (GINA) as:
"a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation causes an associated increase in 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 that is often reversible either spontaneously or with treatment." [1]

1.1.2 Pathogenesis

Asthma is an inflammatory condition of the airways with both acute and chronic phases. An immunoglobulin E (IgE)-type response to common inhaled allergens is the most

common form of the disease in children [2], and is called atopic asthma. The frequency with which asthma is associated with atopy depends on the test used (table 1.1).

Table 1.1 Relationship of asthma and atopy

Test	Population	Percentage asthma associated with atopy	Reference
Skin prick testing	Adults	1-34	3
Serum IgE	Adults	13-67	3
Skin prick test	Children	65	4

In non-atopic asthma, it is likely that there is an immunological basis for disease, as the pathological features and the inflammation observed are similar in atopic and non-atopic asthma [5].

1.1.2.1 Cells of the respiratory immune system

1.1.2.1.a Eosinophils

Bronchial eosinophilia is seen in both atopic and non-atopic asthma, and there is a correlation between sputum eosinophil concentration and asthma exacerbation rates[6]. Eosinophil influx from capillaries into the lungs is controlled by vascular cell adhesion molecule-1 (VCAM-1), eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-4 (IL-4) and IL-5 [5]. When in the lung the eosinophils can be stimulated to release these same proinflammatory mediators, as well as cytotoxic mediators and growth factors, thus amplifying the inflammation. This leads to vascular leakage,

hypersecretion of mucus, smooth muscle contraction, shedding of epithelial cells and bronchial hyperresponsiveness. Eosinophils also regulate airway inflammation and may initiate the process of tissue repair termed 'remodelling' by the release of cytokines [7] and growth factors (see section 1.1.2.4.a below). Corticosteroids, a commonly form of asthma therapy, induce eosinophil apoptosis and phagocytic removal by macrophages [8], which in part explains their therapeutic effect.

1.1.2.1.b T Lymphocytes

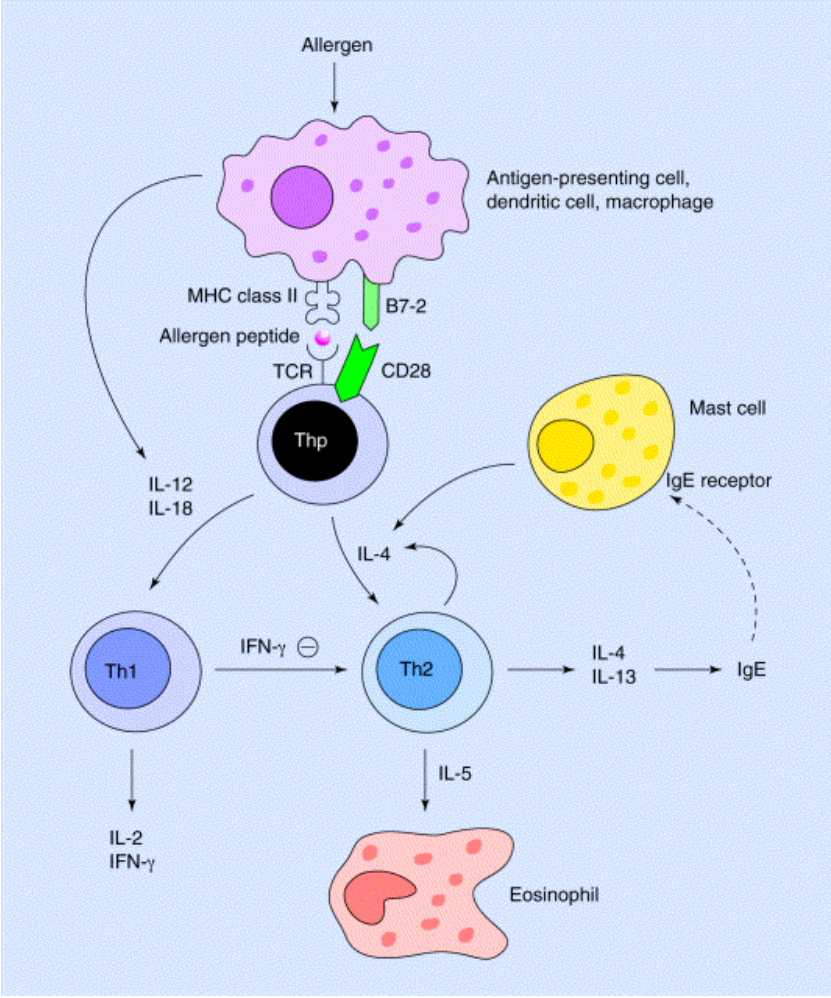
Raised bronchial mucosal lymphocyte numbers are seen in all forms of asthma [9]. The majority of T-lymphocytes bear CD4-receptors whereas CD8-positive cells are more rarely identified, even during exacerbations of asthma [10].

A variety of different factors have been shown to control whether naive $CD4^+$ T cells develop into T_H1 or T_H2 subsets depending on the mediators in their environment (Figure 1.1). The most potent signals in influencing this process are cytokines. If naïve $CD4^+$ T cells are activated by antigen processed and presented by antigen-presenting cells (APCs), and in the presence of IL-12, a T_H1-dominated population will develop, whereas if IL-4 is present in the environment, T_H2 cells are induced. In the respiratory tract, many studies now suggest that T_H2 responses are preferentially stimulated [11]. The cytokines secreted by T_H2 lymphocytes are thought to play a key role in the initiation and perpetuation of this airway inflammation [5]. However, in stable chronic asthmatics T_H0 or

T_H1 cells may also be seen in the airways reflecting chronicity [12]. The cells that line the bronchial airways create the cytokine microenvironment that controls $CD4^+$ T_H subset differentiation. Mast cells secrete IL-4, IL-5, and IL-13 in response to cross-linking of receptor-bound IgE antibody by antigen. In this manner, the early production of cytokines may initiate a shift in the immune responses toward T_H2. Lymphocytes residing near the respiratory tract and secreting IL-4 also influence the cytokine milieu and shift a T-cell response toward T_H2 [13].

Figure 1.1 T helper (CD4+) cells in atopic diseases. Allergen is processed by antigen-presenting cells (dendritic cells) and presented via class II major histocompatibility complex (MHC class II) to T-cell receptors (TCR) on uncommitted T helper cells (Thp). Accessory molecules B7-2 and CD28 amplify this interaction. T_Hp cells differentiate into T_H1 cells in response to IL-12, which, under the influence of IL-12 and IL-18, can release interferon-γ (IFN-γ). Thp cells under the influence of IL-4 differentiate into T_H2 cells, which release IL-4, IL-13 and IL-5. IFN-γ inhibits T_H2 cell differentiation and in this system IL-12, IL-18 and IFN-γ all result in inhibition of T_H2 cells and the release of T_H2 cytokines.

Ig, immunoglobulin; IL, interleukin. Reprinted from Barnes and Lim [5] with permission from Elsevier.



1.1.2.1.b.i T Lymphocyte activation

T cell immune responses are initiated in secondary lymphoid organs, where naïve T cells (T_H0) encounter antigen-presenting cells (APCs, also known as dendritic cells). The interaction between T lymphocytes and APCs creates a specific physical site, termed the "immunological synapse", at which specific ligands and costimulatory molecules trigger and sustain the T cell activation process. Leucocyte Function-associated Antigen-1 (LFA-1) mediates adhesion between T cells and APCs. It also functions to lower T cell activation thresholds [14].

The importance of this initial cellular adherence step is demonstrated by the use of monoclonal antibodies that inhibit the function of LFA-1 that have also been effective in reducing airway eosinophilia in a murine model of atopic asthma [15] and sputum eosinophilia after allergen challenge in asthmatic patients [16].

1.1.2.1.c Macrophages

Alveolar macrophages primarily function to remove particles and apoptotic cells in a non-inflammatory manner. However, when appropriately stimulated they can also process inhaled allergen, which is then presented in a modified and recognisable form to primed T lymphocytes [17]. The macrophage itself then becomes the target of a positive feedback loop by producing macrophage-activation lymphokines, which heighten its capacity to regulate the immunogenic stimulus. They are activated in asthma [18], in particular night-time asthma [19]. Macrophages are responsive to steroid treatment, with

decreased MIP-1α, IFN-γ and granulocyte macrophage – colony stimulating factor (GM-CSF), and increased IL-10, IL-1β [20] and histone deacteylase (HDAC) [21] release after steroid treatment.

1.1.2.1.d Neutrophils

Biopsy, BAL and sputum samples from severe asthmatics often have a high neutrophil count, and a comparatively low eosinophil count [22] particularly during an exacerbation [23]. Indeed, there is an inverse association between FEV_1 and the proportion of sputum neutrophils [24]. As corticosteroids appear to inhibit neutrophil phagocytosis and prolong survival [8], this may in part explain steroid resistance in severe asthma. The eosinophils, which respond to steroids by becoming apoptotic, disappear from the inflamed airway, and the neutrophil may be drawn in and act as a "substitute granulocyte" [25]. However, there is considerable variation between patients.

1.1.2.2 Inflammatory mediators

1.1.2.2.a Cytokines

Cytokines are a large group of glycol-protein mediators that allow communication between cells. They play a critical role in determining the nature of the inflammatory response and its persistence. To date, more than 50 different mediators have been identified in asthma [5]. Some of these (e.g. IL-1, IL-6, TNF-α) are involved in many inflammatory diseases including rheumatoid arthritis and inflammatory bowel disease [11].

Others are more specific to atopy. Airway epithelial cells, smooth muscle cells, endothelial cells, and fibroblasts are all capable of synthesizing and releasing inflammatory mediators including cytokines. Cytokines may also play an important role in antigen presentation and may enhance or suppress the ability of macrophages to act as antigen-presenting cells [5].

The functions of some specific cytokines in the context of asthma are outlined in table 1.2. This is illustrative and not comprehensive.

Table 1.2 Some functions associated with selected cytokines in asthma

Cytokine	General function	Function associated with asthma
Immunoregulatory cytokines		
IL-4	B cell activation Monocyte and macrophage activation T cell growth factor Increased ICAM-1 expression	Promotes immunoglobulin class switching from IgG to IgE. ↑ Eosinophil growth Down-regulates IFN-γ mediated T_H1 responses Promotes AHR (+)
IL-5	Eosinophil activation Mast cell and basophil differentiation and maturation Endothelial adhesion, priming for chemoattractants Cofactor for IgE synthesis	Eosinophil maturation, ↓ apoptosis ↓T_H2 cells Promotes AHR (++)

Cytokine	General function	Function associated with asthma
IL-8	Primes for eosinophil chemotaxis Neutrophil activator Neutrophil and T cell chemoattractant	Down-regulates IgE production
IL-17	T cell proliferation Activates epithelia, endothelial cells, fibroblasts	Promotes airway neutrophilia
IFN-γ	Activates endothelial cells, epithelial cells, alveolar macrophages/ monocytes Inhibition IgE isotype switch Eosinophil activation (late acting) Macrophage activation	↓ Eosinophil influx after allergen ↓ T_H2 cells ↓ IgE ↓ AHR
Pro-inflammatory cytokines		
IL-1	B cell growth factor Neutrophil chemoattractant T cell and epithelial cell activation Increased expression of endothelial adhesion molecule on many cell types	↑Adhesion to vascular endothelium Eosinophil accumulation in vivo Growth factor for T_H2 cells Promotes AHR (+)

Cytokine	General function	Function associated with asthma
TNF-α	Activates epithelium, endothelium, antigen-presenting cells, monocytes/macrophages	Promotes AHR (+)
IL-6	B cell growth factor Macrophage and eosinophil activator T cell growth factor	↑ IgE
GM-CSF	Proliferation and maturation of haematopoetic cells Endothelial cell migration Mast cell, macrophage, epithelial cell, eosinophil and neutrophil differentiation, activation, and in vitro survival	Eosinophil apoptosis, chemotaxis and activation Promotes AHR (+)
LTB_4	Neutrophil and monocyte activator and chemoattractant	Recruits neutrophils and monocytes into airway
CCL2	Monocyte, lymphocyte and basophil recruitment Mast cell/basophil degranulation	Recruits monocytes and lymphocytes into airway
Macrophage inflammatory protein (MIP-1α)	Monocyte and naïve T cell chemoattractant Activates basophils and mast cells	

Cytokine	General function	Function associated with asthma
Inhibitory cytokines		
IL-10	↓ Monocyte and macrophage activation ↑ B cells ↑ Mast cell growth Inhibits T_H1 cytokine production on APC	↓ Eosinophil survival ↓ IgE ↓ T_H1 and T_H2 ↓ AHR
IL-1receptor antagonist (IL-1ra)	↓ T_H2 proliferation	↓ IgE ↓ AHR

IL-4 and IL-5 are key cytokines in asthma, and are over-expressed in asthmatic airways. IL-4 in particular drives antibody class switching to IgE and T_H2 differentiation [5]. IL-4 increases the expression of vascular cell adhesion molecule-1 (VCAM-1) on endothelial and airway epithelial cells, and this may be important in eosinophil and lymphocyte trafficking [5]. Recent work suggests that specifically targeting IL-4 may provide effective new asthma treatments, although the results of early clinical studies have been disappointing [26]. IL-13 is structurally similar to IL-4, and both are recognised by a common receptor chain, and may act as alternative switches for T_H2 activation [5].

Interleukin-5 (IL-5) is essential for the production, maturation, activation and survival of eosinophils and is also important in eosinophil chemoattraction [5]. A clinical trial of a single dose of monoclonal antibody to IL-5 showed decreased blood eosinophils for up to

16 weeks and reduced sputum eosinophils after inhaled allergen challenge, but there was no improvement in clinical outcomes [27].

IL-1 is also important in activating T lymphocytes and is an important co-stimulator of the expansion of T_H2 cells after antigen presentation. For example, IL-4 is expressed by T_H2 clones, once they have been exposed to IL-1 [5].

1.1.2.2.b Apoptosis

Apoptosis is a process of programmed cell death, whereby cells when they have finished their role or become senescent express membrane molecules e.g. Fas which are recognised by macrophages which remove the apoptotic cells by a non-inflammatory mechanism. This is a dynamic process which controls the cell distribution at inflamed sites, limits the progression of inflammation, and promotes resolution [28]. Thus, on-going inflammation may be due to alterations in the regulation of cell apoptosis leading to a chronic and self-perpetuating inflammatory cell survival and accumulation. Activated cells tend to live longer in sites in active inflammation [29] as a consequence of reduced apoptosis [30]. Increased eosinophil survival in asthma is associated with reduced apoptosis [30]. GM-CSF, IL-3, IL-5, and RANTES (regulated upon activation, normal T-cell expressed and secreted), which are over expressed in asthmatic airways, act to inhibit apoptosis [31]. Apoptosis may also be delayed by expression of CD40, a receptor molecule expressed on eosinophils strongly associated with inflammation [32]. Anti-asthmatic

treatments with anti-inflammatory properties may work by promoting apoptosis [33] or reducing cell survival [34].

1.1.2.3 Acute inflammation in asthma

An acute episode of asthma may be triggered by exposure to allergens, viruses, or indoor or outdoor pollutants. From experimental models of atopic asthma, reactions can be divided into early phase and late phase, with this late chronic phase often involving processes of tissue repair called remodelling of the airways [35].

1.1.2.3.a Early phase

Early phase airway reactions against inhaled allergens are triggered by activation of cells bearing allergen-specific IgE. After activation of airway mast cells and macrophages, there is release of proinflammatory mediators such as histamine, and eicosanoids [35]. These induce contraction of airways smooth muscle, mucus secretion and vasodilatation and exudation of plasma into the airways. Plasma proteins may also be responsible for the formation of characteristic bronchial plugs of exudates mixed with mucus produced in increasing amounts in asthma, and inflammatory and epithelial cells [36]. Together, these effects contribute to airflow obstruction.

1.1.2.3.b Late phase

Several hours, usually 6 to 9hr, after allergen exposure, the late-phase inflammatory reaction occurs. This involves the recruitment and activation of eosinophils, $CD4^+$ T cells, basophils, neutrophils, and macrophages. Adhesion molecules such as tumour necrosis factor α (TNF-α) and sVCAM are released, as are proinflammatory mediators such as histamine, prostaglandin D2 (PGD2), and thromboxane B2 [36] and cytokines like IL-1β, IL-4 and IL-5, which are involved in the recruitment and activation of inflammatory cells. T cells are activated after allergen challenge, releasing T_H2 cytokines, which may be a key mechanism of the late-phase response [35].

Cytokines such as IL-5 and GM-CSF enhance eosinophil recruitment, and expression of adhesion molecules. Chemokines such as RANTES and eotaxin also act on eosinophils and T cells to enhance their recruitment and possibly their activation. IL-16 and macrophage inflammatory protein 1α (MIP-1α) are found in BAL fluid of antigen-challenged asthmatics and may also participate in the process [35].

1.1.2.4 Chronic inflammation in asthma

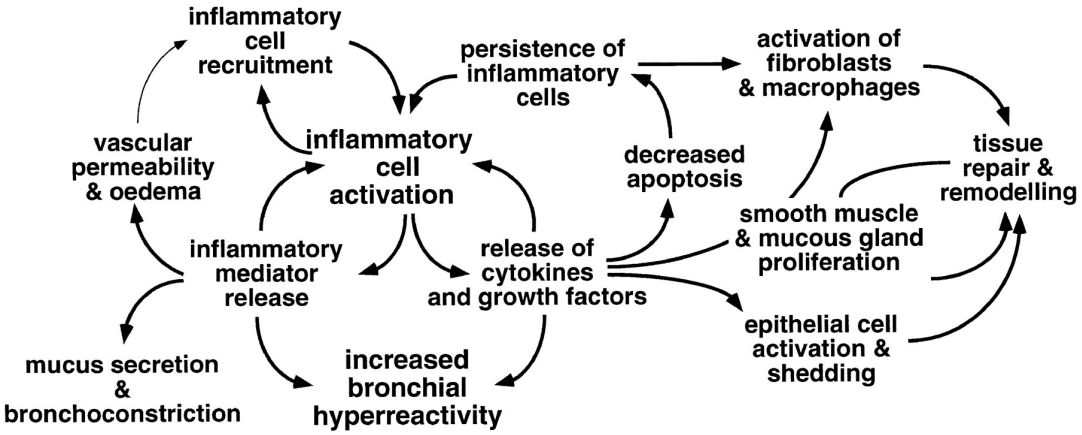
Inflammation in chronic asthma appears to be far more complex than a simple eosinophilic inflammation. All cells of the airways, including T-cells, eosinophils, mast cells, macrophages, epithelial cells, fibroblasts, and even bronchial smooth muscle cells are involved in chronic asthma and become activated.

This chronic inflammation may result in structural changes in the airway, such as fibrosis (particularly under the epithelium), increased thickness of the airway smooth muscle layer (hyperplasia and hypertrophy), hyperplasia of mucus- secreting goblet cells, and new vessel formation (angiogenesis) [35]. There may also be changes in the innervation of the airways [5].Some of these changes may be irreversible, leading to fixed narrowing of the airways, a process known as "remodelling" [35].

1.1.2.4.a Remodelling

The highly complex and inter-dependent interactions involved in remodelling are summarised in figure 1.2. Cells implicated in remodelling include epithelial cells (recruiting and prolonging the survival of inflammatory cells), eosinophils (involved in fibrosis), neutrophils, and smooth muscle cells (hyperplasia and altered myosin function) [35].

Figure 1.2 Mechanisms of acute and chronic inflammation in asthma and remodelling processes. Reproduced from Bousquet *et al* [35], copyright American Thoracic Society 2000



1.1.2.5 Systemic inflammation in asthma

It follows that inflammation in the lungs might be associated with systemic inflammation. Certainly, respiratory symptoms associated with infection, such as those seen in chronic obstructive pulmonary disease (COPD) are associated with a raised CRP, indicative of systemic inflammation [37]. However some studies have shown that CRP is normal in atopic asthma [38], which suggests a different immuno-pathogenesis.

1.1.3 Diagnosis

1.1.3.1 Clinical diagnosis

Diagnosis of asthma is made on the basis of wheezing, chest tightness and episodic breathlessness. There may be a family history, and/or a seasonal pattern to symptoms.

Measurements of symptoms and lung function are important for assessing the characteristics of the patient's asthma.

1.1.3.2 Physical examination

Because asthma is an episodic disorder, physical examination may be normal. The most commonly found abnormality on chest auscultation is wheeze, however normal chest auscultation does not rule out a significant limitation of airflow.

1.1.3.3 Objective testing

Patients often have a poor perception of their own disease, especially in those with long-standing or severe disease [39]. In contrast, measurement of lung function, such as the reversibility of lung function, provides a direct, quantitative, assessment of airflow limitation. Measuring the variability in lung function provides an indirect assessment of airway hyper-responsiveness.

The most commonly used forms of assessment of airway function are the measurement of forced expiratory volume in 1 second (FEV_1) and its accompanying forced vital capacity (FVC), and the measurement of peak expiratory flow (PEF). Both of these measurements rely on the fact that airflow limitation is directly related to the luminal size of the airways (airway calibre) and the elastic properties of the surrounding lung tissue (alveoli). Measurement of FEV_1 and FVC is undertaken during a forced expiratory manoeuvre

using a spirometer. Specific technique for spirometry is discussed in the methodology chapter.

Because a reduced FEV_1 can be caused by diseases other than those causing airflow limitation, a useful assessment of airflow limitation can be obtained as the ratio of FEV_1 to FVC. In the normal lung, flow limitation on forced expiration results in FEV_1/FVC ratios of greater than 80 percent and in children possibly greater than 90 percent. Any values less than these are suggestive of airflow limitation [1].

When making a diagnosis of asthma, a 12% improvement in FEV_1 after inhalation of a bronchodilator, or in response to a trial of glucocorticosteroid therapy is sought [1].

Ideally lung function should be measured first thing in the morning when values are usually close to their lowest and last thing at night when values are usually at their highest.

1.1.3.3.a Airway Hyperresponsiveness

Airway hyperresponsiveness can be assessed directly by stimulating airway smooth muscle with irritants (e.g. methacholine and histamine). Measurement of airway hyperresponsiveness has been standardized for histamine and methacholine administered via aerosol inhalation by tidal breathing or administered in predetermined amounts via a dosimeter. The provocative concentration reduces FEV_1 by 20 percent from baseline

(PC_{20} or PD_{20}). Nocturnal and/or early morning symptoms with a diurnal variation in peak expiratory flow (PEF) (which correlates well with FEV_1) of 20 percent or more are highly characteristic of asthma [1].

Key features of the diagnosis of asthma are detailed in table 1.3.

Table 1.3 Diagnosis of asthma in adults [1, 40]

Consider the diagnosis of asthma in patients with some or all of the following:	
Symptoms	**Signs**
Episodic/variable	None (common)
Shortness of breath	Wheeze – diffuse, bilateral,
Wheeze	expiratory (± inspiratory)
Chest tightness	Tachypnoea
Cough	
Helpful additional information	
Personal or family history of asthma or atopy (eczema, allergic rhinitis)	
History of worsening after use of aspirin/non-steroidal anti-inflammatory drug ingestion, use of β blockers (including glaucoma drops)	
Recognised triggers – pollens, dust, animals, exercise, viral infections, chemicals, irritants	
Pattern and severity of symptoms and exacerbations	
Objective measurements	
>20% diurnal variation on ≥ 3 days in a week for two weeks on Peak Expiratory Flow diary	
or FEV_1 ≥ 12% (and 200ml) increase after short acting β_2 agonist (e.g. salbutamol 400 µg by MDI + spacer or 2.5mg by nebuliser) [1]	
or FEV_1 ≥ 15% (and 200ml) increase after trial of steroid tablets (prednisolone 30mg/day for 14 days) [40]	
or FEV_1 ≥ 15% decrease after 15 minutes of exercise (running) [40]	
Histamine or methacholine challenge in difficult cases	

1.1.4 Asthma control and quality of life

1.1.4.1 Assessment of asthma control

The goals of asthma control (defined by the Global Initiative for Asthma, GINA) [1] are:

Minimal (ideally no) chronic symptoms, including nocturnal symptoms

Minimal (infrequent) exacerbations

No emergency visits

Minimal (ideally no) need for p.r.n. (as-needed) $ß_2$-agonist

No limitations on activities, including exercise

PEF circadian variation of less than 20 percent

(Near) normal PEF

Minimal (or no) adverse effects from medicine.

A telephone survey of 2,803 patients in seven European countries found that less than 25% of patients manage this ideal [41]. Reasons for this can include poor adherence to guidelines, problems with communications, inadequate education and poor motivation. Therefore, there is room for improvement, and new treatments can help with this objective. Patients have also shown a preference for tablet-based medication rather than inhaler if possible [42], an issue which might help compliance.

1.1.4.1.a Asthma control score

An asthma control questionnaire (ACQ) was developed by Juniper *et al* [43] (see appendix 3). In patients whose asthma was stable between clinic visits, reliability of ACQ was

high. The questionnaire includes a survey or important clinical symptoms and short acting β_2-agonist use and well as FEV_1 [43].

1.1.4.2 Quality of Life

Health related quality of life (HRQL) can be measured through generic questionnaires such as the Medical Outcome Survey Short-Form 36 (SF-36) [44]. The strength of generic instruments is that they can be compared across different medical conditions. However, because of their breadth, they have very little depth, and in many conditions, including asthma, generic instruments can be unresponsive to small but clinically important changes in HRQL [45]. An alternative is to use disease specific questionnaires, such as that developed by Juniper *et al.* [46] (see appendix 2). This questionnaire examines different aspects of asthma quality of life – symptoms, activities, emotional well-being and environmental factors – as well as providing an overall score. The developing team determined a Minimal Important Difference (MID) for change in HRQL score, which again was based on patient perceptions. A change of 0.5 is considered to be a clinically significant level [47].

1.1.5 Assessment of airway inflammation

In addition to assessing clinical control of asthma, it can be helpful to measure the extent to which the airways are inflamed. There are several techniques that can be used for this. Non-invasive methods such as measuring cells or mediators in exhaled breath

condensate [48], exhaled nitric oxide [49] and induced sputum [50] are commonly used, and they are well tolerated by patients. More invasive methods such as bronchoscopy with biopsy provide more information with higher attendant risk.

1.1.5.1Invasive investigations

1.1.5.1.a Bronchoscopy

Bronchial biopsy is an invasive technique to directly sample bronchial tissue through bronchoscopy. This enables comparison between animal models and human airway disease. By obtaining a sample of actual lung tissue, one may directly examine cellular pathological events, for example, apoptosis, shedding, and expression of adhesion molecules [51]. Remodelling, expression of specific types of cell and their prevalence ratios can also be directly assessed. Bronchoalveolar lavage (BAL) or airway brushing can also be performed during bronchoscopy. This allows direct sampling of the local environment.

1.1.5.2 Non-invasive investigations

1.1.5.2.a Exhaled Gases

1.1.5.2.a.i Nitric oxide

The presence of exhaled nitric oxide (NO) in the exhaled breath of both animals and humans was first described in 1991 [52]. This was followed by the discovery that exhaled NO levels are higher in asthma compared to normal subjects [53], and fall with treatment

with corticosteroids [54]. There is a correlation between exhaled NO and sputum eosinophil levels [54] (see section 1.1.5.2.b below).

Exhaled NO comes from the nasal mucosa and the lower airways. It is synthesised by inducible NO synthases (iNOS or NOS2), and from non-enzymatic sources from reduction of NO metabolites [54]. Other diseases can be associated with higher exhaled NO, shown in table 1.4 below. Exhaled NO is now being introduced as a method of assessing the response of asthma to treatment [55].

Table 1.4 Factors affecting exhaled nitric oxide

Increased NO	**Decreased NO**
Breath holding [53]	Cigarette smoking [53]
Exercise/hyperventilation [53]	Pulmonary Hypertension [53]
L-arginine (oral) [53]	Kartagener's syndrome [53]
Upper respiratory tract infections [53]	NOS inhibitors [53]
Asthma [53]	Glucocorticoids [53]
Allergen challenge (late response) [53]	HIV infection [56]
Bronchiectasis [53]	
Cystic fibrosis [53]	
Lower respiratory tract infection [53]	
Systemic lupus erythematosis [57]	
Liver cirrhosis [58]	

1.1.5.2.a.ii Carbon monoxide

Levels of exhaled carbon monoxide (CO) rise in atopy and asthma, and rise further during an acute asthmatic reaction independently of airway caliber [59]. There is also an association between exhaled CO and AHR [60].

1.1.5.2.b Induced sputum

Induced sputum as a method for assessment of airway inflammation was introduced in 1958 by Bickerman, using inhalation of hypertonic saline in aerosol form [61]. Early studies found that inhalation of saline could induce bronchospasm in poorly controlled asthmatic subjects, so changes were made to the technique [62]. The modern technique has been shown to be very safe, although bronchospasm can still occur [63]. It is possible to obtain spontaneous sputum from some patients, especially those with more severe disease, but the sample tends to have fewer viable cells [64], and thus be of less value.

Individuals with asthma demonstrate increases in sputum markers of inflammatory cell activation or increased vascular permeability, such as tryptase, IL-5, and fibrinogen as well as sputum cell counts for eosinophils, mast cells and neutrophils [65]. One example of the value of induced sputum as a biomarker in asthma is that there are changes in sputum eosinophil count seen after treatment with prednisolone, correlating with clinical improvement [66].

1.1.5.2.c Exhaled breath condensate

Condensation of some volatiles in gases in exhaled breath can be achieved simply by cooling the tubing through which the patient is exhaling. Successful collection has been reported with a variety of devices with different designs [67]. Most widely used designs include immersion of a Teflon-lined tubing in an ice-filled bucket and a specially designed double-wall glass condenser system. The collected fluid represents the volatile part of aerosolized pulmonary extracellular-lining fluid. The pH of breath condensate fluid of patients with acute asthma has been shown to be over two log orders lower than in control subjects, suggesting that this may be a good indicator of altered airway environment which may be part of the inflammation [68]. Markers in exhaled breath that may prove to be clinically useful in the future include aldehydes, glutathione [69], carbon monoxide, hydrogen peroxide, ethane [70], and nitrate or nitrites [71].

1.1.5.2.d Methacholine or histamine hyperreactivity

Tests of airway hyperresponsiveness are non-specific in isolation, as positive test results have been described in patients with allergic rhinitis, cystic fibrosis, bronchitis, and chronic obstructive pulmonary disease. However, a change in methacholine responsiveness is used to assess response to anti-inflammatory medications such as inhaled corticosteroids, or specific immunotherapy [72].

1.1.5.2.e Blood tests

1.1.5.2.e.i IgE

A study of children with raised IgE levels (at least 10 times the mean) showed a strong link with asthma symptoms [4]. Both total IgE and IgE against *Dermatophagoides farinae* (house dust mite) have been shown to be strongly associated with asthma [73].

1.1.5.2.e.ii Eosinophils

There is a link between raised serum eosinophils and asthma symptoms in children [74]. However, in severe asthma the bronchial eosinophils tend to be reduced relative to raised neutrophils [24], so it is unlikely that their peripheral eosinophil count would be an accurate reflection of disease.

1.1.5.2.e.iii C-Reactive Protein

C-reactive protein (CRP) is the prototype acute phase protein and is a major systemic inflammatory marker synthesised by hepatocytes in response to IL-6 [75]. CRP is a very non-specific marker of inflammation: levels may be transiently elevated for 2 to 3 weeks after a major infection or trauma, and is transiently raised after exercise [76]. CRP may also be of limited value among patients with chronic inflammatory conditions such as rheumatoid arthritis and SLE. Finally, adipocytes also release IL-6, and obesity itself is related to a raised CRP [77]. Some studies have shown that CRP is elevated in asthmatic subjects [78], although this association was weakened when obesity was controlled for. Other studies have suggested that CRP is similar between asthmatic and non-asthmatic

non-smokers [38]. However, if CRP is measured using very sensitive techniques (high sensitivity or hs-CRP), a significant difference can be seen between asthmatic and healthy people (mean±SD 1.33±1.48mg/L compared with 0.21±0.30mg/L) [79]. This difference is not seen in patients taking inhaled corticosteroids. Steroid-naive patients also showed a negative correlation between clinical indices such as FEV_1 and hs-CRP, and a positive correlation relative to sputum eosinophils [79].

1.1.6 Asthma treatment

1.1.6.1 Pharmacological treatments

Some of the drugs that are effective in asthma can only be used via inhalation because they are not absorbed when given orally. Medications taken for asthma fall into two groups, relievers and preventors. Relievers are rapid-acting bronchodilators that act to relieve bronchoconstriction and its accompanying acute symptoms such as wheezing, chest tightness, and cough. Inhaled β_2 agonists such as salbutamol are bronchodilators, and act principally to dilate the airways by relaxing airway smooth muscle. They reverse bronchoconstriction and related symptoms of acute asthma, but do not reverse airway inflammation or reduce airway hyperresponsiveness [80]. Long-acting β_2 agonists (LABAs), such as formoterol, salmeterol provide relief of symptoms in addition to a reduction in exacerbations [81].

Preventors are medications taken on a long-term basis to keep persistent asthma under control. Of all single medications, inhaled glucocorticosteroids (ICS) are at present the most

effective controllers [1]. Oral steroid medication is indicated as treatment of an acute exacerbation of asthma not responding to other treatment, or for longer term treatment of unresponsive asthma [40]. Leucotriene receptor antagonists are another oral medication that can improve asthma control [40]. More recently, Omalizumab (a recombinant humanised monoclonal antibody against IgE) has been shown to be useful in patients with atopic asthma and concomitant allergic rhinitis [82].

1.1.7 Summary

Asthma is a highly complex inflammatory disorder with many potential therapeutic approaches. Treatments aim to modify many aspects of the disease, usually with the intention of decreasing inflammation, the most commonly used being corticosteroids. Future developments in asthma medication will focus on alternative anti-inflammatory agents.

1.2 Atheroma and Inflammation – parallels for asthma

Atherosclerosis has been described as "a chronic inflammation induced and perturbed by lipid accumulation" [83]. The primary site of inflammation is in the arteries. T lymphocytes and monocytes are early progenitors of this inflammation [84]. The T lymphocytes involved show a predominantly T_H1 cytokine picture, with expression of IL-2 and IFN-γ in a large proportion of plaques [83]. A much lower proportion of plaques show T_H2 cytokines such as IL-4. The natural consequence of atherosclerosis is plaque rupture, which can cause myocardial ischaemia or infarctions or cerebrovascular events, and can lead to death.

Post mortem studies of patients who have died suddenly of coronary ischaemia suggest that the typical causal lesion is often a small, unnoticed plaque, causing minimal clinical symptoms. These plaques have a thin or fragmented fibrous cap, which has a poor connective tissue skeleton (see table 1.5). This cap ruptures, exposing the blood in the lumen to the procoagulant effects of a lipid-rich core infiltrated with inflammatory cells [85-87]. Cytokines released by the lipid core attract smooth muscle cells into the subintimal space. Both cell types trigger further matrix break down within the lesion by releasing metalloproteases [88]. This process is exacerbated by IFNγ secretion, which suppresses collagen formation by intimal smooth muscle cells [89] and in addition may lead to their apoptosis [88].

Table 1.5 Characteristic features of the vulnerable atherosclerotic plaque (from Sattar & Gaw [85]).

Thin, fragmented fibrous cap
Underdeveloped connective tissue skeleton
Lipid enrichment
Inflammatory cell infiltration
Evidence of proteolytic enzyme release
Apoptosis of smooth muscle cells

1.2.1 Inflammatory markers and coronary heart disease

Systemic levels of acute phase markers such as white cell count, serum amyloid A (SAA) and C-reactive protein (CRP) have been shown to predict the risk of coronary heart disease

(CHD) events independently in men and women [90 - 93]. Markers such as VCAM-1 may be released by vascular endothelium in very early stages of atherosclerosis [94].

The answer to how circulating cytokines enhance CHD risk likely lies in the dual functions of cytokines, for in addition to their role in regulating immune responses, cytokines mediate numerous metabolic effects. One consequence of this functional pleiotropy is that the intensity of the metabolic adaptations parallels other cytokine effects [85] Cytokine-induced metabolic effects, such as temporary changes in lipids and peripheral insulin resistance, function as part of the physiological reaction to infection and acute inflammation, to ensure the transport of specific metabolic fuels to and from essential organs [95]. In the short term, these changes are beneficial. However, when these same cytokines are chronically elevated, even modestly as in the case of obese individuals, the effects are harmful, and may promote accelerated atherogenesis. Indeed, CRP concentrations in population studies correlate with levels of many classical and novel CHD risk factors [96].

1.2.2 Statins

Statins are a class of cholesterol-lowering drugs that reduce cholesterol biosynthesis through competitive inhibition of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Figure 1.3) Statins occupy a portion of the binding site in HMG-CoA, thus blocking access to the active site [97]. They were initially introduced to the market in 1987 and there are now several commercially available drugs with similar

properties groups under the umbrella term “statin” (table 1.6). In 2004 atorvastatin was the best selling drug in the world [98]. Their main clinical application is in cardiovascular disease, where they have been consistently shown to decrease mortality from ischaemic heart disease [99 - 102] and stroke [103, 104]. These studies also showed that treatment with statins provided greater protection than predicted from cholesterol reduction [105], therefore further anti-inflammatory functions of these drugs were suggested, for example preventing essential substitution (e.g. prenylation) of signalling molecules.

Figure 1.3 Cholesterol biosynthesis pathway showing potential effects of inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase by statins, causing decreased prenylation of signalling molecules, as well as derivatives from mevalonate and cholesterol.

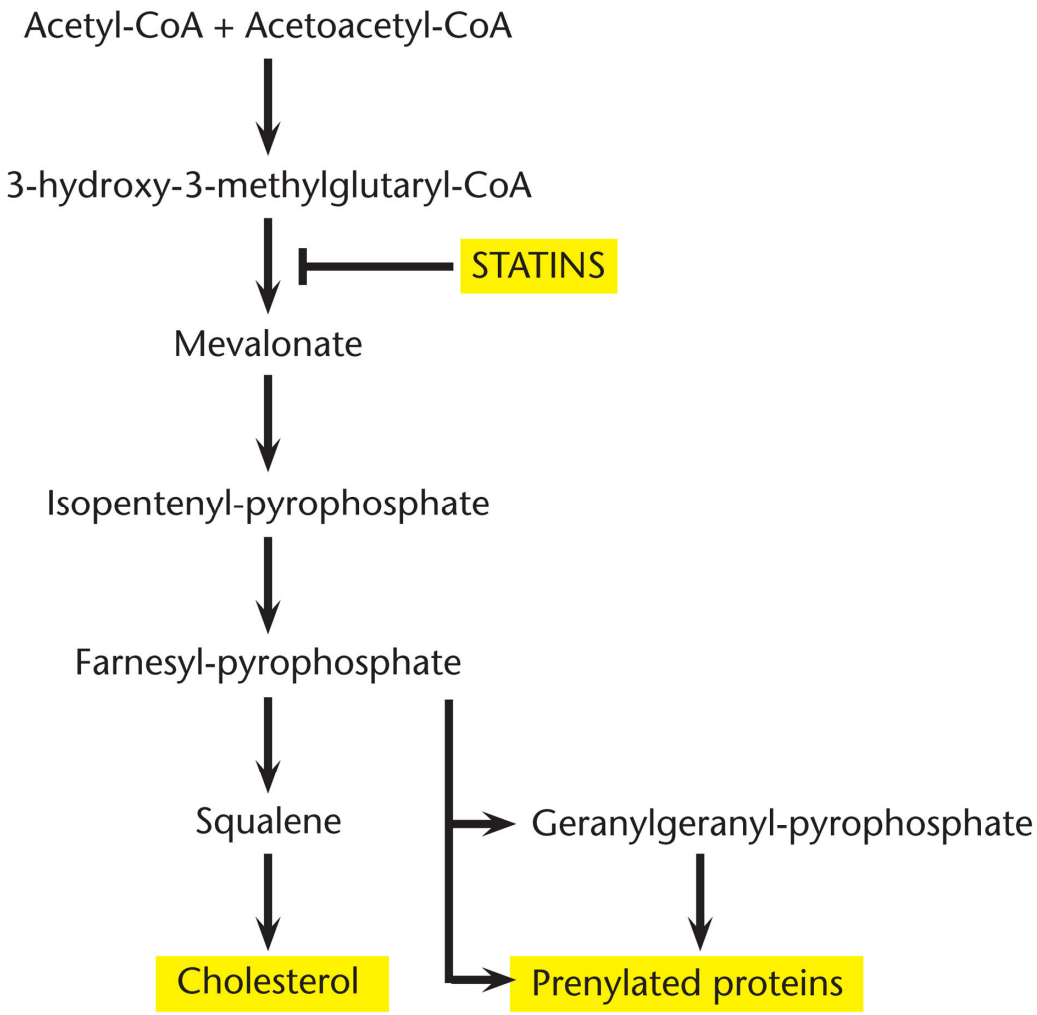


Table 1.6 The statin family of drugs

Drug name	Dose range (mg)	Comment
Atorvastatin	10 – 80	Completely synthetic origin
Fluvastatin	20 – 80	
Lovastatin	Not prescribed in the UK	The original medication, derived from *Aspergillus terreus*. No longer prescribed in the UK
Mevistatin	Not prescribed in the UK	Original molecule derived from *Penicillium citrinum*. Never used clinically due to side effects
Pitavastatin	Not prescribed in the UK	Also known as Itavastatin. Not yet commercially available
Pravastatin	10 – 40	
Rosuvastatin	5 – 20	New drug
Simvastatin	10 – 80	Discovered from *Aspergillus terreus*
Cerivastatin	Not prescribed in the UK	Removed from the market in 2001 due to severe adverse reactions

Evidence has accumulated that statins lower C-reactive protein (CRP) [106 – 109], a key indicator of inflammation, which itself is an independent risk factor for cardiovascular mortality and morbidity [110, 111]. This reduction in CRP is likely a consequence of the ability of statins to reduce the production of IL-6 [112, 113]; the cytokine which activates the acute-phase CRP response [75]. Based on these observations it has been proposed that the

clinical effectiveness of statins might be due to a combination of functions including cholesterol reduction, anti-inflammatory, antithrombotic and immunomodulatory effects.

1.2.3 Effect of statins in vascular endothelium

Statin therapy has been shown to have impact upon many processes in atheroma, helping to reduce the likelihood of atherosclerotic plaque rupture, or limiting thrombus formation should rupture occur. Comparative investigations suggest that the lipid soluble statins are capable of modulating vascular smooth muscle cell growth, independently of their cholesterol lowering capability [114]. The statins may also directly suppress platelet activation, limiting platelet thrombus formation [115]. In addition, they may stabilise lesions through a change in the composition of the plaque [116, 117], and a reduction in the number of inflammatory cells within the plaque [118].

1.2.3.1 Plaque stability

Ultrasound studies have shown that statin therapy does not result in extensive plaque regression [119]. This seems to contradict the findings of large intervention trials, which indicated that the plaques of treated patients carried a lower risk for acute coronary events [99, 101, 120, 121]. It has been shown from experimental work in primates [122] and in a clinical trial of patients undergoing carotid endarterectomy that statins have a plaque-stabilising effect [123]. Histological examination in the trial showed that the lesions in the treated patients contained a significantly lower concentration of lipids (-66%), less

oxidized LDL (-40%), and lower macrophage and T-cell counts (-41% and -54%, respectively) than did arteries from untreated control individuals.

1.2.4 Mechanism of action

Statins have several possible mechanisms of action that may be inter-related which result in the reduction of inflammation. These include a) modulating the cholesterol content, and thus reducing the stability of lipid raft formation and subsequent effects on the activation and regulation of immune cells, and b) preventing the prenylation of signalling molecules and subsequent down-regulation of gene expression; both resulting in reduced cytokine, chemokine and adhesion molecule expression, with effects on cell apoptosis or proliferation.

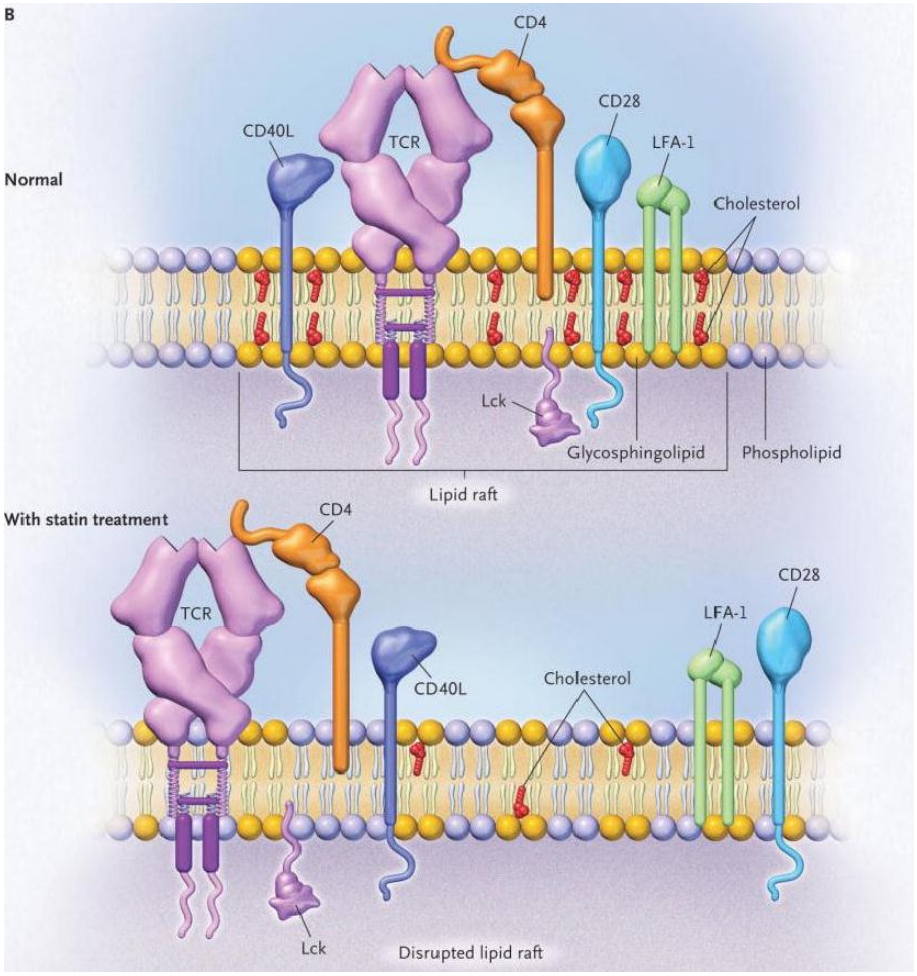
There are additional less well described anti-inflammatory properties of statins including antioxidant effects which have been described for some statins related to their ability to scavenge oxygen-derived free radicals [124].

1.2.4.1 Lipid raft formation

Lipid rafts are small cell membrane structures, or microdomains, rich in cholesterol and glycosphingolipid, which house intracellular enzymes, mainly kinases. These lipid rafts can be translocated by the actin cytoskeleton, which controls their specific redistribution, clustering and stabilisation within the cell membrane. When these rafts are assembled they form critical sites for processes such as cell movement, intracellular transport or

signal transduction. Lipid rafts act as platforms, bringing together molecules essential for the activation of immune cells, but also separating such molecules when the conditions for activation are not appropriate. Several strands of evidence suggest that the inhibition of cholesterol synthesis by statins disrupts these lipid rafts and thereby influences the function of lymphocytes [125, 126] (figure 1.4). A central component of the interaction between lymphocytes and antigen presenting cells, which results in T-cell activation, is interferon-γ (IFN-γ) induced up-regulation and assembly of the major histocompatibility complex class-II (MHC-II). Statins reduce IFN-γ production by T_H1 cells [127] and thus act as repressors of MHC-II-mediated T-cell activation [128, 129]. This effect may, however, decrease over time [130].

Figure 1.4 Lipid rafts and statins. The T-cell receptor (TCR) and costimulatory molecules, including lymphocyte function-associated antigen 1 (LFA-1), CD28, CD4, and CD40 ligand (CD40L), are recruited to lipid rafts after activation. Statins interfere with the activation of T cells by depleting membrane cholesterol and disrupting the integrity of lipid rafts. Statin treatment causes the exclusion from lipid microdomains of raft-associated molecules such as the Lck protein tyrosine kinase, the inhibition of actin polymerization, and the formation of a stable immunologic synapse and therefore disrupts T-cell activation From Ehrenstein, *et al*.[125]



1.2.4.2 Prenylation and regulation of cytokine synthesis

Altered cytokine synthesis observed with statin therapy may be a consequence of altered lipid raft formation. However, there is an alternative or additional pathway of cytokine synthesis that may be affected by statins. The mevalonate synthetic pathway mediated by HMG-CoA reductase is crucial for the biosynthesis of isoprenoids (Figure 1.3, above), which are essential for normal cellular proliferation and activity. Farnesyl pyrophosphate is a later intermediate on this pathway and serves as a precursor for the synthesis of various isoprenoids, for example geranylgeranyl or farnesyl groups, which prenylate proteins through covalent links. These can anchor these proteins to lipid rafts. Many prenylated proteins play important roles in the regulation of cell growth, cell secretion and signal transduction. Thus, by inhibiting prenylation, statins affect many cell processes involved in inflammation.

1.2.5 Anti-inflammatory effects of statins on non-respiratory cells and diseases

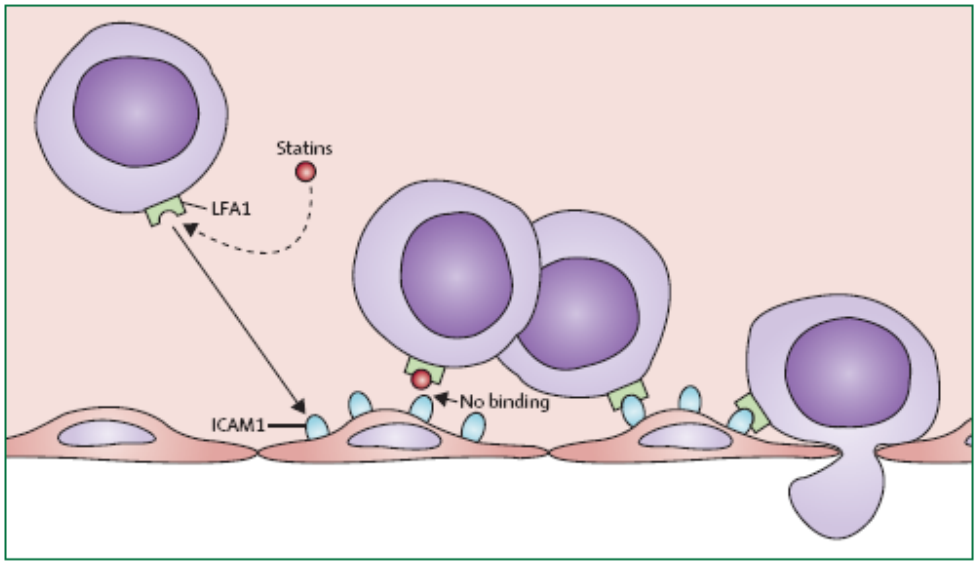
These two complementary mechanisms of prenylation and lipid raft stability allow statins to affect the function of many different cells and to attenuate inflammation in experimental models of disease.

1.2.5.1 Cell adhesion molecules

Statins interfere with cell binding by reducing leukocyte–endothelial cell adhesion [131, 132]. This occurs because statins attenuate the up-regulation of P-selectin normally seen on activated endothelial cells [133], and they also interfere with monocyte [134] and lymphocyte

attachment to endothelium by suppressing intercellular adhesion molecule-1 (ICAM-1) and lymphocyte-function-associated antigen 1 (LFA-1) interactions [135] (figure 1.5). Statins have been shown to decrease the expression of the receptor for chemoattractant chemokine ligand 2 (CCL2) expression on endothelial cells in rats [136, 137] and thereby reduce monocyte adhesion to vascular endothelium. Interference with the same chemokine in mice causes them to be unable to mount a T_H2 cell response [138].

Figure 1.5 Interruption of leucocyte–endothelium interaction. Leucocyte function-associated antigen 1 (LFA1) is important for lymphocyte binding to endothelial cells and for lymphocyte activation. Various statins directly inhibit this endothelial interaction by attaching to LFA1, thereby blocking its binding to intercellular adhesion molecule 1 (ICAM1). From Terblanche *et al* [139]. Reprinted with permission from Elsevier.



1.2.5.2 Cytokine and mediator release

Statins alter protein expression, which is reflected in altered cytokine release. *In vitro* experiments looking at spontaneous and lipopolysaccharide-induced secretion of interleukin (IL)-6 and tumour necrosis factor (TNF)-α in human cell lines showed reduced output due to statins in both cases [112, 140]. Fluvastatin and simvastatin but not pravastatin reduce production of IL-6 and interleukin-1 β (IL-1β) in human umbilical vein endothelial cells (HUVECs) [141]. Atorvastatin has also been shown to inhibit production of TNF-α [142]. Lovastatin induces T_H2 production of IL-4, IL-5 and IL-10 *in vitro* [127]. Increased prostacyclin [143] and decreased endothelin [144] production are seen in human endothelial cells after statin treatment.

1.2.5.3 Cellular apoptosis or proliferation

Statins increase apoptosis as demonstrated in human vascular endothelial cells [145], and in plasma cell lines from patients with multiple myeloma [146]. Statins can then enhance the clearance of apoptotic cells by human and mouse macrophages, a statin-specific effect reversible with mevalonate, through modulation of Rho-GTPases [147, 148]. Lovastatin and simvastatin have also been shown to block Fc receptor-mediated phagocytosis by cultured human monocytes [149].

Proliferation of T lymphocytes and B lymphocytes is inhibited by statins [150, 151], and statins can alter the ratio of T_H1 to T_H2 lymphocytes; cerivastatin, simvastatin, lovastatin, and atorvastatin can promote T_H2 polarisation through suppression of T_H1 lymphocyte

development and augmentation of T_H2 lymphocyte development from naive CD4+ T cells when primed *in vitro* [152]. Statins also reduce the proliferation of cardiac fibroblasts in rat and rabbit models [153].

1.2.5.4 Antioxidant effects

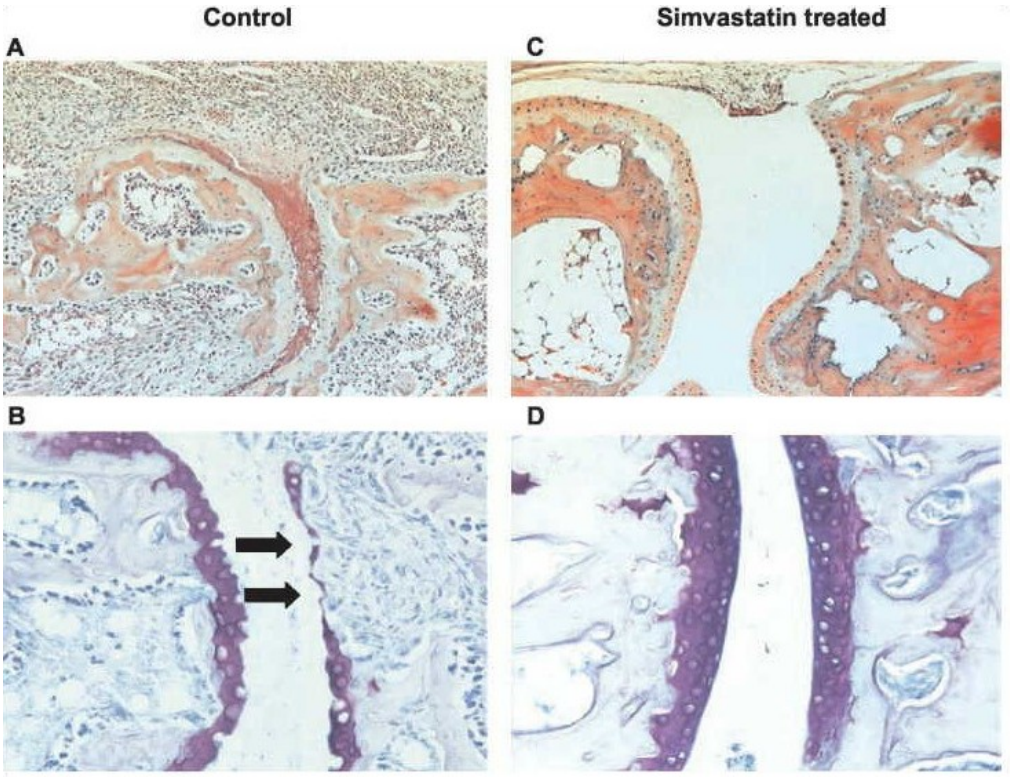
Metabolites of atorvastatin have been shown to possess potent antioxidative properties [154, 155], and protect very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) from oxidation [156]. Simvastatin acts as an antioxidant in rat liver microsomes [157] and vascular smooth muscle [158], and human lipoprotein particles [159], which may contribute to its anti-atherogenic effect**.**

1.2.5.5 Experimental models of disease

Statins have diverse effects on many chronic animal models of auto-immune disease. In models of systemic lupus erythematosus (SLE) the administration of atorvastatin resulted in a significant reduction in serum IgG anti-dsDNA antibodies and decreased proteinuria, reduced glomerular immunoglobulin deposition and glomerular injury. Disease improvement was paralleled by decreased expression of MHC class II on monocytes and B lymphocytes. T cell proliferation was impaired by atorvastatin *in vitro* and *in vivo* and a significant decrease in glomerular MHC class II expression was also observed [160]. Cerivastatin and simvastatin have also been shown to inhibit human neutrophil response to ANCA *in vitro* [161].

In experiments with collagen-induced arthritis in mice, simvastatin was given intraperitoneally either before (prophylactically) or after (therapeutically) induction of arthritis and a marked reduction in serum IL-6 and IFN-γ was seen, with a significant histological improvement [162] (figure 1.6).

Figure 1.6 Simvastatin in collagen-induced arthritis. Simvastatin administration to mice with after onset collagen-induced arthritis resulted in significantly reduced joint pathology. *A–D*, Mice were treated with phosphate-buffered saline or simvastatin (40 mg/kg) following detection of arthritis. After 14 days of simvastatin administration, arthritic paws were removed and stained with Haemotoxylin & Eosin or toluidine blue. Profound cartilage surface erosion and loss of proteoglycan was observed in PBS controls (arrows), whereas simvastatin recipients exhibited significantly reduced histologic evidence of destruction. From Leung *et al* [162] Copyright 2003 The American Association of Immunologists, Inc.



In a mouse model of autoimmune retinal disease, treatment with 20 mg/kg/day intraperitoneal lovastatin over 7 days, suppressed clinical ocular pathology, retinal vascular leakage, and leukocytic infiltration into the retina [163]. Efficacy was reversed by co administration of mevalonolactone, the downstream product of HMG-CoA reductase.

1.2.5.6 Clinical studies

A double-blind, randomised, placebo-controlled trial examined the efficacy of atorvastatin 40 mg daily for six months in rheumatoid arthritis. At the end of that period, patients who had received statin were found to have decreased plasma levels of lipids, fibrinogen and viscosity. Disease activity score improved significantly on atorvastatin treatment compared with placebo. CRP and erythrocyte sedimentation rate reduced by 50% and 28% respectively, relative to placebo [164].

A trial of atorvastatin during tetanus toxoid (TT) vaccination showed a marked increase in anti-TT antibodies 15 days post-vaccination, with a suppression of lymphocyte and platelet count [165]. This complex immunological picture raises more questions about the effect of statins on the immune system.

1.2.5.7 Different statins may have different anti-inflammatory properties

It has recently become apparent that the different families of statins may have different biochemical functions. Kiener and colleagues [166] showed that lipophilic statins such as atorvastatin and simvastatin have a much greater effect on inflammatory responses in

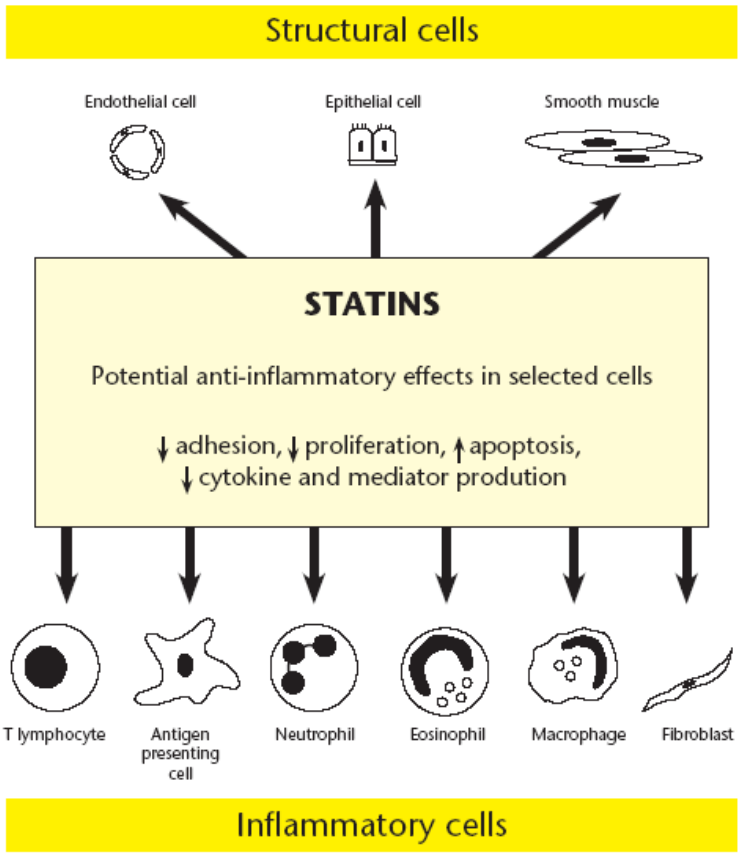
human and mouse models than the hydrophilic pravastatin. Similarly, when looking at sensitisation of human smooth muscle cells to apoptotic agents, lovastatin and simvastatin showed a powerful sensitising effect, whereas atorvastatin showed less of an effect, and pravastatin had no activity [167]. There is also a dose-response effect seen for example where cerivastatin is much more potent than fluvastatin in blocking NF-κB activation in human blood monocytes [168]. Some statins have differing effects on protein expression, for example in monocytes stimulated by lipopolysaccharide (LPS), pravastatin and fluvastatin may induce production of TNF-α, IFN-γ, and IL-18 [169, 170], whereas atorvastatin and simvastatin inhibit production of TNFα [112, 113, 142, 171].

It is therefore important to recognise that all statins may not have the same therapeutic potential. For example, a clinical study in 27 healthy volunteers found significant differences between the *ex vivo* immunological responses after atorvastatin and simvastatin therapy. Atorvastatin led to a significant down-regulation of the expression of human leukocyte antigen (HLA)-DR and of the CD38 activation marker on peripheral T cells, whereas simvastatin up-regulated both these molecules. In contrast, superantigen-mediated T cell activation was inhibited by simvastatin and enhanced by atorvastatin [172]. However, initial experimental work in inflammatory lung disease has used both simvastatin [173] and pravastatin [174] with clear effect.

1.2.6 Potential therapeutic role for statins in respiratory disease

The therapeutic effect of statins on cardiovascular and auto-immune disease seem to be broadly anti-inflammatory, which are also likely to apply to lung diseases in which there is an inflammatory component (figure 1.7).

Figure 1.7 Potential anti-inflammatory effects of statins on different structural and inflammatory cells within the lungs.



1.2.6.1 Possible effects of statins on cellular inflammatory processes in the lung

There are several inflammatory processes in the lung that may be susceptible to the effects of statins.

Statins could affect the chemokine and adhesion molecule-directed migration of inflammatory cells from blood into the airways [169, 175 - 177]. Since both eosinophils and macrophages express the adhesion molecule LFA-1, this offers a potential target for modification of airway inflammation. Treatments other than statins targeted at reducing the expression of LFA-1 have been effective in decreasing airway eosinophilia in a mouse model of atopic asthma [15] and have reduced sputum eosinophilia after allergen challenge in asthmatic patients [16]. Since statins can inhibit LFA-1/ICAM-1 interaction, as seen in HIV [135], there is potential for statins to have an equivalent effect in asthma, where the pathophysiology is associated with eosinophil accumulation. Lovastatin has recently been shown to inhibit human alveolar epithelial production of IL-8 [178], which might also contribute a beneficial effect of statins in the treatment of neutrophil associated inflammatory diseases of the lungs.

The observation that statins increase eosinophil apoptosis in humans [179] suggests a further therapeutic role. The mechanism of this is likely due to the rapid reduction of cellular expression of CD40 after statin administration and this strongly inhibits eosinophil survival [32]. Similarly, the neutrophilia associated with a mouse model of acute lung injury is markedly reduced with lovastatin treatment [180], and this modulation of neutrophil

apoptosis may prove beneficial in other inflammatory lung diseases, such as smokers with asthma or COPD where neutrophils are present and where corticosteroid treatment may be of limited benefit. In addition to induction of apoptosis, statins, in this case lovastatin, also enhance the clearance of apoptotic cells by human and mouse macrophages, a statin-specific effect reversible with mevalonate [147].

Statins could affect the activation and proliferation of a variety of cells associated with lung inflammation. For example, statins suppress T_H1 cell activation, and IFN-γ production, as seen in a recent trial in rheumatoid arthritis [164], and by analogy this treatment could decrease the IFN-γ dependent pathology of chronic asthma and pulmonary tuberculosis. Similarly, statins decrease natural killer (NK) cell activity in treated transplant patients [181] and this might be relevant to the pathogenesis of asthma in which NK cells may have a pathogenic role [182, 183]. The decrease in expression of MHC-II induced by statins has been observed on monocytes, macrophages, and on B lymphocytes in mice [160], which implies a widespread down-regulatory effect on presentation and immune response to inhaled or lung-associated antigens.

Statins may also have a role in attenuating the tissue repair and remodelling consequences of chronic aberrant immune activation and inflammation. For example statins inhibit the proliferation of airway smooth muscle in human cell lines [184] and lower the expression of the profibrogenic cytokine transforming growth factor (TGF)-β1 [185]. Statins also reduce the tissue damage and cellular changes associated with cigarette

smoking. The mechanism of this appears to be related to statins reducing the production of matrix metalloprotease (MMP)-9 and airway remodelling in smoking rats [186, 187], and rabbits [188] and in human macrophages [189] and monocytes [142] from smokers. Other MMPs may also be reduced [188 - 192]. By targeting this key aspect of remodelling, this indicates a potential therapeutic role for statins in fibrotic lung diseases.

Finally, it is worth bearing in mind the different pharmacological properties between statins. For example, lovastatin seems to increase lymphocyte secretion of IL-4 and IL-5 in a mouse model of multiple sclerosis [127], and therefore this particular statin may be of limited use in asthma, where these cytokines are directly implicated in the pathogenesis.

1.2.6.2 Statin treatment of human and experimental respiratory diseases

1.2.6.2.a Age-related deterioration in lung function

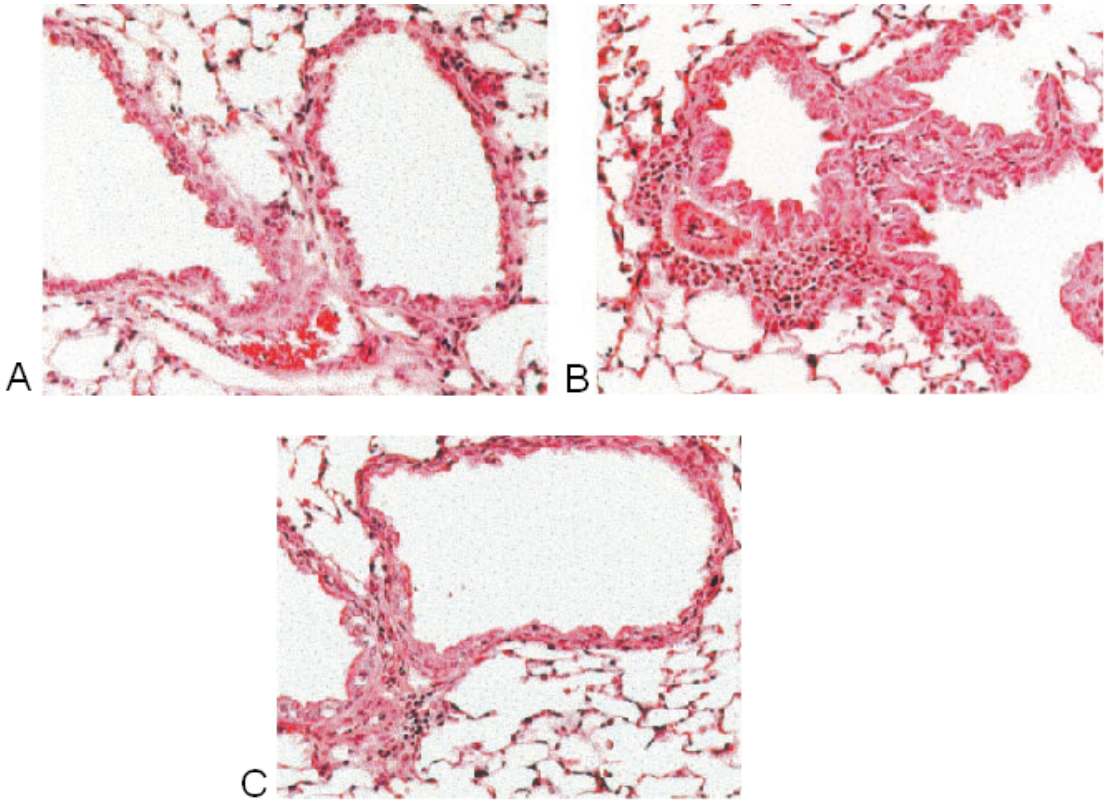
A cohort study of 803 patients suggested that statins significantly reduced the deleterious effect of age on lung function, with patients taking a statin exhibiting a mean decline in FEV1 of 10.9mL/year, compared with 23.9mL/year in patients who were not [193]. This protective effect was reduced in smokers.

1.2.6.2.b Asthma

In a mouse model of atopic airways disease, the potential benefits of statin therapy on inflammatory airway disease were demonstrated [173]. In this model, airway eosinophilia

was elicited using ovalbumin (OVA) as the allergen. Simvastatin given before each OVA challenge caused a reduction in inflammatory cell infiltrate and eosinophilia in broncho-alveolar fluid, and a decrease in the OVA-specific production of IFN-γ, IL-4 and IL-5 by thoracic node lymphocytes in vitro (figure 1.8). The same anti-inflammatory effects of pravastatin have been reported in a similar experimental model of atopic airway inflammation [174]. The anti-inflammatory properties of statins observed in animal models of atopic asthma [173] and in smoking-induced lung disease [186] suggests that statin treatment could improve asthma control in smokers with asthma who are insensitive to treatment with corticosteroids [194].

Figure 1.8 Histological evidence of decreased lung inflammation in mice treated with simvastatin. A, Naive mouse, given saline challenge. B, ovalbumin antigen challenged mouse; peribronchial and perivascular inflammatory infiltrates are seen, with eosinophils present and mucosal hyperplasia. C, ovalbumin-challenged mouse plus treatment with simvastatin; a reduction in inflammatory infiltrates is seen compared with B. Hematoxylin & Eosin staining. Reproduced from MacKay *et al*.[173] Copyright 2004 The American Association of Immunologists, Inc.



Separately, fluvastatin has been shown to inhibit proliferation of human peripheral blood mononuclear cells (PBMC) from asthmatic patients, and to reduce expression of IL-5 and IFN-γ, in response to both allergen-specific (house dust mite) and non-allergen specific stimulation [195].

1.2.6.2.c Emphysema and COPD

In rat and mouse models of emphysema [186, 196] found that simvastatin inhibited lung parenchymal destruction, reduced mRNA expression of IFN-γ, TNF-α and MMP-12, and peribronchial and perivascular inflammatory cell infiltration. Induction of MMP-9, a major inflammatory mediator, was reduced in the same model when the experiment was repeated using human lung microvascular endothelial cells *in vitro* [186]. A nested case-control study in humans has also suggested that statins may decrease respiratory mortality in patients with COPD [197].

1.2.6.2.d Pulmonary hypertension

Statins induce Rac 1 expression while suppressing Rho A in a rat model of pulmonary hypertension [198], and induce apoptosis of pulmonary vascular cells [198, 199]. A rat model also shows improved pulmonary artery pressure, ventricular and blood vessel remodelling, and polycythaemia [200], suggesting a significant survival advantage following treatment with simvastatin [186, 201]. An open-label clinical case series of patients with

pulmonary hypertension showed that simvastatin delays disease progression and may improve survival [202].

1.2.6.2.e Idiopathic pulmonary fibrosis (IPF)

Early experimental data suggests that simvastatin could modify critical determinants of the profibrogenic machinery responsible for the aggressive clinical profile of IPF, and could potentially prevent adverse lung parenchymal remodelling associated with persistent myofibroblast formation [203]. This hypothesis has recently been tested in a clinical trial of lovastatin in IPF but preliminary data showed no improvement in survival [204].

1.2.6.2.f Acute lung injury

In a model of acute lung injury, mice treated with simvastatin showed decreased lung permeability, along with significant reduction in NF-κB mediated gene transcription, suggesting a potential role for statins in the management of this disease [205].

1.2.6.2.g Community acquired pneumonia

This concern for an adverse role for statins in reducing resistance to lung infection was partly addressed in a retrospective cohort study which showed that statins were associated with a 22% decrease in overall 30-day mortality (from 28% to 6%) from community-acquired pneumonia. This remained significant even after adjustment for potential confounders such as previous co-morbidity, which would normally be expected to increase mortality [206]. A case-

control study of diabetic patients concurred [207]. However, there is still a need to monitor the effects of statin therapy prospectively on the immune response. Interestingly, a study of statin therapy of *Chlamydia pneumoniae* in mice suggested an increase in inflammatory cell infiltration into the lungs during acute infection [208].

1.2.6.2.h Lung transplantation

The outcomes in lung transplantation were compared between 39 patients taking statins for hyperlipidemia (mainly atorvastatin and pravastatin) and 161 who were not. Acute rejection was less frequent, bronchoalveolar lavage (BAL) showed lower total cellularity, as well as lower proportions of neutrophils and lymphocytes, and survival was 91% compared with 54% in controls [209].

1.3 Summary

Asthma is a chronic, degenerative disease, with serious negative effects on quality of life. At present, many therapies exist, but many patients still do not have complete control. Statins may be a possible novel adjunct to current therapies: their anti-inflammatory credentials are well established. The evidence from animal models is strongly suggestive of a beneficial effect in terms on improving inflammation. The logical next step would be a proof of concept clinical trial.

2. HYPOTHESIS

This randomised controlled trial will test the hypothesis that statins improve asthma control and airway inflammation of patients with chronic allergic asthma.

2.1 Primary End Point

The primary end point is an improvement in morning PEF. It is expected that an improvement of 20l/min will be seen after 8 weeks of atorvastatin.

2.2 Secondary End Points

2.2.1 Clinical

It is expected that an improvement will be seen in indicators of asthma control, including:

Asthma control questionnaire score

Asthma Quality of Life Questionnaire

PC_{20} methacholine

2.2.2 Inflammatory

Markers of inflammation in blood and sputum are expected to show a reduction inflammation. This would be seen in a reduction in inflammatory cells in sputum cell count, such as macrophages, neutrophils and eosinophils. This would be accompanied by a reduction in inflammatory cytokines in sputum supernatant and serum. In particular, a reduction in CRP and IL-6 would be expected.

3. RANDOMISED CONTROLLED TRIAL

3.1 Recruitment

54 adult allergic asthmatic patients were recruited from hospital respiratory clinics and from general practice. Potentially eligible patients were identified by scrutiny of hospital records and from computerised General Practice records. Their hospital doctor or GP then invited them to take part and volunteers were screened for eligibility in the research unit.

3.1.1 GPASS

The General Practice Administration system for Scotland (GPASS) is the national Primary Care System and is one of Britain's leading general practice systems. It is used in over 890 Scottish practices (www.show.scot.nhs.uk/gpass/), and allows searching of patient records by a number of parameters, including age, diagnosis and/or prescriptions.

A shortcoming of any patient record system is that the information on the database has been entered by a number of people and many of them may not have any clinical background. Consequently, it is prudent to keep the original search wide. For example, a search based on prescription is more likely to be accurate than a search based on documented diagnosis.

Once a list of broadly suitable patients was produced, the details of each patient were examined for other exclusion criteria. The resultant list was then left for the GP and

practice nurse to examine so that they could remove any patients they deemed unsuitable, e.g. housebound patients.

3.1.1.1 Search strategy

A search was performed for patients age between 18 and 70 who had been prescribed a β2-agonist inhaler and an inhaled corticosteroid in the last year, see figure 3.1

Figure 3.1 GPASS search strategy for patients

GROUP (and) -

Registration age in years is between 18 and 70 inclusive

And

Prescription drug BNF code is 301011 (SELECTIVE BETA2 AGONISTS)

And

Prescription weeks since issued is less than or equal to 52

And

GROUP (and) -

Prescription drug BNF code is 302000 (CORTICOSTEROIDS)

And

Prescription weeks since issued is less than or equal to 52

3.2 Subjects

3.2.1 Inclusion criteria

The inclusion criteria are shown in figure 3.2.

Figure 3.2 Inclusion criteria

Diagnosis of asthma:
Objective:
Confirmed by methacholine airway hyperresponsiveness (PC20 ≤ 8 mg/mL) *or*
by evidence of variable airflow obstruction with an increase in FEV1 of > 12% following nebulised salbutamol (2.5 mg) [210] *or*
Diurnal peak flow variability of > 20% for 3 days in a week (with a minimum change of 60 L) during the run-in period of the study [40]
AND
Symptomatic:
Episodic wheezing, chest tightness and/or dyspnoea [211]
AND
Asthma control questionnaire score of ≥ 1 (range 0-7) prior to randomisation *or*
Use of inhaled $beta_2$-agonist on 5 or more days in the week before randomisation *or*
FEV1 reversibility >12% *or* diurnal peak flow variability of >20% during the run-in period of the study for at least 3 days of a week
Age range 18-70 years
Duration of asthma ≥ 1 year and on stable medication for 4 weeks
Receiving regular inhaled steroid treatment (≤ 1000 mcg Beclomethasone equivalent daily) and no other medication for their asthma other than a short-acting bronchodilator
Stable asthma medication for at least 4 weeks prior to randomisation
Written informed consent

3.2.2 Exclusion criteria

Exclusion criteria are listed in figure 3.3.

Figure 3.3 Exclusion criteria

Current smokers or ex-smokers of < 1 year or ex-smokers who have smoked > 5 pack years
Patients with unstable asthma; defined as the presence of 1 or more of the following events, due to asthma, in the month prior to randomisation: Emergency/"out of hours" visit of patients to the GP GP visit to patient at home A & E hospital attendance Hospital admission
Patients in whom cardiovascular risk requires statin therapy
Any known sensitivity or adverse reaction to statin, or previous evidence of myopathy or myositis plus creatinine kinase and liver function tests > x2 upper limit of normal range
Non-atopic asthma; defined as skin test wheal ≤ 3mm over negative control saline
Pregnancy or lactation
Patients who require medications known to interact with statins, such as azole antifungal agents, erythromycin, clarithromycin, cyclosporine, gemfibrozil, verapamil and amiodarone
Inability to fully comprehend the patient information sheet
Inability to demonstrate correct use of peak flow meter after instruction

Patients who showed specific IgE sensitivity or were skin test positive to grass pollen allergen were not recruited from mid May to the end of July (grass allergen season in UK) if they were symptomatic of hayfever.

3.3 Structure of Study

The study was a 24-week randomised double blind crossover study comparing the effect on asthma control of oral atorvastatin therapy (40 mg daily) with that of a matched placebo. Each treatment was administered for 8 weeks separated by a 6-week washout period and a 2-week run-in period prior to randomisation. Randomisation was performed in sequential blocks of four.

Patients were assessed on 9 visits (13 occasions, as some visits were performed over 2 days) (figure 3.4):

Screening visit

Obtain written informed consent

General medical history & physical examination

ACQ

Spirometry and reversibility testing

Blood sampling

Skin prick testing

Issue diary card and peak flow meter

Adjustment of asthma medication to BTS guidelines if required. If any changes were made, randomisation visit was deferred an extra two weeks to allow four weeks on stable medication

Randomisation visit

Baseline clinical measurements

2 weeks after screening visit (or 4 weeks if any change made to baseline asthma medication)

ACQ

AQLQ [43](See appendix 2)

ATS Score to assess severity of asthma

Spirometry and reversibility testing

Induced sputum

Exhaled NO

Methacholine responsiveness

Randomise patient if all criteria met

Issue medication container A.

Issue diary card

Follow up visits (Phase A)

2 and 4 weeks after randomisation

Spirometry and reversibility testing

Issue new diary

End of medication A visit (Phase A)

8 weeks after randomisation

ACQ

AQLQ [43](See appendix 2)

ATS Score to assess severity of asthma

Spirometry and reversibility testing

Induced sputum

Exhaled NO

Blood sampling

Methacholine responsiveness

Issue new diary

End of washout visit (Phase B)

At least 6 weeks after end of medication A

ACQ

AQLQ [43](See appendix 2)

ATS Score to assess severity of asthma

Spirometry and reversibility testing

Induced sputum

Exhaled NO

Blood sampling

Methacholine responsiveness

Issue medication container B

Issue new diary

Follow up visits (Phase B)

2 and 4 weeks after starting medication B

Spirometry and reversibility testing

Issue new diary

End of medication B visit (Phase B)

8 weeks after starting medication B

ACQ

AQLQ [43](See appendix 2)

ATS Score to assess severity of asthma

Spirometry and reversibility testing

Induced sputum

Exhaled NO

Blood sampling

Methacholine responsiveness

Patients recorded morning and evening PEF measurements and daily symptoms throughout the study.

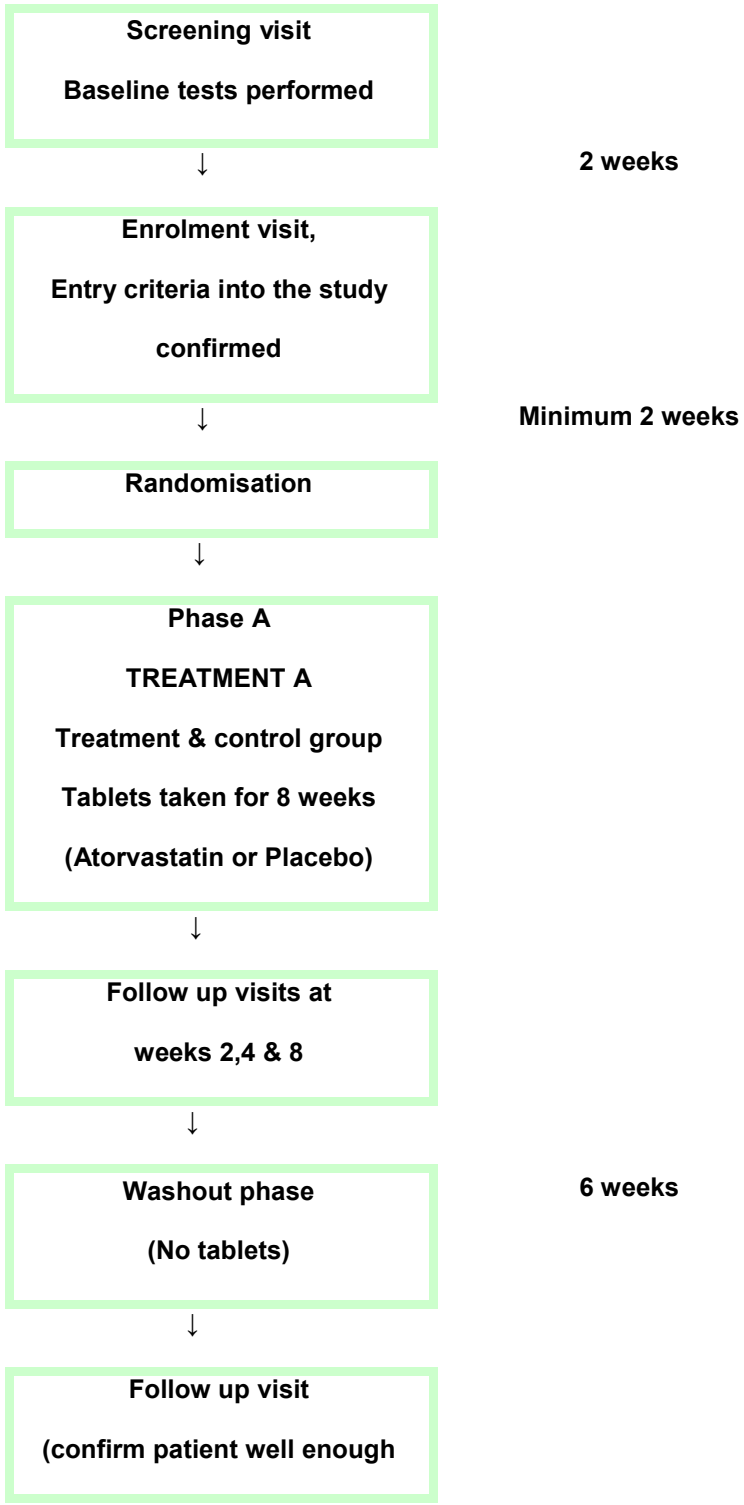
3.3.1 Pregnancy testing

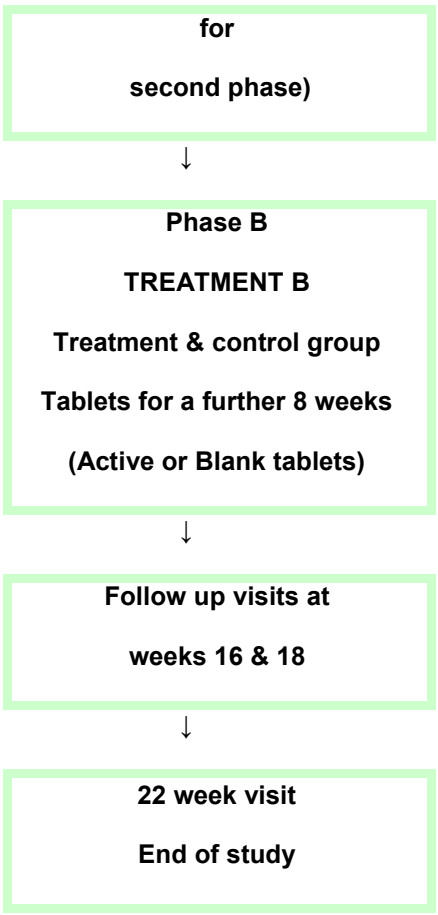
Pregnancy test was performed in all women of child-bearing age before all methacholine tests and subjects were informed about adequate contraception during and for one month after the study.

3.3.2 Exacerbations during washout phase

If a subject had an exacerbation during the wash-out phase of the study, visit 6 was delayed until the patient had been stable for 4 weeks.

Figure 3.4 Flow chart for structure of study





3.4. Statistical analysis

3.4.1 Power calculations

A sample size of 44 has 90% power to detect a difference in means of 20L/min in peak expiratory flow (PEF) (primary endpoint), assuming a standard deviation of differences of 40L/min, using a paired t-test with a 0.05 two-sided significance level. A total of 54 patients were recruited to ensure that 44 patients completed the study.

3.4.2 Analysis Sets

As this was a crossover study, patients only contribute to an analysis if the data point from both treatment periods was available. The Full Analysis Set therefore consists of those patients who attended the final visit of treatment Period B. All of the available data is present in the Full Analysis Set.

3.4.2.1 Baseline Data

The baseline demographic and other characteristics are presented by sequence group (i.e. AB or BA treatment sequences), and in total, using the minimum, maximum, mean and standard deviation for continuous variables, and counts and percentages for categorical variables.

3.4.3 Endpoints

3.4.3.1 General Principles

Each of the two treatment periods lasts for eight weeks, and Week 8 data was analysed with statistical tests. The Week 0, Week 2 and Week 4 visits were summarised where data was available, but was not analysed by statistical tests. In all trials the later data is usually most important, but this is particularly true of crossover studies, where the end of Period B is the furthest from the washout period. The summaries of the earlier visits were considered as extra information for general interest regarding the timing of the treatment

effect, and are not considered to cause problems with multiplicity. Descriptions of endpoints are summarised in Table 3.1.

Results were analysed as the difference between means (treatment difference) or medians of endpoints, without controlling for baseline variables, as these are considered irrelevant in crossover trials. Comparison is therefore not made between baseline values of variables.

Table 3.1 Description of end points

End point	Derived from	Time of measurement
Primary end point		
Mean morning peak flow (PEF)	Patient diary cards	Just before the Week 8 visits of each treatment period
Secondary end points		
Mean evening peak flow (PEF)	Patient diary cards	Just before the Week 8 visits of each treatment period.
Daily Asthma Control Score	Patient diary cards	Just before the Week 8 visits of each treatment period.
Mean number of puffs of relief β_2 agonist as measured in the morning	Patient diary cards	Just before the Week 8 visits of each treatment period.
Mean number of puffs of relief β_2 agonist as measured in the evening	Patient diary cards	Just before the Week 8 visits of each treatment period.
Exacerbation rates	Patient diary cards	Just before the Week 8 visits of each treatment period.
Asthma Control Score	Clinic questionnaire	Week 8 visit of each treatment period
ATS Score	Clinic questionnaire	Week 8 visit of each treatment period
Asthma Quality of Life Questionnaire	Clinic questionnaire	Week 8 visit of each treatment period
Use of Oral Steroids	Patient diary cards	Throughout study

End point	Derived from	Time of measurement
Pre-salbutamol: FEV_1 PEF FVC FEF_{25-75} FEV_1 percentage predicted PEF percentage predicted FVC percentage predicted FEF_{25-75} percentage predicted	Clinic Spirometry	Week 8 visit of each treatment period
Post-salbutamol: FEV_1 PEF FVC FEF_{25-75} FEV_1 percentage predicted PEF percentage predicted FVC percentage predicted FEF_{25-75} percentage predicted PC_{20} methacholine	Clinic Spirometry	Week 8 visit of each treatment period
Asthma Related Events - Out of hours visits to GP - GP home visits - Visits to Accident and Emergency - Hospitalisations	Patient diary cards	Throughout study

End point	Derived from	Time of measurement
Exhaled NO (FE_{NO}) Exhaled CO	Clinic testing	Week 8 visit of each treatment period
Immunological Tests in Blood: - ICAM-1 - IL-6 - hsCRP - TNF-α - Lymphocyte proliferation	Clinic sample	Week 8 visit of each treatment period
Lipids in Blood - Cholesterol - Triglycerides - HDL cholesterol	Clinic sample	Week 8 visit of each treatment period
Serum Biochemistry Safety Checks Renal function: - Urea - Potassium - Sodium Liver function tests: - Bilirubin - AST - ALT	Clinic sample	Week 8 visit of each treatment period
Sputum Cell Counts - Total cell count - Macrophages	Clinic sample	Week 8 visit of each treatment period

End point	Derived from	Time of measurement
- Neutrophils		
- Eosinophils		
- Lymphocytes		
- Viability		
- Epithelial cells		
Macrophage activation indices	Clinic sample	Week 8 visit of each treatment period
(sputum):		
- MPO		
- LTB_4		
- IL-1b		
- IL-1RA		
- IL-6		
- IL-8		
- IL-17		
- TNF-α		
- IFN-γ		
- GM-CSF		
- MIP-1a		
- CCL2		

3.4.3.2 Treatment of Diary Cards

The diary cards had space for up to eight weeks' data. However, we analysed only the last seven days data that were filled in prior to each visit. A card was declared null and void if there was no morning PEF data. The last day's data was calculated by counting back to the last non-missing day. For each of morning PEF, evening PEF, daily control

scores and puffs of relief medication separately, the number of non-missing days in the seven-day period up to (and including) the identified last day of data was counted. At least three days' data for each variable had to be available separately for that variable to be considered as evaluable. The means of the seven days were calculated for each of the evaluable variables.

3.4.3.3 Treatment of ACQ Scores (Clinic & Diary Versions)

For the standard clinic version of the ACQ score there are seven questions each scored on a seven-point scale (0=good control, 6=poor control). The overall score is the mean of the seven responses.

The daily diary ACQ is slightly different. The morning PEF data is used instead of FEV_1, and converted to a percentage predicted value which is scored like the FEV_1 version on the standard version (i.e. >95% = 0, 95%-90% = 1, 80%-89% = 2, 70%-79% = 3, 60%-69% = 4, 50%-59% = 5 <50% = 6). $Beta_2$ agonists puffs are totalled for the morning and afternoon (to calculate the number of puffs used in last 24 hours). The scores for this are: 0 puffs, 1-2 puffs = 1, 3-4 puffs = 2, 5-8 puffs = 3, 9-12 puffs = 4, 13-16 puffs = 5, >16 puffs = 6. Once this is done the mean of the seven questions can be calculated.

3.4.3.4 Treatment of Use of Oral Steroids

The use of oral steroids was calculated by examining the CRF at each study visit, to see if a patient used oral steroids at any time during the treatment period (i.e. unlike the other endpoints Week 2 and Week 4 visits were included as well).

3.4.3.5 Treatment of Asthma Related Events

The total number of these events (out of hours visits to GP, GP home visits, visits to accident and emergency, hospitalisations) was calculated across each of the visits in a treatment period (i.e. Weeks 2, 4 and 8). The totals were also calculated separately for each of the four different types of event.

3.4.4 Analysis Techniques

The main analyses were carried out by Normal Linear Models that include parameters for patient, period and treatment.

3.4.5 Software

All analyses were carried out using SAS version 9 (SAS Institute, Cary, NC).

3.5 Location of work

All the clinical work for the study was undertaken at the Asthma Research Centre, Department of Respiratory Medicine, Gartnavel General Hospital, Glasgow. Sputum and blood analysis were performed within the department of Immunology, Western Infirmary, Glasgow and Department of Biochemistry, Royal Infirmary, Glasgow. Statistical analyses were performed in conjunction with the Robertson Centre for Biostatistics at the University of Glasgow.

3.6 Patient Safety

3.6.1 Drug interactions and side effects associated with atorvastatin

Statins should be administered with caution if there is a history of liver disease, a history of alcohol excess, renal impairment/failure or hypothyroidism. In the screening process the renal and hepatic function were assessed using routine biochemistry testing. Patients with a history of hereditary muscular disorders or previous history of muscular toxicity whilst using statin medication subjects were excluded from this study.

Side effects with statins are rare [212], but include headache, myalgia, abdominal pain, flatulence, diarrhoea, nausea, vomiting, insomnia, anorexia, alopecia, peripheral neuropathy, urticaria, pruritis, impotence, chest pain, hypoglycaemia or hyperglycaemia [213]. Atorvastatin has recently been identified as causing nightmares [214], a feature also seen in other statins.

Patients concurrently taking digoxin, oral contraceptives, amlodipine, colestipol, antacids or warfarin were closely monitored (and appropriate blood levels measured) as atorvastatin may enhance or decrease the effect of these drugs. It is also been shown that the level of atorvastatin in the body may be reduced if the patient is on any of the drugs listed above [212]. Patients on azole antifungal agents, erythromycin, clarithromycin, azithromycin, cyclosporine, gemfibrozil, verapamil and amiodarone were excluded from this study and general practitioners were asked to omit the study medication if any of these medications were required during the course of the study.

As per the recommendations of the MHRA [215], subjects were asked to avoid fresh grapefruit juice while on the study and to report muscle pain, weakness or cramps and to stop treatment if this was severe. Creatine kinase (CK) levels were checked and treatment did not re-commence if levels were elevated >2 times normal.

3.6.2 Unused medication

All unused medication was returned to the pharmaceutical company (Pfizer) for destruction at the end of the study.

3.6.3 Current anti-asthma drug treatment

Patients were asked to continue on their usual anti-asthma drug therapy throughout the study.

3.6.4 Adverse Events

An adverse event (AE) is defined by the UK Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031) as:

> An exacerbation, or unexpected increase in the frequency or intensity of a pre-existing condition (other than asthma), including intermittent or episodic conditions.
>
> Significant or unexpected worsening or exacerbation of asthma
>
> A suspected drug interaction
>
> Any clinically significant laboratory abnormality

Adverse events are graded according to their severity as follows:

Mild: Awareness of signs or symptoms, but easily tolerated. There is no loss of time from normal activities. Symptoms resolve easily with no medical treatment (other than short-acting bronchodilators). Signs and symptoms are transient.

Moderate: Discomfort severe enough to cause interference with the patient's usual activities. Symptomatic treatment is possible.

Severe: Incapacitating with inability to do work or usual activities, signs and symptoms may be of a systemic or require medical intervention and/ or treatment. Hospitalisation may be required.

A reasonably related event is one that is in the opinion of the investigator, possibly, probably or definitely related to the study product.

3.6.4.1 Serious Adverse Events

A serious adverse event (SAE) is any adverse event, which results in:

Death

Is life-threatening (this refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)

In-patient hospitalisation or prolongation of inpatient hospitalisation. (Hospitalisation for a pre-existing condition, including elective procedures, which has not worsened, does not constitute a serious adverse event)

Persistent or significant disability/incapacity that interferes with the person's ability to conduct normal activities of daily living

Congenital anomaly or birth defect

In addition, an important medical event may be considered a SAE when, based on appropriate medical judgment, it may jeopardise the subject and/or may require medical or surgical intervention to prevent one of the outcomes listed.

Any SAE that was ongoing on completion of the trial would have been followed until it

resolved or stabilised,

returned to baseline condition or value (if baseline value is available) or

could be attributed to agent(s) other than the study agent/ to factors unrelated to study conduct

3.6.4.2 Suspected Unexpected Serious Adverse Reactions (SUSAR)

SUSARs are suspected adverse reactions related to an investigational medicinal product that are both unexpected and serious.

3.6.4.3 Method for the reporting of Adverse Events

Serious Adverse Events: were collected in the CRF and a SAE form completed for each one. These were held in the site file and formed part of the Annual Safety Report which was sent to the MHRA, Ethics and the sponsor.

Serious Adverse Reactions (SARs): are reactions judged by the chief investigator to be related to the study drug, although listed in the protocol as expected drug reactions. These were collected in the CRF and a SAE form completed for each one. All SARs were held in the site file and formed part of the Annual Safety Report sent out as above.

Suspected Unexpected Serious Adverse Reactions: are reactions judged by the chief investigator to be related to the study drug, and are *unexpected* study drug reactions according to the protocol. If they occur, they are collected in the CRF and an SAE form completed for each one. SUSARs should be sent to the MHRA, ethics committee and the sponsor within 7 days for all fatal or life-threatening SUSARs and 15 days for all others.

3.6.5 Clinical Trial Obligations

3.6.5.1 Ethics and the MHRA

In 2004, the introduction of EU directive 2001/20/EC means that in order to conduct a clinical trial of a medicinal product, the following must be obtained:

3.6.5.2 Sponsorship

A sponsor is an individual, company, institution or organisation which takes responsibility for the initiation, management or financing of a clinical trial. For this trial, the North Glasgow University Directorate NHS Trust Research and Development agreed to act as sponsor.

3.6.5.3 Ethical approval

An ethics committee is an independent body consisting of health care professionals and non-medical members. The function of an ethics committee is to provide an opinion before a clinical trial starts having been given details of the following particulars:

• The relevance of a clinical trial and the trial design

• Evaluation of the expected benefits and risks

• The protocol

• Suitability of the investigator and supporting staff

• The investigator's brochure

• The quality of facilities available

• The process for obtaining informed consent and the alternative procedures for doing so on behalf of those incapable of giving informed consent

• Details of any indemnity or compensation in the event of injury or death attributable to the clinical trial

• Insurance to cover the liability and the investigator and sponsor

• Details of payments to be made to investigators and participants in the trial

• Arrangements for the recruitment of clinical trial subjects

Only if the opinion of the ethics committee on all the above points is favourable will the trial be permitted to start. The legislation requires that the decision from the ethics committee must be supplied within 60 days of the date of receipt of the application. If the ethics committee requires further information to make a final judgment as to its opinion, the time taken for that further information to be supplied by the applicant is not included in the 60-day schedule.

To ensure the effective functioning of ethics committees, a new UK Ethics Committee Authority (UKECA) has been created to establish, recognise and monitor ethics committees. The executive procedures of the UKECA are carried out by the Central Office of Research Ethics Committee (COREC).

Ethics committee approval was granted on the 18th January 2005.

3.6.5.4 MHRA Approval

Applying to the MHRA for Clinical Trial Approval (CTA) is a complex undertaking. The amount of information required for the application was substantial. With assistance from Karen Dunlop at Pfizer, we eventually, in March 2005, submitted:

Application form

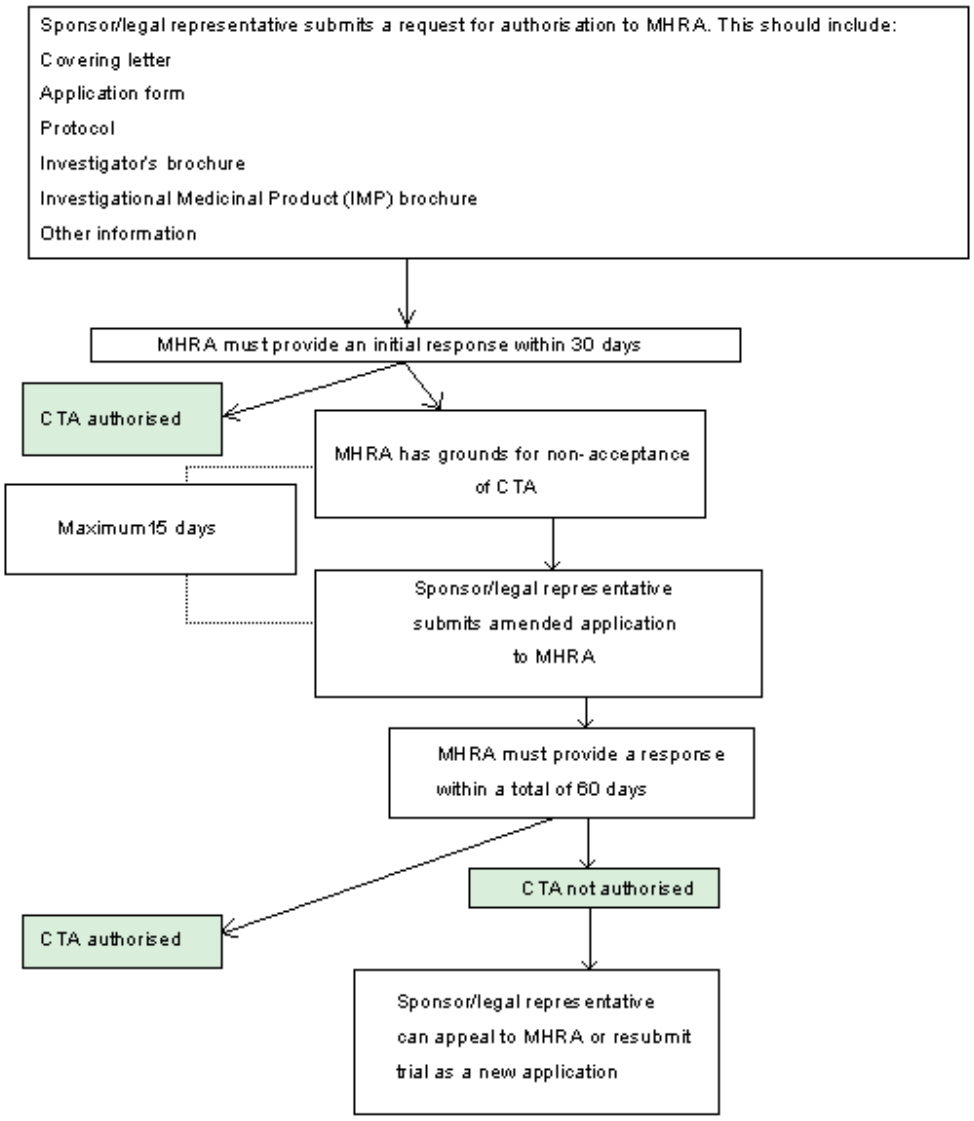
Protocol

Investigational Medicinal Product (IMP) brochure

The MHRA then has 30 days to consider its response, during which time it may ask one lot of questions. If it does so, the 30 day clock is "on hold" until a reply is obtained (figure 3.5).

We received no questions, and on day 30 (9th April 2005), we received permission to proceed.

Figure 3.5 Flowchart for Application to MHRA for Clinical Trial Authorisation (CTA)



3.6.5.5 Amendments

Where a change must be made to the protocol, this must first be approved by the ethics committee and the MHRA, unless the change relates to patient safety, in which case the amendment may be submitted after the change has taken place. Normally the MHRA will issue approval subject to ethics committee agreement. Copies of both agreements are then forwarded to the sponsor. Patients were asked to re-consent if there were any amendments to the protocol.

3.6.5.6 Annual Safety Reports

It is the legal responsibility of the sponsor to submit, once a year throughout the clinical trial, (or on request), a safety report to the MHRA and the Ethics Committee, taking into account all new available safety information produce along the reporting period. Safety reports were issued on the 18th January 2006 and at the close of the study on the 27th February 2007.

3.6.5.7 Other obligations

3.6.5.7.a Trial registration

In line with recent requirements by International Committee of Medical Journal Editor (ICMJE) journals [216 - 218], clinical trials hoping for future publication are required to be registered on an international, searchable database. In addition, this database must hold specific information about the trial, as specified by a WHO registration advisory group [219] (see table 3.2, below). For trials recruiting after the 1st of July 2005, the deadline for this

registration was the 13th September 2005 [219]. Trials must be registered before the first patient is recruited [218]. Accordingly, the trial was registered with www.clinicaltrials.gov on 30th June 2005. Clinicaltrials.gov was established by the US National Institute of Health and is one of the largest research databases currently in existence [216].

Table 3.2 Minimum required information for Clinical Trial Database, as defined by the ICMJE [219]

Item	Comment
Unique trial number	The unique trial number will be established by the primary registering entity (the registry)
Trial registration date	The date of registration will be established by the primary registering entity
Secondary IDs	May be assigned by sponsors or other interested parties (there may be none)
Funding source(s)	Name of the organization(s) that provided funding for the study
Primary sponsor	The main entity responsible for performing the research
Secondary sponsor(s)	The secondary entities, if any, responsible for performing the research
Responsible contact person	Public contact person for the trial, for patients interested in participating
Research contact person	Person to contact for scientific inquiries about the trial
Title of the study	Brief title chosen by the research group (can be omitted if the researchers wish)
Official scientific title of the study	This title must include the name of the intervention, the condition being studied, and the outcome (e.g. The International Study of

Item	Comment
	Digoxin and Death from Congestive Heart Failure)
Research ethics review	Has the study at the time of registration received appropriate ethics committee approval (yes/no)? (It is assumed that all registered trials will be approved by an ethics board before commencing.)
Condition	The medical condition being studied (e.g. asthma, myocardial infarction, depression)
Intervention(s)	A description of the study and comparison/control intervention(s). (For a drug or other product registered for public sale anywhere in the world, this is the generic name; for an unregistered drug the generic name or company serial number is acceptable.) The duration of the intervention(s) must be specified
Key inclusion and exclusion criteria	Key patient characteristics that determine eligibility for participation in the study
Study type	Database should provide drop-down lists for selection. This would include choices for randomized vs. nonrandomized, type of masking (e.g. double-blind, single-blind), type of controls (e.g. placebo, active), and group assignment, (e.g. parallel, crossover, factorial)
Anticipated trial start date	Estimated enrolment date of the first participant
Target sample size	The total number of subjects the investigators plan to enroll before closing the trial to new participants
Recruitment status	Is this information available (yes/no)? (If yes, link to information.)
Primary outcome	The primary outcome that the study was designed to evaluate. Description should include the time at which the outcome is

Item	Comment
	measured (e.g. blood pressure at 12 months)
Key secondary outcomes	The secondary outcomes specified in the protocol. Description should include time of measurement (e.g. creatinine clearance at 6 months)

3.6.5.7.b Confidentiality

All investigators ensured that patient confidentiality was maintained at all times adhering to the Data Protection Act (1998) and according to the Clinical Trials Directive 2001/20/EC 'Good Clinical Practice' (G.C.P) guidelines.

3.6.5.7.c Monitoring of the study

The study was intended to be monitored within 6 months of the first patient being recruited. However, due to staffing constraints within the Research and Development, full monitoring was delayed until July 2007. An audit of documentation was carried out in April 2006. 10% of all patient information documented in the case report file was checked and verified for completeness and adherence to the protocol. The Chief Investigator and the research team reviewed recruitment on a fortnightly basis.

4. METHODOLOGY

4.1 Asthma-specific measurements

4.1.1 Diary card recordings

A diary card was used (Appendix 1) to measure asthma symptoms [220], PEF recordings and inhaled $beta_2$-agonist use. PEF measurements were be undertaken by patients at home using a mini-Wright peak flow meter (Clement Clarke, Harlow, UK). On return visits, the diary was analysed for variability and exacerbations (see below). The best of three measurements was recorded twice daily (prior to treatment with salbutamol) in the diary. Peak flow variability was calculated from the difference between the highest and lowest daily reading divided by the mean PEF reading multiplied by 100 (amplitude % mean).

One of the reasons that PEF is collected in clinical trials is that for the individual patient, regular daily measurements often provide the clinician with a much clearer picture of the patient's clinical status that does a single measure of FEV_1 or PEF made in the clinic. Frequent measurement of PEF provides valuable information about diurnal variation in airway calibre and evidence of day-to-day fluctuation in the patient's status [221].

4.1.1.1 Asthma quality of life (AQLQ)

Quality of life was assessed using the Juniper Asthma Quality of Life Questionnaire [222], before and after taking each medication (on visits 2, 5, 6 and 9). A sample of this questionnaire is in Appendix 2.

4.1.1.2 Asthma Control

Asthma control was assessed using the Juniper Asthma Control Questionnaire (ACQ) [43] on each visit. The patient answered the first 6 questions, and the final question was completed by a member of the research team from spirometry. A sample of this questionnaire is in Appendix 3.

4.1.2 Spirometry (FEV_1, FVC), reversibility testing

Baseline pre-bronchodilator spirometric measurements were recorded from the best of three attempts using a dry wedge spirometer (Vitalograph, Buckingham, UK) (figure 4.1), with measurements not varying by more than 5% or 0.2 mL. Spirometric measurements were made before and after nebulised salbutamol (2.5 mg). Measurements were performed at the same time of day (am or pm) for each patient.

Figure 4.1 Spirometry

4.1.3 Asthma severity

Asthma severity, or impairment, is defined by physiological and clinical parameters [223]. The degree of impairment is calculated as the sum of the scores for post-bronchodilator FEV_1, reversibility of FEV_1, or PC_{20}, and medication need:

- Postbronchodilator FEV_1: this is scored from zero (no evidence of airflow limitation) to 4 (severe degree of airflow limitation, FEV_1 < 50% predicted).
- Reversibility to salbutamol or hyperresponsiveness to methacholine (defined as PC_{20} <8 mg/mL: scored from zero (reversibility <10% or PC_{20}>8 mg/mL) to 3 (reversibility >30% or PC_{20}<0.125 mg/mL).
- Minimum medication needed to control symptoms: from none (scored zero) to high dose systemic steroid daily (scored 4).

4.1.4 Exacerbations of asthma and Adverse Events

Mild exacerbation was defined as one of the following for 2 consecutive days:

- a drop in PEF > 20% below baseline value,
- use of more than 3 additional puffs of reliever bronchodilator over 24 hours (excluding prophylactic puffs for exercise) as compared with baseline value, or
- night awakening due to asthma.

A severe exacerbation was defined as:

- any worsening of asthma control considered by the investigator or GP to require a short course of oral corticosteroids/hospitalization, or

- decrease in morning PEF to more than 30% below the baseline value on 2 consecutive days [224].

The following were monitored to assess adverse events:

- Emergency or "out of hours" visit of patients to the GP;
- GP visit to patient at home;
- GP or investigator prescribing extra treatment;
- A & E hospital attendance;
- Hospital admission and length of stay.

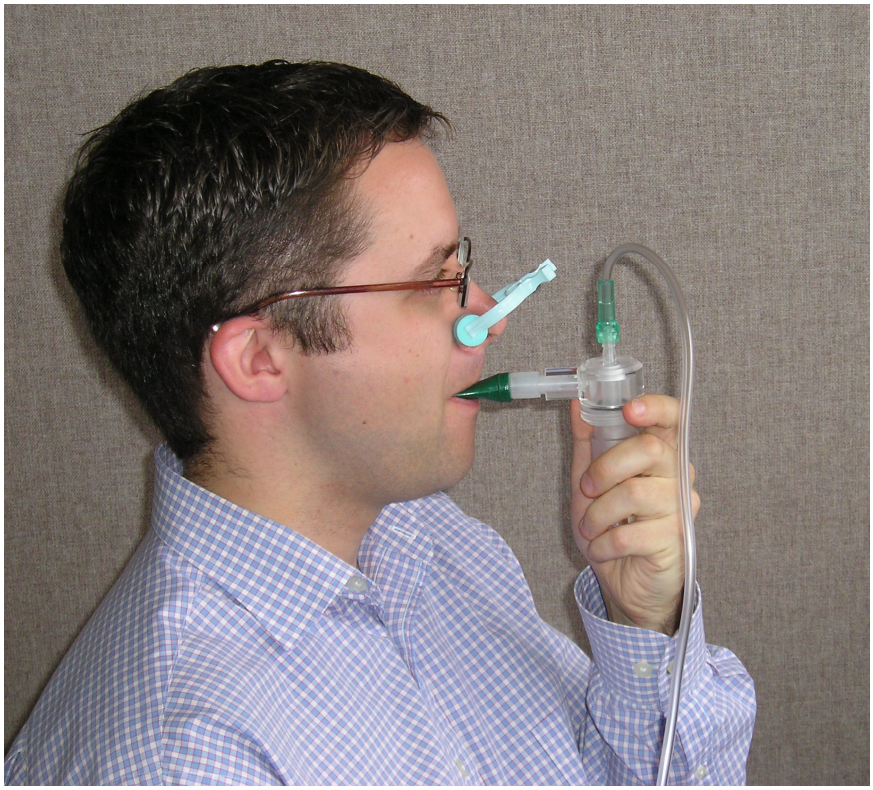
Patients recorded events that they would consider "out of the ordinary" for them, such as headaches, nausea or muscle cramps, that might be attributable aside-effects of the medications. These were also documented in the adverse events section.

4.1.5 Airway responsiveness

Recent guidelines for bronchial challenge testing with methacholine have been published [225]. The tidal breathing method described is a version of Cockcroft's technique [226], which has been shown to produce slightly better responses that the alternative breath-hold with dosimeter method [227]. The nebuliser was calibrated before the study began (Appendix 4). After initial spirometry, patients received 2 minutes of nebulised 3% saline. Further spirometry was then performed. Patients received 2 minutes of increasing concentrations of methacholine, from 0.03mg/mL to 16 mg/mL. After each period, spirometry was preformed at 30 seconds and 90 seconds, with a third test at 180

seconds if a fall in FEV_1 was recorded (figure 4.2). Once a drop in FEV_1 of 20% from the lowest post-saline FEV_1 was recorded, all measurement stopped and the patient was given a nebuliser of salbutamol to reverse the effects of the methacholine. The patient was then monitored until the FEV_1 returned to normal.

Figure 4.2 Methacholine administration



Methacholine hyperreactivity is measured by the PC_{20}, a function of the concentration required to bring about a drop in FEV_1 of 20%. This is calculated using the following equation:

$$PC_{20} = \text{antilog}\left\{ \frac{(\log C_2 - \log C_1)(20-R_1)}{\log C_1 + R_2 - R_1} \right.$$

Where

C_1 = second to last methacholine concentration (i.e. the concentration preceding C_2)

C_2 = final methacholine concentration (the concentration resulting in a 20% or greater fall in FEV1)

R_1 = percent fall in FEV_1 after C_1 and

R_2 = percent fall in FEV_1 after C_2

This was inserted into a Microsoft Excel package for ease of calculation:

Post Saline FEV_1 (mL)	**2nd last FEV_1 (mL)**	**Last FEV_1 (mL)**	**Concentration at 2nd last FEV_1 (mg/mL)**	**Concentration at last FEV_1 (mg/mL)**
3190	2930	2520	4	8

1st % fall	**2nd % fall**	**PC_{20} (mg/mL)**
8.15047	21.0031	7.5787

A value of methacholine hyperreactivity >16 mg/mL was read as 16 mg/mL. Bronchial hyper-reactivity is defined as a PC_{20} FEV_1 of <8 mg/mL, so patients who had a level below this were suitable for inclusion in the trial. Methacholine challenge testing was

only performed in subjects who had a baseline FEV_1 of greater than 60% predicted. Patients who had a baseline FEV_1 lower than this were still included in the trial, but without methacholine testing.

4.2 Measurement of atopy

Atopy is the predisposition to produce IgE antibody to common aeroallergens. This can be identified and quantified by either skin-prick testing or serum immunoassay. Allergen skin prick tests give a more sensitive and more repeatable diagnosis of atopy than measurement of serum IgE antibody [228, 229]. At the screening visit, skin prick tests for allergy to cat dander, house dust mite (*Dermatophagoides farinae*) and mixed grass pollen (wheat, timothy grass, foxtail grass, rye-grass, meadow grass, cocksfoot) was performed (Soluprick, ALK, Horsholm, Denmark), with a positive control (histamine) and a negative control (saline and glycerine) [230]. A positive result was a wheal reaction after 15 minutes of 3mm greater than the control wheal diameter [231].

In the event of a negative skin prick test, but a strong history of atopy (e.g. hay fever), a serum sample was sent to the hospital immunology laboratory for IgE antibody testing.

In addition, serum samples from each patient were saved for batch analysis at the end of the study. Total IgE and specific IgE to house dust mite, and grass pollen were assayed by automated fluorescent-immunoassay (Unicap 100, Pharmacia UK, Ltd, Milton

Keynes, UK,). Total IgE >120 International Units/L and specific IgE >0.35 Arbitrary Units/L were considered positive.

4.3 Induced sputum

Sputum was induced using an ultrasonic nebuliser (Sonix 2000, Medic Ltd, Harlow, Essex, UK), initially filled with 3% saline. Subjects inhaled the nebulised solution for 7 minutes (nebuliser output 0.9 mL/min; mass median diameter 5.5 micrometer) following pre-treatment with 2.5mg nebulised salbutamol. After this, the nebuliser was filled with 4% saline and the nebulised solution inhaled for a further 7 minutes (figure 4.3). Finally, the nebuliser was filled with 5% saline and the nebulised solution was inhaled for a final 7 minutes. At each 7-minute interval the inhalation was stopped to allow expectoration into a polypropylene container, and for spirometry checks. The test was not performed on subjects with an FEV_1 less than 1 L. If FEV_1 fell by 20% or more, or if troublesome symptoms occurred, the test was stopped. If the FEV_1 fell by 10-20%, the concentration of saline was not increased. The sample was kept on ice until processed for cell counts and centrifugation to harvest the soluble phase of sputum. This was always done within 2 hours [50]. Cell counts were performed and the supernatant stored for analysis of inflammatory mediators.

Figure 4.3 Induced sputum technique

4.3.1 Analysis of sputum

Analysis was performed in a procedure similar to that described by Popov *et al*[232]. Sputum was poured into a petri dish and mucous plugs were selected from surrounding saliva (figure 4.4). Plugs were transferred into a pre-weighed bijou bottle and weighed.

A 1:10 dilution of dithiothreitol (DTT) (Calbiochem, Merck Biosciences Ltd, Beeston): Phosphate buffered saline (PBS) (VWR International Ltd, Poole) 4 times the weight of sputum was added to the sample and mixed in. A further 4 times the original weight of sputum of PBS was then added. The sample was then passed through a sterile nylon

(nitex) mesh to filter out clumps, into a pre-weighed centrifuge tube, which was then weighed again. An aliquot of 20 μL was removed then diluted 1:1 with 0.1% trypan blue (Sigma Aldrich Company Ltd, Gillingham). A manual total cell count and a viability count were then performed using a haemocytometer (figure 4.5). Samples were assessed at this point and excluded if they failed to meet the criteria listed in table 4.1.

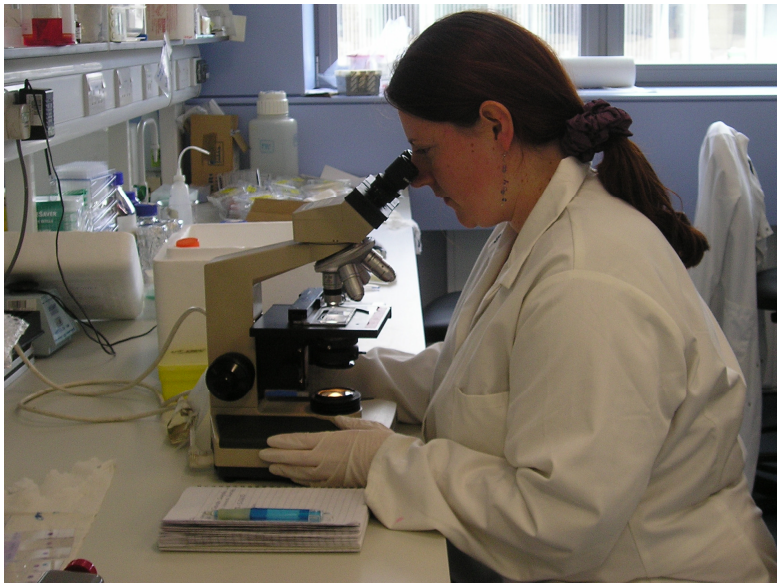
Table 4.1 Sputum cell count – criteria for exclusion of sample

Parameter	Threshold for exclusion
Cell viability	<40%
Total cells to be counted	<400
Proportion of squamous cells	>80%

The sample was then centrifuged at 1000 rpm for 10 minutes, at 4 °C. Multiple 1 mL aliquots of supernatant were stored at –20 °C. If only 1 mL was available, it was split into 2 x 0.5 mL.

Figure 4.4 Selection of mucus plugs

Figure 4.5 Cell counting



4.3.1.1 Differential cell counting by staining of cytocentrifuge cell smear preparations

The cell pellet was resuspended at a concentration of 0.6 x 10^6/mL in a volume of 200 μL which was kept on ice. 70 μL was pipetted into each funnel of a cytocentrifuge to obtain 7 x 10^4/mL, insert into a cytospin holder. 2 slides (VWR International Ltd, Poole) were prepared for cytospins, using filter cards, funnels and cytoclips (Thermo Electron Corporation, Basingstoke) as per manufacturer's instructions. They were then spun at 450 rpm for 6 minutes (Shandon Cytospin 4 Cytocentrifuge, Thermo Electron Corporation, Basingstoke), then air dried for 1 – 2 hours. Samples were then fixed in methanol for 10 minutes. Fixed slides were then stained using Romanowsky staining – Rapi Diff II Stain Pack (Triangle Biomedical Sciences Ltd, Skelmersdale).

A differential cell count of greater than 400 inflammatory cells was performed. In addition to this the squamous epithelial cell count was performed in order to estimate saliva contamination.

4.3.1.2 Analysis of sputum biomarkers

4.3.1.2.i Microbead fluorescent assay: Luminex technology

The inflammatory cytokines IL-1β, IL-6, IL-8, TNF-α, GM-CSF IL-1RA, IL-17, IFN-γ, MIP-1α and CCL2 were measured using an assay kit (Biosource, Invitrogen, Paisley, UK). The format of luminex allows the measurement of a combination of various

cytokines or other mediators at the same time in a small volume, which makes it particularly suitable for sputum samples.

Luminex technology consists of a uniform batch of polystyrene microbeads dyed internally with a combination of two fluorescent markers, which give this batch a unique spectral address for identifying that bead set on a fluorescent reader. Using different ratios of markers with different batches of beads allows them to be detected separately when they are mixed. A batch of beads with a characteristic marker identity is coated with a capture antibody specific for the cytokine or mediator of interest, thus each cytokine or mediator has a specific set of beads that can be identified simultaneously in a mix.

The beads are prepared by washing twice using the supplied buffer; after this, they are ready to use. The sputum supernatant samples are incubated with the antibody-coated beads on an orbital shaker for 2 hours. Internal quality control and quantification is achieved using standard samples containing known amounts of these mediators. After this time, any unbound sample is washed from the well by washing 3 times with the supplied wash buffer, which is aspirated using a vacuum manifold. A cocktail of cytokine-specific biotinylated detection antibodies are added and incubated for a further hour. The plate is the washed 3 times to remove any unbound detection antibody, and streptavidin conjugated to the fluorescent protein, R-phycoerythrin, is added then incubated for 30 minutes. An additional 3 washes later, the beads are analysed by

fluorimetry with the Bio-plex System instrument (Bio-Rad Laboratories, Hertfordshire, UK). As the analyte forms an essential component of the sandwich between the microbead and the fluorescent reporter dye, the amount of fluorescence associated with each bead set can be directly attributed to the concentration of cytokine in each sample. Multiple beads are read for each analyte giving a mean value of fluorescence intensity, which is directly proportional to the concentration of the cytokine or mediator in each sputum supernatant sample.

Occasionally while using this test, the beads become stuck together. This unpredictable occurrence means that the beads are thus unable to be counted properly. These results are discarded and not included in the final analysis.

Validity of this technique is examined using a technique known as "spiking"[233]. This involves adding a known amount of mediator to unprocessed sputum, processing the sputum as usual and then measuring recovery by immunoassay. Unspiked sputum is simultaneously processed and assayed so that percentage recovery can be calculated. This technique is however, extremely expensive and due to the limited resources of this study it was not possible to run spiking experiments specifically for these mediators. However, previous work in our department has shown good levels of recovery, consistent with those described by other centres[233, 234]. Despite this, measuring mediators in sputum supernatant remains difficult and controversial, and all results must be interpreted with considerable caution.

4.3.1.2.ii Enzyme-linked immunoassay, ELISA

4.3.1.2.ii.a Leukotriene (LT)B_4

This assay is based a competitive binding technique during which any LTB_4 present in a sample competes with a set amount of alkaline phosphatase-labelled LTB_4 conjugate for sites on a mouse monoclonal antibody (R&D Systems, Abingdon, UK). The microplate has been coated with goat anti-mouse antibody, and during incubation the mouse monoclonal antibody will become bound to the coating antibody. The sample and conjugate compete for binding sites on the antibody. Following a wash step to remove excess conjugate and any unbound sample, a substrate solution is added to the wells in order to determine the bound enzyme activity. Following colour development the absorbance is read immediately at 405 nm. As the assay is a competitive ELISA, the intensity of the colour in a given well is inversely proportional to the concentration of LTB_4 in that sample.

The sputum supernatants were diluted 1:2 with the supplied assay buffer before use in this assay. The microplate was marked into sections to contain the samples and standards, and wells for total activity, non-specific binding, maximum binding and substrate blank were also included. Assay buffer was added to all wells, excluding the wells reserved for total activity and substrate blank. The standards and samples were then added to the allocated wells, with the LTB_4 conjugate being added directly afterwards. The LTB_4 conjugate was not added to the total activity or substrate blank wells. LTB_4 antibody solution was then added to the wells, excluding the non-specific binding, total activity

and substrate blank wells. The plate was left to incubate at room temperature for 2 hours, on a horizontal orbital microplate shaker. A wash removed any unbound sample or excess conjugate. At this stage, LTB_4 conjugate was added to the total activity wells. The substrate para-nitrophenyl phosphate was added, and allowed to incubate for 1 hour at room temperature on the benchtop. When the colour had developed sufficiently, a solution of 1N sulphuric acid stopped the reaction and stabilized the colour in each well, and the plate was read immediately.

A standard curve was generated which allowed the calculation of the concentration of LTB_4 in each sample, after the non-specific binding optical density was subtracted from each result.

LTB_4 is a competitive ELISA, and requires the use of various internal controls mentioned above (total activity, non-specific binding, and substrate blanks) in duplicate, as the manufacturer states that results are only valid in the presence of these controls. This meant that a smaller total number of samples could be run in the analysis, so not all patient samples could be included.

4.3.1.2.ii.b Myeloperoxidase

Myeloperoxidase (MPO) was measured using a test kit developed for the quantitative measurement of natural human MPO (Cambridge Bioscience, Cambridge, UK). The assay was based on the sandwich ELISA technique. The sputum supernatants were

diluted 1:5 with the supplied dilution buffer before use in this assay. Samples and standards are incubated in microtitre wells coated with antibodies recognising human MPO. During this incubation step, the solid-bound antibody captures any MPO present in the sample. Unbound material present in the sputum supernatant sample is removed by washing. Biotinylated second antibody to human MPO is then added to the wells. If human MPO was present in the sample, the biotinylated-antibodies will bind to the captured MPO. Excess biotinylated-antibodies are removed by washing. Streptavidin-peroxidase conjugate is applied to the wells. This conjugate reacts specifically with the biotinylated-antibody bound onto the detected MPO. Excess streptavidin-peroxidase conjugate is removed by washing, and the substrate, tetramethylbenzidine is added to the wells. A coloured product develops, and the amount of colour that forms is directly proportional to the concentration of MPO present in the sample. The enzymatic reaction is stopped by the addition of citric acid and the absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance values versus the corresponding concentrations of defined standards.

4.4 Measurement of exhaled Nitric Oxide

Exhaled NO (FE_{NO}) is detected using chemiluminescence and detection depends on the photochemical reaction between NO and ozone generated in the analyser [235]. The specificity of exhaled NO measurements by chemiluminescence has been confirmed by gas chromatography-mass spectrometry [236]. FE_{NO} was measured on a chemiluminescence analyser (LR2149, Logan Research Ltd., Rochester, Kent). In 2005 the American

Thoracic Society and European Respiratory Society published joint guidelines for measurement of FE_{NO} [49]. These superseded previous guidelines [237, 238].

Measurement of FE_{NO} requires slow expiration against resistance, which creates back pressure to close the soft palate and thus eliminates contamination of exhaled NO by nasal NO [239] (figure 4.6).

Figure 4.6 Exhaled nitric oxide testing

4.5 Allergen-driven blood lymphocyte proliferative response in vitro.

Peripheral blood mononuclear cells were separated by density gradient centrifugation over lymphoprep, ficol-isopaque SG 1.088. The mononuclear cells were harvested, washed, counted and at 10^6 cells/mL cultured in vitro with a growth medium of RPMI, 10% autologous plasma, under penicillin and streptomycin cover for 3 days with or without mitogen (phytohaemagglutinin, PHA), or solid-phase anti-CD3/28 bound onto microbeads, in a humidified atmosphere at 37 °C with 5% CO_2. Proliferation was measured by incorporation of tritiated thymidine (0.5 uCi) for 16 hours before harvest onto glass-fibre filters and counting in a beta counter.

4.6 Other immunological tests in blood

Serum was analysed for C-reactive protein (CRP) concentration in the Routine Lipids section of the Biochemistry Department of Glasgow Royal Infirmary (high sensitivity assay, Roche/Hitachi, Roche Diagnostics GmbH, Mannheim, Germany). High sensitivity commercial ELISA kits were used for a variety of serum mediators according to manufacturers' instructions (IL-6 and TNF-α, Quantikine; sICAM-1, Parameter; both R & D Systems Europe Ltd, Abingdon).

4.7 Measurement of renal and liver safety parameters, and lipids in blood

Routine biochemistry laboratory samples were sent to the hospital laboratory for screening, at the end of treatment periods A and B, and before starting treatment period B.

Blood for renal function (urea and electrolytes) and liver function tests (LFTs) were performed before and after each treatment period and creatinine kinase (CK) was performed at baseline. If a patient complained of muscle pain or excessive muscle fatigue, CK was repeated at that time. Cholesterol, triglycerides and HDL-cholesterol were measured in the Gartnavel General Biochemistry Laboratory at screening, at the end of treatment periods A and B, and before starting treatment period B.

4.8 Equivalent steroid dose

Equivalent steroid dose was calculated according to GINA guidelines [1] (see table 4.2).

Patients with a beclomethasone equivalent of 1000mcg or less were eligible for the trial.

Table 4.2 Equivalent inhaled corticosteroid doses (from GINA [1])

Drug	Multiplication factor to calculate beclomethasone equivalent
Beclomethasone	1
Budesonide	1
Qvar	2
Fluticasone	2

5. RESULTS

5.1 Introduction

4303 patients were invited to participate in the trial, from 39 practices and hospital outpatients. Of these, 439 expressed an interest in taking part. 140 were excluded prior to clinical screening by telephone, for reasons such as pregnancy or lactation, current statin medication, diagnoses of COPD, non-attendance at appointments or because they were unable to be contacted. Of the 137 subjects that attended screening at Gartnavel General Hospital, 54 were recruited to the trial between August 2005 and August 2006 (table 5.1).

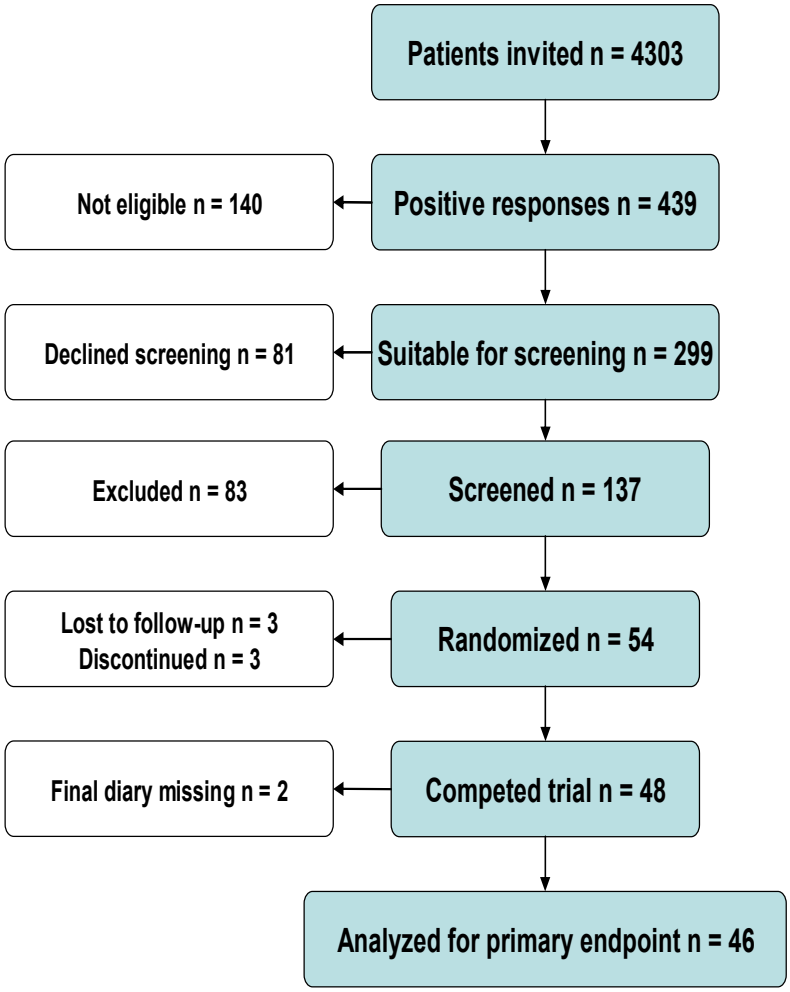
Table 5.1 Reasons for exclusion of screened patients from study

Reason for exclusion from study	Number of patients
Withdrew or unable to provide consent	20
Negative allergy test	24
Negative spirometry	16
Unsuitable medication	3
Declined to take part	20

5.2 Flow of participants

The progress of participants through the trial is shown in the CONSORT diagram (figure 5.1).

Figure 5.1 CONSORT diagram showing flow of patients through study



5.3 Baseline characteristics

5.3.1 Demographics

Baseline demographic characteristics of the 54 subjects who underwent randomisation were similar (table 5.2).

Patients taking atorvastatin before placebo were younger than the group taking placebo first, mean age 39.4 years compared with 45.9 years. This difference was non-significant (p=0.082). There were equal numbers of women in both groups (42.9% vs. 42.3%) and all patients were white. 10.7% in the atorvastatin and 11.5% in the placebo group were former smokers. The mean duration of asthma was 24.9 years.

Mean alcohol consumption was 8.2 units per week, this was similar in both groups. 88.9% of patients had positive IgE serology (total IgE >120 IU/L or specific IgE for house dust mite or grass pollen >0.35 IU/L), similar in both groups (85% for atorvastatin, 92.3% for placebo). Finally, mean equivalent beclomethasone dose was 476 mg (SD 283), with no significant differences between groups.

Table 5.2: Demography at baseline mean (SD) in all patients, and sub divided into those randomised to received atorvastatin then placebo, and those randomised to receive placebo then atorvastatin

Variable	Total (n=54)	Atorvastatin > Placebo (n=28)	Placebo > Atorvastatin (n=26)	t-test (p value)
Male sex, n (%)	23 (42.59)	12 (42.9)	11 (42.3)	0.968
Ex-smoker, n (%)	6 (11.11)	3 (10.7)	3 (11.5)	0.923
Age in years (SD)	42.5 (13.7)	39.4 (13.4)	45.9 (13.5)	0.082
Asthma duration years (SD)	24.9 (16.6)	24.1 (15.0)	25.7 (18.4)	0.730
Equivalent beclomethasone dose, mg (SD)	476 (283)	441 (309)	513 (251)	0.351
Positive IgE testing, n (%)	48 (88.9)	24 (85.7)	24 (92.3)	0.438
Alcohol units (SD)	8.2 (8.0)	9.1 (8.3)	7.2 (7.7)	0.387

5.3.2 Baseline outcome measures

5.3.2.1 PEF

Baseline PEF measurements of randomised patients are listed in table 5.3. Mean (SD) morning PEF measurements were 390.3 L/min (103.5) (Table 5.3). Values in the group commencing on atorvastatin and placebo were similar (395.6 L/min (107.8) vs. 384.6 L/min (100.5)). Evening PEF measurements were also similar (overall mean 403.3 L/min (101.4), atorvastatin 408.9L/min (109.4), placebo 397.4 L/min (93.9)).

Table 5.3 Baseline PEF

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
Morning PEF (L/min)	54	390.3	103.5	28	395.6	107.8	26	384.6	100.5
Evening PEF (L/min)	52	403.3	101.4	27	408.9	109.4	25	397.4	93.9

5.3.2.2 Reliever inhaler use

Baseline use of reliever inhalers was similar between groups. Total mean (SD) number of puffs of reliever inhaler was 2.3 (2.0).

Table 5.4 Baseline reliever inhaler use

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
Puffs of reliever inhaler used daily	51	2.3	2.0	27	2.1	2.1	24	2.5	2.0

5.3.2.3 Asthma severity

Mean (SD) ATS severity score was 3.3 (1.3) (Table 5.5). There was no significant difference in score between groups.

Table 5.5 Baseline ATS severity score

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
ATS severity score	54	3.3	1.3	28	3.1	0.9	26	3.5	1.5

5.3.2.4 Asthma Control Questionnaire (ACQ) Score

Mean (SD) ACQ score was 1.5 (0.8) at baseline (Table 5.6). There was no significant difference between groups.

Table 5.6 Baseline Asthma Control Score

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
ACQ	54	1.5	0.8	28	1.5	0.8	26	1.6	0.8

5.3.2.5 FEV_1

Table 5.7. Mean (SD) FEV_1 pre-salbutamol was 2.78 L (0.85) with no difference between groups, mean post-salbutamol was 3.18 L (0.84). This equates to a mean reversibility of 14.9% (11.7). Mean percentage predicted $FEV_1$was 85.7% (19.3), comparable between groups.

Table 5.7 Baseline FEV_1 and reversibility

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
FEV_1 pre-salbutamol (L)	54	2.78	0.85	28	2.96	0.91	26	2.59	0.74
FEV_1 post-salbutamol (L)	54	3.18	0.84	28	3.33	0.98	26	3.02	0.65
Predicted FEV_1 (%)	54	85.7	19.3	28	86.9	17.3	26	84.4	21.6
FEV_1 reversibility (%)	54	14.9	11.7	28	13.3	9.4	26	17.0	13.8

5.3.2.6 PEF (spirometry)

Mean (SD) PEF when measured at the clinic by spirometry was 426.6 L/min (130.4) pre-salbutamol, and 499.8 L/min (133.4) post-salbutamol. Mean percentage predicted PEF (pre-salbutamol) was 92.3% (22.9), which was similar in both groups (Table 5.8).

Table 5.8 Baseline PEF (spirometry)

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
Spirometry PEF pre-salbutamol (L/min)	54	426.6	130.4	28	434.2	122.2	26	418.3	140.8
Spirometry PEF post-salbutamol (L/min)	54	499.8	133.4	28	505.9	138.9	26	493.3	129.7
Predicted PEF (%)	54	92.3	22.9	28	91.7	19.2	26	93.0	26.7

5.3.2.7 FVC

Mean (SD) FVC pre-salbutamol was 3.70L (1.10), post-salbutamol was 4.03L (1.04).

Mean percentage predicted FVC was 95.8% (20.2) (Table 5.9).

Table 5.9 Baseline FVC

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
FVC pre-salbutamol (L)	54	3.70	1.10	28	3.98	1.26	26	3.41	0.82
FVC post-salbutamol (L)	54	4.03	1.04	28	4.20	1.23	26	3.85	0.80
Predicted FVC (%)	54	95.8	20.2	28	98.5	20.6	26	92.8	19.6

5.3.2.8 FEF_{25-75}

Mean (SD) FEF_{25-75} pre-salbutamol was 2.35 L/sec (1.09) (Table 5.10). Post-salbutamol was 2.87 L/sec (1.11). Mean percentage predicted FEF_{25-75} was 60.6% (25.0), which was comparable between groups.

Table 5.10 Baseline FEF_{25-75}

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
FEF_{25-75} pre-salbutamol (L/sec)	54	2.35	1.09	28	2.40	1.04	26	2.23	1.15
FEF_{25-75} post-salbutamol (L/sec)	54	2.87	1.11	28	3.03	1.18	26	2.70	1.04
Predicted FEF_{25-75} (%)	54	60.6	25.0	28	59.4	20.2	26	61.8	29.7

5.3.2.9 FEV_1/FVC ratio

Mean (SD) pre-salbutamol FEV_1/FVC ratio was 75.5% (9.8) (Table 5.11). Mean post-salbutamol FEV_1/FVC ratio was 79.1% (8.2).

Table 5.11 Baseline FEV_1/FVC ratio

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Mean	Std Dev	n	Mean	Std Dev	n	Mean	Std Dev
FEV_1/FVC ratio pre-salbutamol (%)	54	75.5	9.8	28	75.2	8.8	26	75.7	10.9
FEV_1/FVC ratio post-salbutamol (%)	54	79.1	8.2	28	79.6	8.0	26	78.6	8.6

5.3.2.10 Methacholine responsiveness

Geometric mean (SD) methacholine responsiveness as measured by PC_{20} was 2.23mg/mL (3.65) (Table 5.12). Initial results were slightly lower in the atorvastatin group compared with the placebo group [1.95 mg/mL (4.03) vs. 2.69 mg/mL (4.23)].

Table 5.12: Baseline methacholine responsiveness

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Geometric mean	Std Dev	n	Geometric mean	Std Dev	n	Geometric mean	Std Dev
Methacholine responsiveness (PC_{20}, mg/mL)	43	2.23	3.65	22	1.95	4.03	21	2.69	4.23

5.3.2.11 Asthma Quality of Life Questionnaire (AQLQ)

AQLQ score was similar between groups at baseline (Table 5.13). Total score was 5.55 (range 1-7). Total score and sub-scores of symptoms (5.38), activities (5.88), emotional well-being (5.39) and environmental quality of life (5.34) showed no obvious differences between groups.

Table 5.13 Baseline AQLQ scores (One patient in the placebo arm completed their questionnaire incorrectly).

Variable	Total			Atorvastatin > Placebo			Placebo > Atorvastatin		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
AQLQ	53	5.75	5.03, 6.19	28	5.77	4.92, 6.17	25	5.75	5.09, 6.28
AQLQ- symptoms	53	5.38	4.83, 6.08	28	5.50	4.58, 6.08	25	5.67	5.33, 6.08
AQLQ - activities	53	5.88	5.50, 6.45	28	6.18	5.38, 6.45	25	6.27	5.55, 6.55
AQLQ - emotions	53	5.39	4.60, 6.40	28	5.70	4.4, 6.3	25	5.80	4.80, 6.60
AQLQ - environment	53	5.34	4.75, 6.25	28	5.63	4.63, 6.13	25	5.50	4.75, 6.25

5.3.3 Inflammatory markers

The baseline values for various inflammatory markers in the groups randomised to start with either atorvastatin or placebo are described below.

5.3.3.1 Sputum markers

5.3.3.1.a Sample quality

Median total filtrate volume was 18.5 mL (IQR 8.0 to 35.5). This was similar between groups. Additionally, indicators of sputum quality such as total cells recovered (2 x10^6/mL, 0.9 to 6.7), and viability (77.0%, 65.5 to 85.5), were also comparable (Table 5.14).

Table 5.14 Baseline sputum sample quality. The lower number of samples in absolute number of cells counted represents samples discarded due to inadequate quality, as discussed in the methodology section.

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
Filtrate volume (mL)	36	18.5	8.0, 35.5	19	20.0	8.0, 41.0	17	17.0	8.0, 35.0
Total number of cells recovered from sample (x10^6/mL)	36	2.0	0.9, 6.7	19	1.8	0.9, 4.8	17	2.7	0.9, 7.0
Viability (%)	36	77.0	65.5, 85.5	19	75.0	67.0, 85.0	17	78.0	64.0, 88.0
Total cell count per ml sputum filtrate (x10^6/mL)	36	0.14	0.07, 0.28	19	0.14	0.07, 0.22	17	0.12	0.07, 0.34
Total viable cells recovered from sample (x10^6/mL)	36	1.4	0.8, 4.3	19	1.4	0.7, 3.0	17	1.9	0.9, 4.9
Absolute number of cells counted per slide	30	576	501, 643	16	581	520, 671	14	566	481, 609

5.3.3.1.b Cell counts

No significant difference was seen between groups for all cell counts (Table 5.15). Median macrophages were 196.5 x10^4 cells per slide (IQR 101.3 to 235.0). Neutrophils were 129.8 x10^4 cells per slide (60.0 to 186.5), eosinophils were 6.4 x10^4 cells per slide (2.0 to 16.3) and lymphocytes were 2.5 x10^4 cells per slide (1.0 to 5.5). Bronchial epithelial cells accounted for 44.0 x10^4 cells per slide (12.0 to 60.3) and total non-squamous cells were 405.3 x10^4 cells per slide (401.5 to 410.0).

Table 5.15 Baseline values for sputum cell counts

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
Macrophages ($x10^4$ cells)	30	196.5	101.3, 235.0	16	191.0	167.3, 217.0	14	213.0	95.5, 254.0
Neutrophils ($x10^4$ cells)	30	129.8	60.0, 186.5	16	129.8	57.5, 177.8	14	146.0	66.0, 268.0
Eosinophils ($x10^4$ cells)	30	6.4	2.0, 16.3	16	4.9	1.3, 18.9	14	6.4	4.0, 13.5
Lymphocytes ($x10^4$ cells)	30	2.5	1.0, 5.5	16	2.6	1.6, 8.3	14	2.5	1.0, 4.5
Bronchial epithelial cells ($x10^4$ cells)	30	44.0	12.0, 60.3	16	50.8	23.5, 71.1	14	19.5	11.5, 50.0
Total non-squamous cells ($x10^4$ cells)	30	405.3	401.5, 410.0	16	407.8	402.5, 413.3	14	403.5	401.5, 409.5
Squamous epithelial cells ($x10^4$ cells)	30	178.3	110.5, 257.0	16	181.3	118.0, 269.5	14	173.5	86.0, 212.0

5.3.3.1.c Cell count proportions

There was no significant difference between patients starting with atorvastatin and those starting with placebo for any of the different cell types in sputum analysis (Table 5.16).

Median macrophages represented 49.5% (IQR 30.5 to 57.5) of total cell count. Neutrophils accounted for 35.5% (14.5 to 48.3), eosinophils 1.5% (0.6 to 5.0), lymphocytes 0.7% (0.3 to 1.1) and bronchial epithelial cells 10.8% (3.0 to 16.0). Median squamous cells as a proportion of absolute cell count were 30.8% (20.8 to 38.5).

Table 5.16 Baseline values for sputum cell count proportions

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
Macrophage %	30	49.5	30.5, 57.5	16	47.3	40.5, 53.8	14	52.8	24.5, 61.5
Neutrophil %	30	35.5	14.5, 48.3	16	35.5	14.0, 43.8	14	35.8	14.8, 67.5
Eosinophil %	30	1.5	0.6, 5.0	16	1.1	0.3, 5.0	14	2.0	0.8, 3.0
Lymphocyte %	30	0.7	0.3, 1.1	16	0.8	0.5, 1.9	14	0.6	0.3, 1.0
Bronchial epithelial cells %	30	10.8	3.0, 16.0	16	12.5	5.5, 21.8	14	5.0	2.5, 13.5
Squamous cells as % of absolute count	30	30.8	20.8, 38.5	16	31.0	22.8, 39.8	14	30.0	20.0, 37.0

5.3.3.1.d Supernatant analysis

More variability was observed in the supernatant samples than other baseline values. Sputum LTB_4 was higher in the atorvastatin group than the placebo group [atorvastatin median 216.8 ng/mL (IQR 66.5 to 303.5) vs. placebo median 162.0 ng/mL (73.5 to 222.5), as was MPO [54.4 ng/mL (15.4 to 85.8) vs. 44.2 ng/mL (37.0 to 80.4)]. In contrast, IL-1b was higher in the placebo group [atorvastatin median 41.2 pg/mL (28.6 to

91.9) vs. 162.8 pg/mL (61.9 to 223.2), as was IL-1RA [22.8 ng/mL (13.3 to 40.7) vs. 41.2 ng/mL (25.8 to 52.6), IL-6 [30.6 pg/mL (15.0 to 59.2) vs. 91.9 pg/mL (21.7 to 161.2), IL-17 [15.9 pg/mL (3.6 to 49.9) vs. 97.7 pg/mL (32.8 to 159.7), TNF-α [7.7 pg/mL (2.8 to 17.7) vs. 43.3 pg/mL (9.2 to 61.0), IFNγ [9.7 pg/mL (3.3 to 23.9) vs. 40.8 pg/mL (19.2 to 85.5), GM-CSF [126.9 pg/mL (77.6 to 184.2) vs. 304.0 pg/mL (134.3 to 488.3), MIP1α [118.2 pg/mL (61.6 to 276.7) vs. 363.9 pg/mL (139.0 to 769.6), and CCL2 [195.1 pg/mL (123.2 to 368.5) vs. 332.5 pg/mL (220.7 to 616.9). Finally, IL-8 was similar between groups [1.4 ng/mL (0.7 to 9.6) vs. 2.0 ng/mL (1.0 to 4.7)] (Table 5.17).

Table 5.17 Baseline values for sputum supernatant inflammatory markers

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
LTB_4 (ng/mL)	37	179.0	73.5, 276.0	20	216.8	66.5, 303.5	17	162.0	73.5, 222.5
MPO (ng/mL)	37	47.8	32.6, 80.4	20	54.4	15.4, 85.8	17	44.2	37.0, 80.4
IL-1b (pg/mL)	37	76.4	38.9, 167.2	20	41.2	28.6, 91.9	17	162.8	61.9, 223.2
IL-1RA (ng/mL)	37	27.8	18.8, 47.0	20	22.8	13.3, 40.7	17	41.2	25.8, 52.6
IL-6 (pg/mL)	37	35.2	19.5, 105.6	20	30.6	15.0, 59.2	17	91.9	21.7, 161.2
IL-8 (ng/mL)	37	1.9	0.8, 5.8	20	1.4	0.7, 9.6	17	2.0	1.0, 4.7
IL-17 (pg/mL)	37	32.8	3.6, 137.9	20	15.9	3.6, 49.9	17	97.7	32.8, 159.7
TNF-α (pg/mL)	37	11.4	4.6, 46.5	20	7.7	2.8, 17.7	17	43.3	9.2, 61.0
IFN-γ (pg/mL)	37	19.8	4.9, 71.6	20	9.7	3.3, 23.9	17	40.8	19.2, 85.5
GM-CSF (pg/mL)	37	149.5	93.0, 367.9	20	126.9	77.6, 184.2	17	304.0	134.3, 488.3
MIP1α (pg/mL)	37	167.4	74.5, 483.8	20	118.2	61.6, 276.7	17	363.9	139.0, 769.6
CCL2 (pg/mL)	37	257.5	143.7, 477.3	20	195.1	123.2, 368.5	17	332.5	220.7, 616.9

5.3.3.2 Serum markers

No obvious differences were seen between groups for CRP [median 1.2 mg/L (IQR 0.6 to 2.6)], ICAM-1 [209.4 ng/mL (185.7 to 245.2)], TNF-α [1.1 pg/mL (0.7 to 1.8)], IL-6 [1.1 pg/mL (0.7 to 3.1)], IL-1 [101.0 pg/mL (7.6 to 172.1)] or IL-8 [14.3 pg/mL (0.6 to 24.0)]. IL-5 was higher in the atorvastatin group than the placebo group [median 11.2 pg/mL (1.9 to 20.2) vs. 3.3 pg/mL (1.9 to 31.2)]; as was IL-10 [median 49.5 pg/mL (10.5 to 102.5) vs. 33.9 pg/mL (10.5 to 77.4)] (table 5.18).

Table 5.18 Baseline values for serum inflammatory markers. Different sample sizes reflect availability of results for different tests, as discussed in the methodology

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
CRP (mg/L)	53	1.2	0.6, 2.6	28	1.3	0.9, 2.9	25	0.9	0.5, 2.3
ICAM-1 (ng/mL)	54	209.4	185.7, 245.4	28	210.0	170.5, 244.8	26	209.4	191.2, 245.4
TNF-α (pg/mL)	54	1.1	0.7, 1.8	28	1.1	0.8, 1.9	26	1.0	0.7, 1.8
IL-6 (pg/mL)	54	1.1	0.7, 3.1	28	1.3	0.7, 3.0	26	1.1	0.7, 3.4
IL-1 (pg/mL)	47	101.0	7.6, 172.1	25	101.2	20.5, 164.9	22	99.5	7.6, 172.1
IL-5 (pg/mL)	47	7.3	1.9, 26.4	25	11.2	1.9, 20.2	22	3.3	1.9, 31.2
IL-8 (pg/mL)	47	14.3	0.6, 24.0	24	14.1	4.4, 22.9	23	14.3	0.6, 29.3
IL-10 (pg/mL)	46	36.1	10.5, 87.0	25	49.5	10.5, 102.5	21	33.9	10.5, 77.4

5.3.3.3 Lymphocyte proliferation

There was no difference between groups for the control lymphocyte proliferation [median 412 counts/min/well (IQR212 to 652). In contrast, testing using PHA gave lower results for the group starting with atorvastatin [36672 counts/min/well (2288 to 104773) vs. 20721 counts/min/well (724 to 96458) for placebo], as did anti-CD3/28 [15681 counts/min/well (2097 to 65625) vs. 7407 counts/min/well (487 to 50815)] (table 5.19).

Table 5.19 Baseline values for lymphocyte proliferation

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
Control (counts/min/well)	47	412	212, 652	25	440	267, 641	22	340	210, 652
PHA (counts/min/well)	47	36518	894, 104642	25	36672	2288, 104773	22	20721	724, 96458
Anti-CD3/28 (counts/min/well)	47	13880	658, 65625	25	15681	2097, 65625	22	7407	487, 50815

5.3.3.4 Exhaled gases

Total baseline median (IQR) exhaled Nitric Oxide (FE_{NO}) was 18.33 ppb, (10.05 to 33.07). Exhaled carbon monoxide (CO) median was 4.38 ppm (3.83 to 5.07) (Table 5.20). There was no difference between groups.

Table 5.20 Baseline values for exhaled gases

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
Exhaled NO (ppb)	53	18.33	10.05, 33.07	28	18.71	11.33, 44.1	25	17.1	9.13, 32.05
Exhaled CO (ppm)	53	4.38	3.83, 5.07	28	4.43	3.82, 5.13	25	4.35	3.93, 4.83

5.3.3.5 Biochemical indices

5.3.3.5.a Lipids

No difference between groups was seen in baseline serum triglycerides [median 1.3 mmol/L (IQR 0.8 to 1.7)], cholesterol [5.1 mmol/L (4.5 to 5.9)] or HDL [1.3 mmol/L (1.1 to 1.5)] (table 5.21).

Table 5.21 Baseline serum lipids. One value is missing for triglycerides in the atorvastatin group due to a lab error.

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
Triglycerides (mmol/L)	52	1.3	0.8, 1.7	26	1.3	0.7, 1.7	26	1.2	0.8, 1.7
Cholesterol (mmol/L)	53	5.1	4.5, 5.9	27	5.0	4.3, 6.1	26	5.1	4.6, 5.8
HDL (mmol/L)	53	1.3	1.1, 1.5	27	1.3	1.1, 1.7	26	1.3	1.2, 1.5

5.3.3.5.b Renal function

There was no difference in baseline urea [median 4.6 mmol/L (IQR 4.0 to 6.1)], potassium [4.0 mmol/L (3.9 to 4.2)] or sodium [140 mmol/L (138 to 141)] (table 5.22).

Table 5.22 Baseline values for renal function. One value is missing for potassium in the placebo group due to a haemolysed sample.

Variable	Total			Atorvastatin			Placebo		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
Urea (mmol/L)	53	4.6	4.0, 6.1	27	4.6	4.1, 6.4	26	4.6	3.9, 6.1
Potassium (mmol/L)	52	4.0	3.9, 4.2	27	4.0	3.9, 4.2	25	4.1	3.9, 4.2
Sodium (mmol/L)	53	140	138, 141	27	140	139, 141	26	140	138, 141

5.3.3.5.c Liver function

No difference was observed between groups in baseline values for bilirubin [median 9 μmol/L (IQR 7 to 11)], AST [22 IU/L (19 to 27)] or ALT [21 IU/L (17 to 34)] (table 5.23).

Table 5.23 Baseline values for liver function. One value is missing for AST in the placebo group due to a haemolysed sample

Variable	Total			Atorvastatin			Placebo		
	n	Media n	IQR	n	Media n	IQR	n	Media n	IQR
Bilirubin (µmol/L)	53	9	7, 11	27	9	6, 11	26	9	8, 11
AST (IU/L)	52	22	19, 27	27	22	19, 26	25	23	18, 28
ALT (IU/L)	53	21	17, 34	27	20	17, 33	26	23	16,35

5.4 Loss to follow-up

3 patients (5.5%) were lost to follow-up. 3 prematurely discontinued, with a further 2 patients failing to return their final diary. Thus for the primary end point, 14.8% were effectively lost to follow-up. Loss to follow up of 5% or lower is usually of little concern, whereas a loss of greater than 20% means that readers should be concerned about the possibility of bias. Losses between 5 and 20% can still be a source of bias [240].

5.5 Compliance

5.5.1 Peak flow measurements

Compliance with measuring PEF can be a problem in all clinical trials. We attempted to minimise this by emphasising the importance of diary card completion. In this study, the number of missing results was equal in both placebo and atorvastatin arms at analysis (table 5.24).

Table 5.24 Summary of incomplete data from peak flow diaries (morning PEF). Note 6 patients were prematurely excluded from the trial, 3 lost to follow-up and 3 discontinued.

	Stage of trial	Included results (n)	Missing results (n)
Atorvastatin	**Randomisation**	49	4
	Week 8	46	7
Placebo	**Randomisation**	54	0
	Week 8	47	7

5.5.2 Tablets

All patients were asked to return any unused medication and the container at the end of each course of treatment (2 per patient). 75.7% of containers were returned. Compliance was calculated by comparing the number of tablets returned with the number issued. Mean compliance was 94.5% for atorvastatin and 96.6% for placebo.

Compliance from medication was also inferred by alteration in serum cholesterol and LFTs, discussed in the results section, below.

5.6 Carry over effect

Crossover trials are elegant because they remove patient variation by allowing the patient to be compared with themselves. This makes them more efficient than a parallel group study[241]. The principle drawback of a crossover trial is the possibility of a "carry over" effect. This is minimized by the use of a "washout period" (in the case of this study, six

weeks). Treatment-period interaction analysis shows no evidence of carry over effect for any outcome (Appendix 5).

5.7 Results – Clinical

5.7.1 Peak expiratory flow

The primary outcome was morning PEF at 8 weeks. At 8 weeks, the difference in mean morning peak expiratory flow, for atorvastatin as compared with placebo, did not differ between groups [treatment difference -0.5 L/min, 95% CI -10.6 to 9.6, p=0.921]. There were no significant changes at 2 or 4 weeks (table 5.25 and 2.26, figure 5.2 and 5.3). There was also no difference in evening PEF [treatment difference 4.6 L/min, -5.8 to 14.9, p=0.377] (table 5.25 and 2.26, figure 5.4 and 5.5).

Table 5.25 Morning and evening PEF at weeks 0, 2, 4 and 8

Variable	Atorvastatin											
	Week 0			Week 2			Week 4			Week 8		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Δ Diary morning PEF, L/min	49	389.6	115.7	47	384.3	111.4	39	383.9	111.0	46	387.0	106.5
Δ Diary evening PEF, L/min	48	402.6	114	47	392.6	110.5	39	389.3	112.1	46	395.3	101.0
	Placebo											
	Week 0			Week 2			Week 4			Week 8		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Δ Diary morning PEF, L/min	54	386.4	106.9	52	387.1	110.0	45	380.5	105.8	47	393.9	114.2
Δ Diary evening PEF, L/min	53	401.0	105.6	52	399.5	114.4	45	395.7	109.5	47	403.8	116.3

Table 5.26 Morning and Evening PEF at week 8 for atorvastatin and placebo treatment, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Mean	SD	n	Mean	SD	difference		
Δ Diary morning PEF, L/min	46	387.0	106.5	47	393.9	114.2	-0.5	-10.6, 9.6	0.921
Δ Diary evening PEF, L/min	46	395.3	101.0	47	403.8	116.3	4.6	-5.8, 14.9	0.377

Figure 5.2 Morning PEF at 2, 4 and 8 weeks for atorvastatin and placebo. Bars indicate standard deviation. No significant difference is seen between groups at any point.

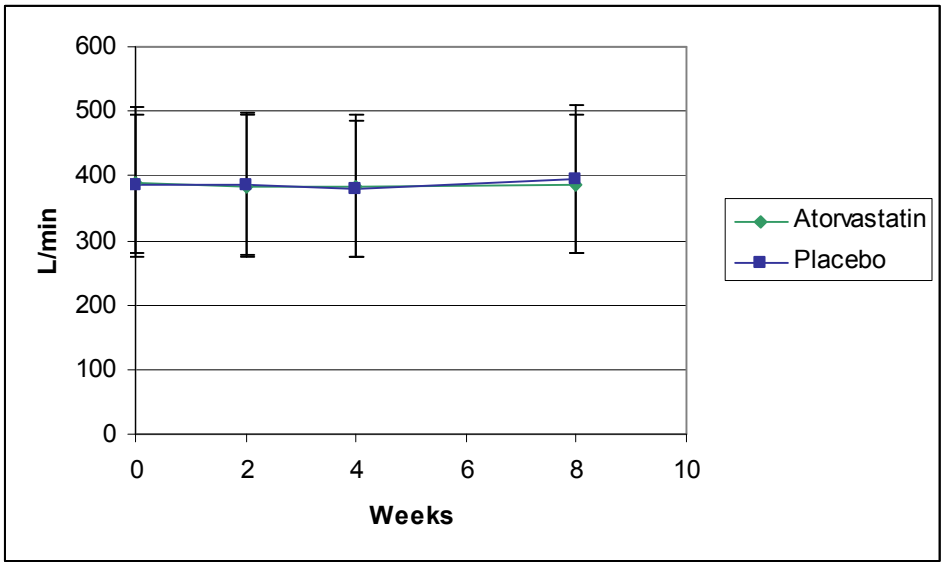


Figure 5.3 Box and whisker plot for morning PEF after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.921).

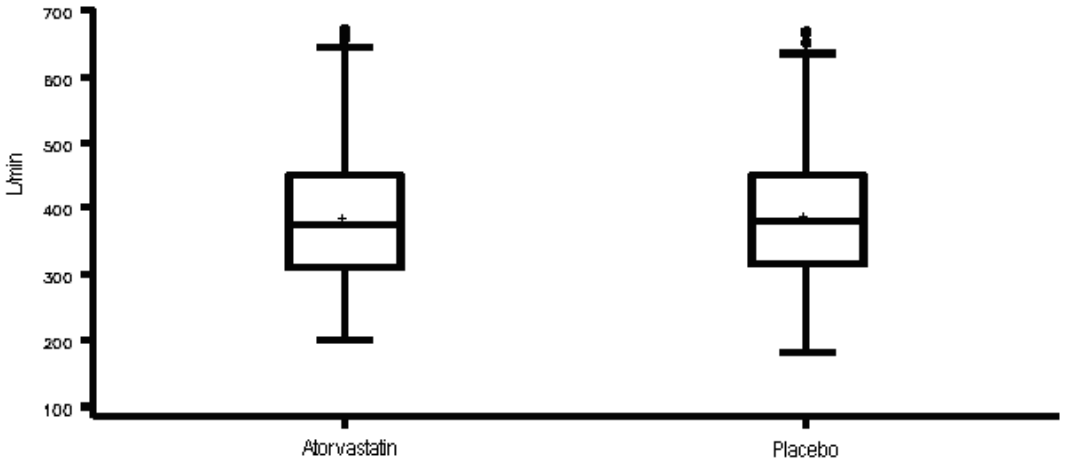


Figure 5.4 Evening PEF at 2, 4 and 8 weeks for atorvastatin and placebo. Bars indicated standard deviation. No significant difference is seen between groups at any point.

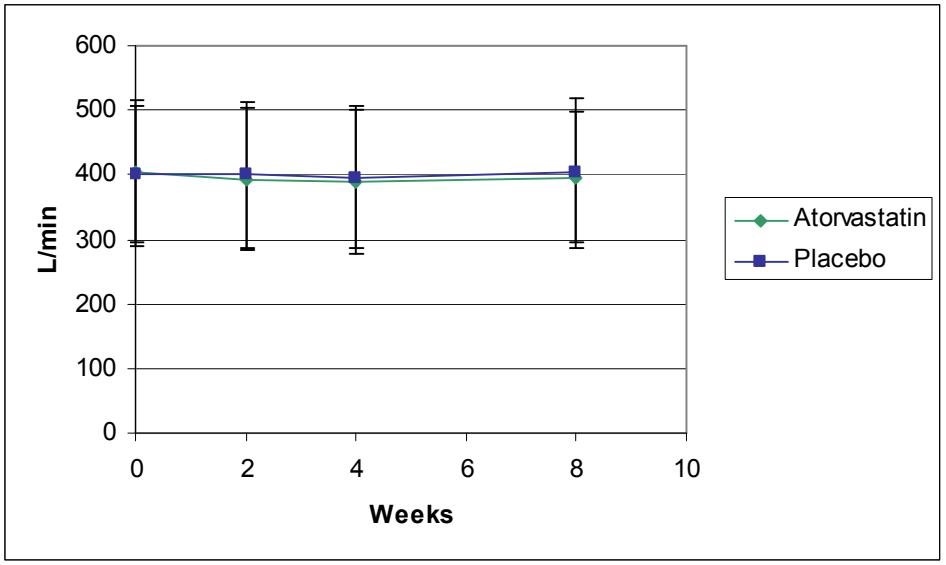
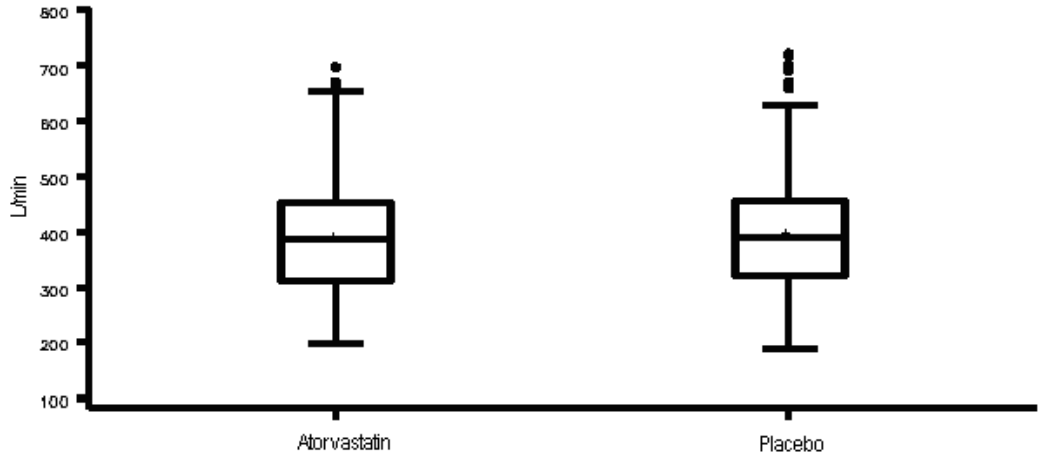


Figure 5.5 Box and whisker plot for evening PEF after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.377).



5.7.2 Reliever inhaler use

There was no difference in reliever inhaler use at 2, 4 or 8 weeks [treatment difference at 8 weeks 0.08 puffs of inhaler, 95% CI -0.41 to 0.57, p=0.745] (Table 5.27, Figure 5.6 and 5.7).

Table 5.27 Reliever inhaler use at week 8 for atorvastatin and placebo treatment, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Mean	SD	n	Mean	SD	difference		
Puffs of reliever inhaler	47	2.1	2.0	47	2.1	1.9	0.08	-0.41, 0.57	0.745

Figure 5.6 Reliever inhaler use at 0, 2, 4 and 8 weeks. Bars indicate standard deviation. No significant difference is seen between groups at any point.

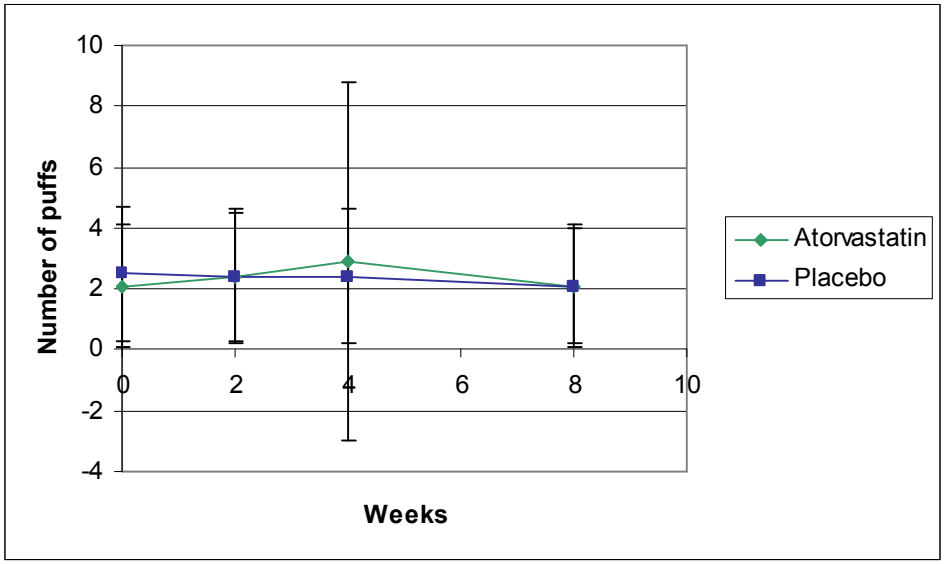
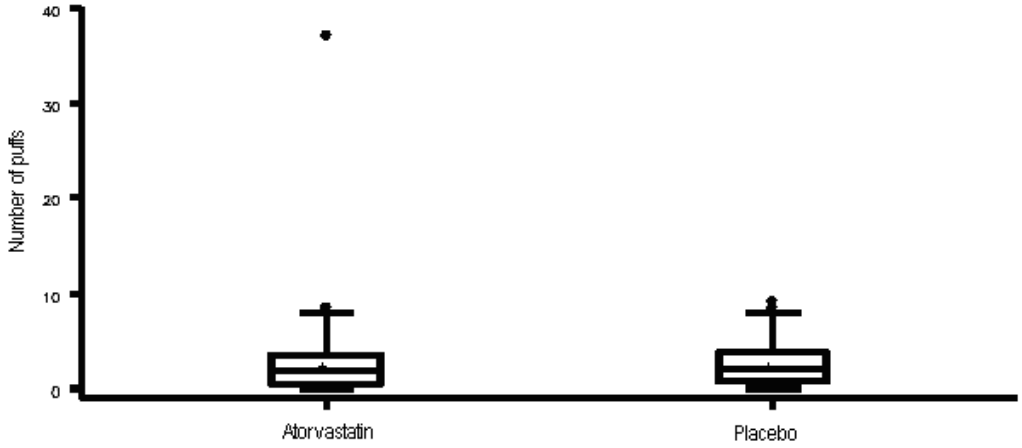


Figure 5.7 Box and whisker plot for use of reliever inhaler after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.745).



5.7.3 Spirometry

5.7.3.1 FEV_1

FEV_1 did not show a statistically significant difference after 8 weeks of atorvastatin [treatment difference 0.01 L (95% CI -0.08 to 0.10), p=0.815 pre-salbutamol, and -0.05 L (-0.12 to 0.03), p=0.205, post-salbutamol](table 5.28 and figure 5.8 and 5.9). The values were also considered as percentage predicted for an adult of the same age and height. Mean FEV1 % predicted (pre-salbutamol), did not differ between the atorvastatin and the placebo group [treatment difference -0.05% (-3.0 to 2.9), p=0.973] (table 5.28 and figure 5.10). There was a non-significant trend towards lower reversibility in the atorvastatin group [treatment difference -3.0% (-6.4 to 0.3) p=0.074] (table 5.28 and figure 5.11).

Table 5.28 FEV_1 results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Mean	SD	n	Mean	SD			
FEV_1 pre salbutamol, L	48	2.77	0.83	50	2.71	0.79	0.01	-0.08, 0.10	0.815
FEV_1 post salbutamol, L	49	3.06	0.83	50	3.12	0.80	-0.05	-0.12, 0.03	0.205
FEV_1 % predicted	48	83.0	17.3	50	82.6	18.2	-0.05	-3.0, 2.9	0.973
FEV_1 reversibility, %	48	15.0	11.7	50	17.5	15.6	-3.0	-6.4, 0.3	0.074

Figure 5.8 Box and whisker plot for FEV_1 pre-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.815).

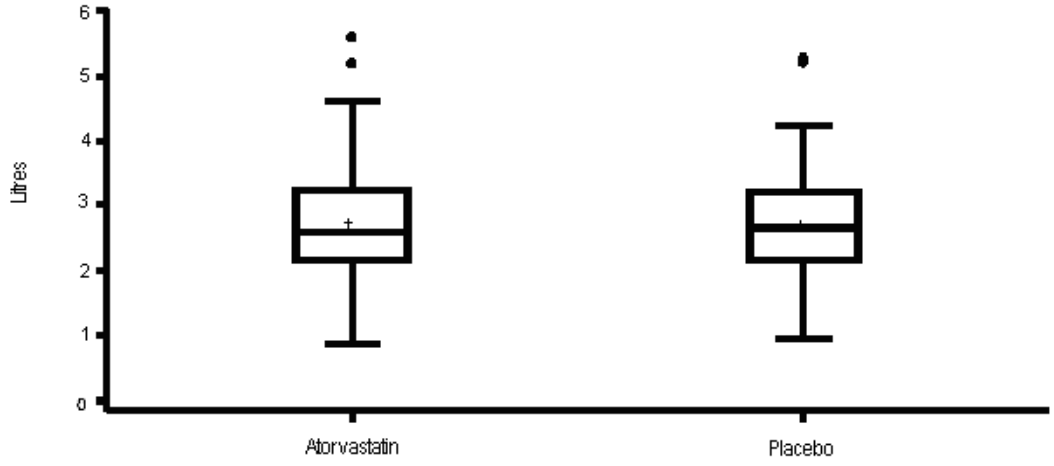


Figure 5.9 Box and whisker plot for FEV1 post-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.205).

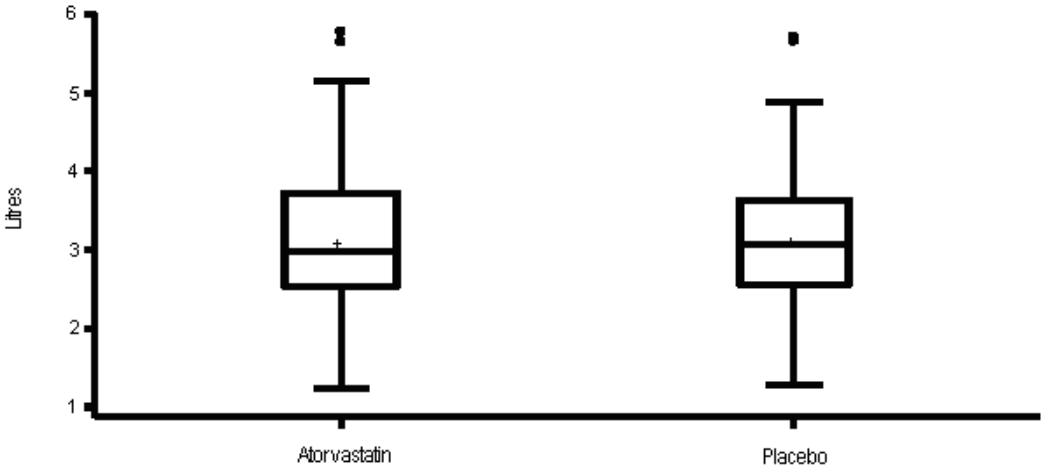


Figure 5.10 Box and whisker plot for percentage predicted FEV_1 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.973).

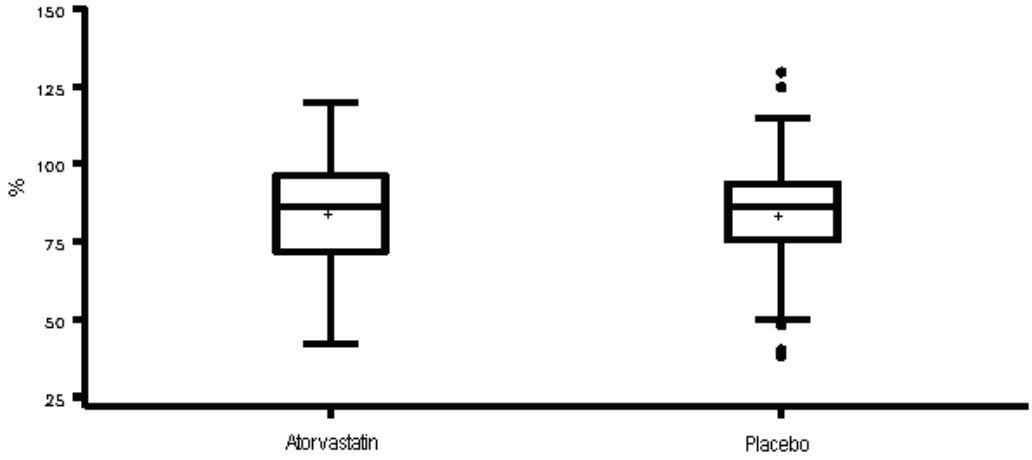
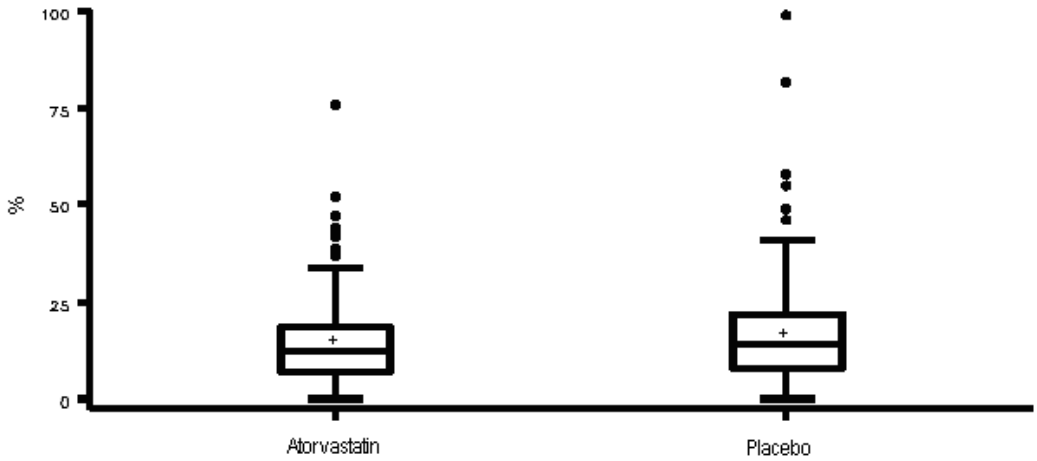


Figure 5.11 Box and whisker plot for FEV_1 reversibility after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. A trend of borderline significance is seen between groups (p=0.074).



5.7.3.2 Spirometry PEF

There was no difference in PEF measured during spirometry pre-salbutamol [treatment difference 4.8 L/min (95% CI -11.9 to 21.6) p=0.563] (table 5.29, figure 5.12). Post-salbutamol PEF was slightly lower in the atorvastatin group [treatment difference -12.4 L/min (25.5 to -0.8) p=0.064] (table 5.29, figure 5.13). Percentage PEF predicted pre-salbutamol did not vary between groups [treatment difference 0.2% (-3.3 to 3.6) p=0.927] (table 5.29, figure 5.14).

Table 5.29 Spirometry PEF results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Mean	SD	n	Mean	SD			
PEF during spirometry, L/min, pre-salbutamol	48	427.1	129.6	50	417.8	127.9	4.8	-11.9, 21.6	0.563
PEF during spirometry, L/min, post-salbutamol	49	482.4	135.5	50	491.4	128.9	-12.4	-25.5, 0.8	0.064
PEF % predicted	48	92.0	22.1	50	90.6	23.5	0.2	-3.3, 3.6	0.927

Figure 5.12 Box and whisker plot for spirometry PEF pre-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.563).

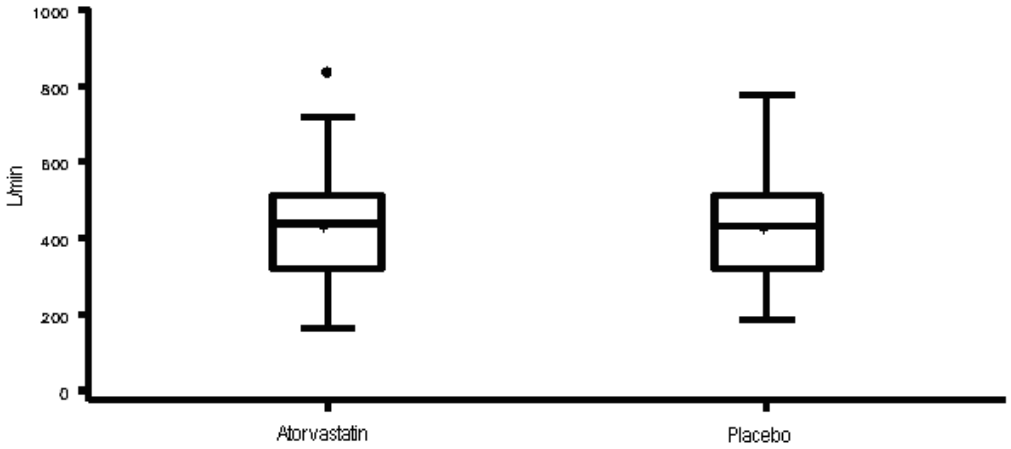


Figure 5.13 Box and whisker plot for spirometry PEF post-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. A trend of borderline significance is seen between groups (p=0.064).

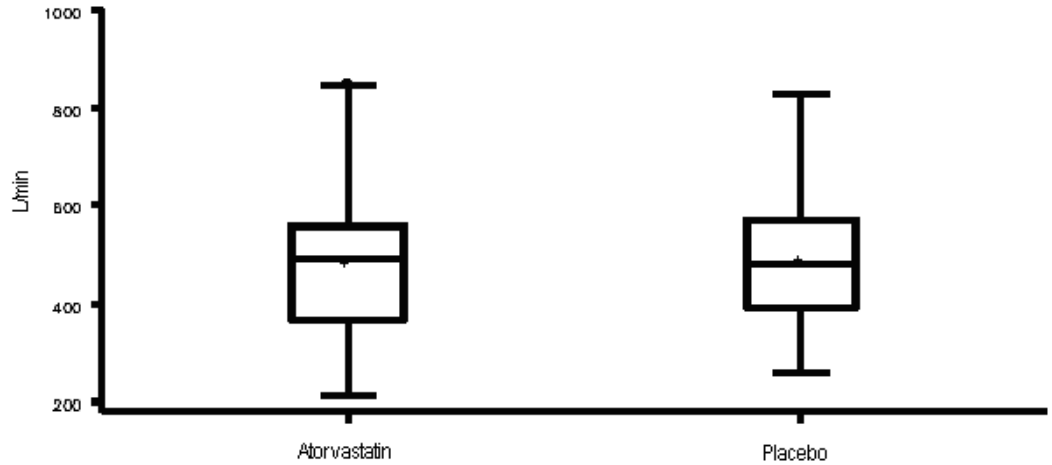
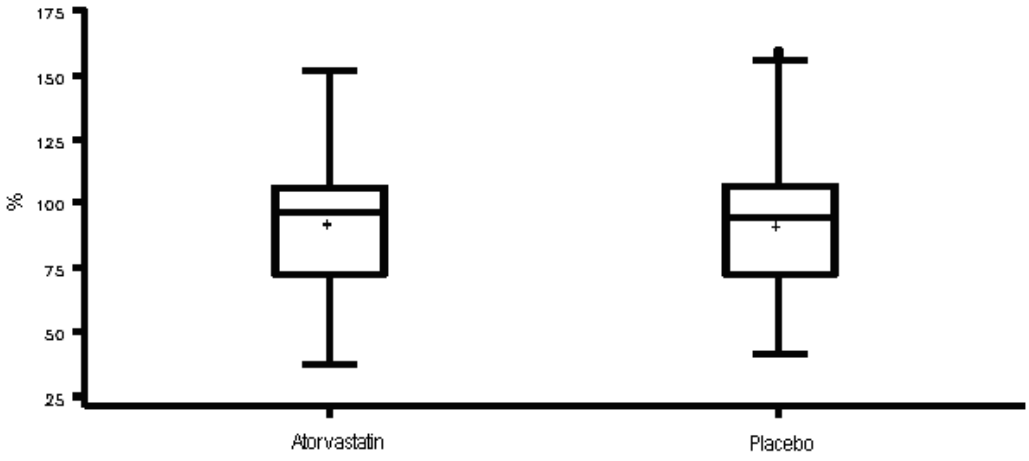


Figure 5.14 Box and whisker plot for percentage predicted spirometry PEF pre-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.927).



5.7.3.3 FVC

No effect of atorvastatin was seen in pre-salbutamol mean (SD) FVC [treatment difference 0.0 L (95% CI -0.1 to 0.1), p=0.627] (table 5.30, figure 5.15). Post-salbutamol FVC was slightly lower in the atorvastatin group [treatment difference -0.1 L (-0.2 to 0.0), p=0.037] (table 5.30, figure 5.16). Percentage FVC predicted pre-salbutamol did not vary between groups [treatment difference -1.3% (-4.5 to 2.0) p=0.431] (table 5.30, figure 5.17).

Table 5.30 FVC results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Mean	SD	n	Mean	SD			
FVC, pre-salbutamol, L	48	3.6	1.1	50	3.7	1.0	0.0	-0.1, 0.1	0.627
FVC, post-salbutamol, L	49	3.9	1.04	50	4.00	1.0	-0.1	-0.2, 0.0	0.037
FVC % predicted	48	92.9	16.5	50	93.8	18.1	-1.3	-4.5, 2.0	0.431

Figure 5.15 Box and whisker plot for FVC pre-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.627)

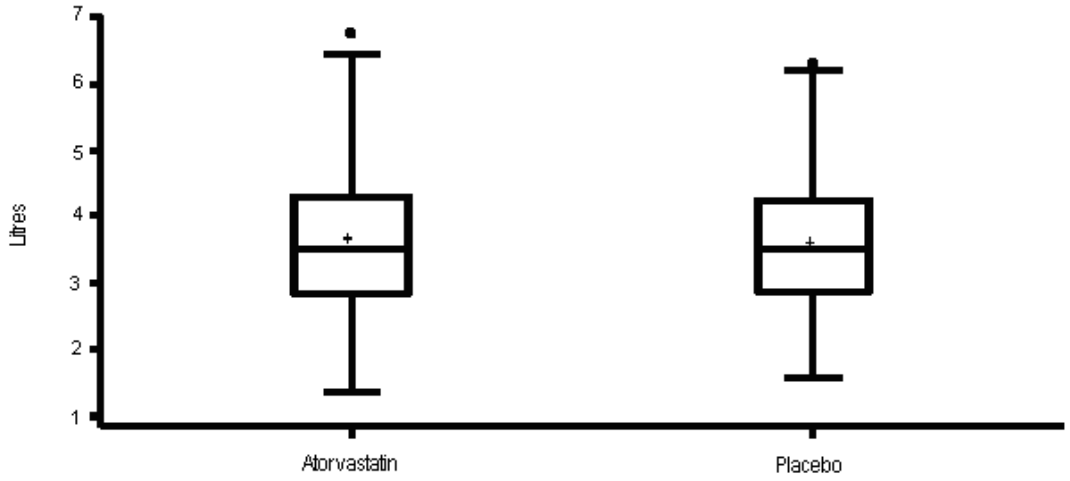


Figure 5.16 Box and whisker plot for FVC post-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. A significant difference is seen between groups (p=0.037)

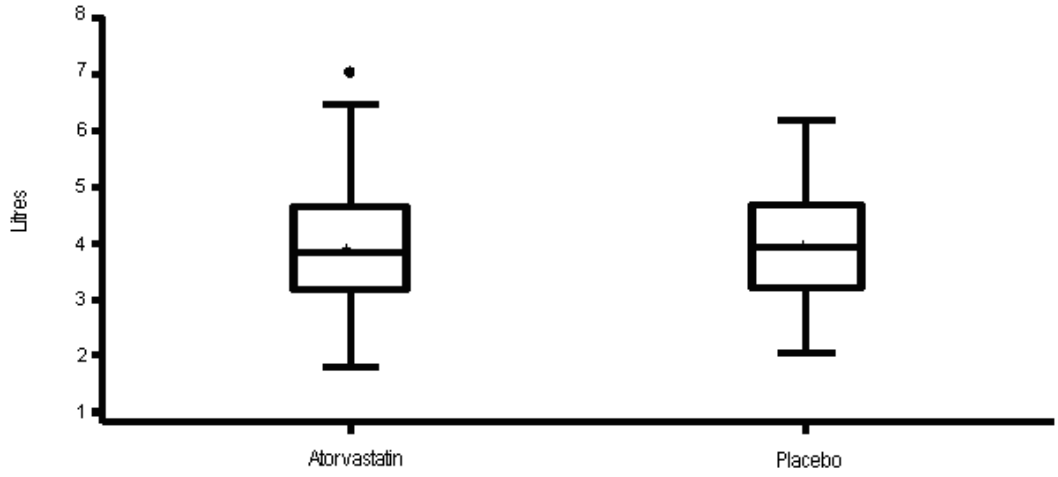
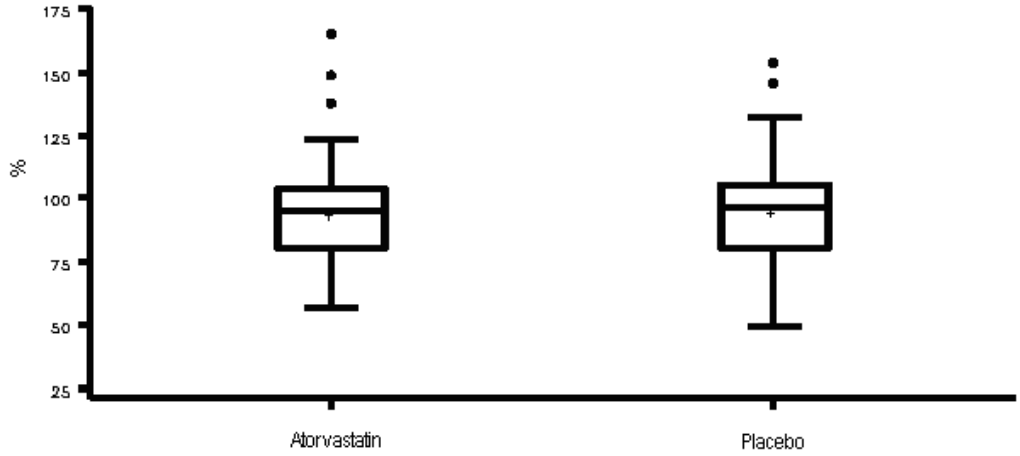


Figure 5.17 Box and whisker plot for percentage predicted FVC pre-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.431).



5.7.3.4 FEF_{25-75}

No effect could be seen on mean FEF_{25-75} either pre-salbutamol [treatment difference 0.0 L/sec (95% CI -0.2 to 0.2), p=0.871] (table 5.31, figure 5.18) or post-salbutamol [treatment difference 0.1 L/sec (-0.1 to 0.2), p=0.484] (table 5.31, figure 5.19). Percentage FEF_{25-75} predicted pre-salbutamol did not vary between groups [treatment difference 1.2% (-2.5 to 4.9) p=0.512] (table 5.31, figure 5.20).

Table 5.31 FEF_{25-75} results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Mean	SD	n	Mean	SD			
FEF_{25-75} pre-salbutamol, (L/sec)	48	2.3	1.0	48	2.3	1.2	0.0	-0.2, 0.2	0.871
FEF_{25-75} post-salbutamol, (L/sec)	49	2.9	1.2	48	2.8	1.2	0.1	-0.1, 0.2	0.484
FEF_{25-75} % predicted	47	57.9	21.4	48	56.8	23.3	1.2	-2.5, 4.9	0.512

Figure 5.18 Box and whisker plot for FEF_{25-75} pre-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.871).

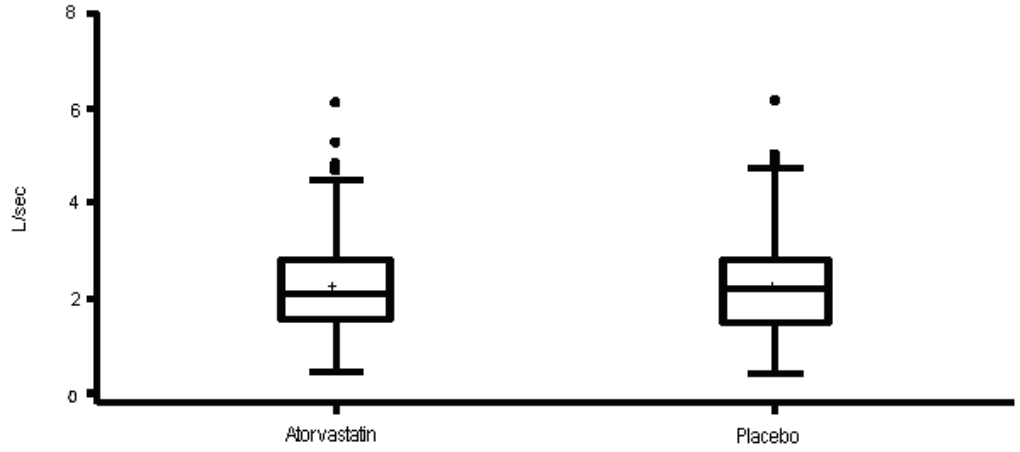


Figure 5.19 Box and whisker plot for FEF_{25-75} post-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.484).

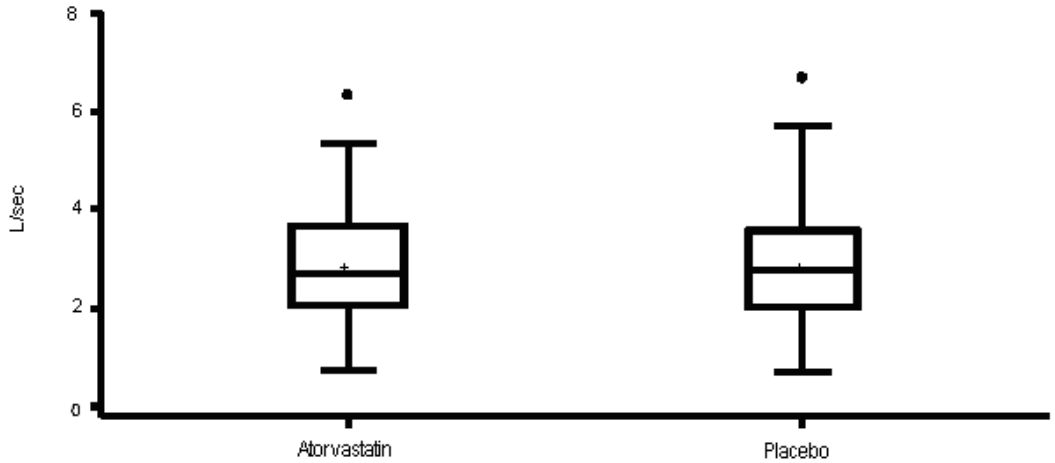
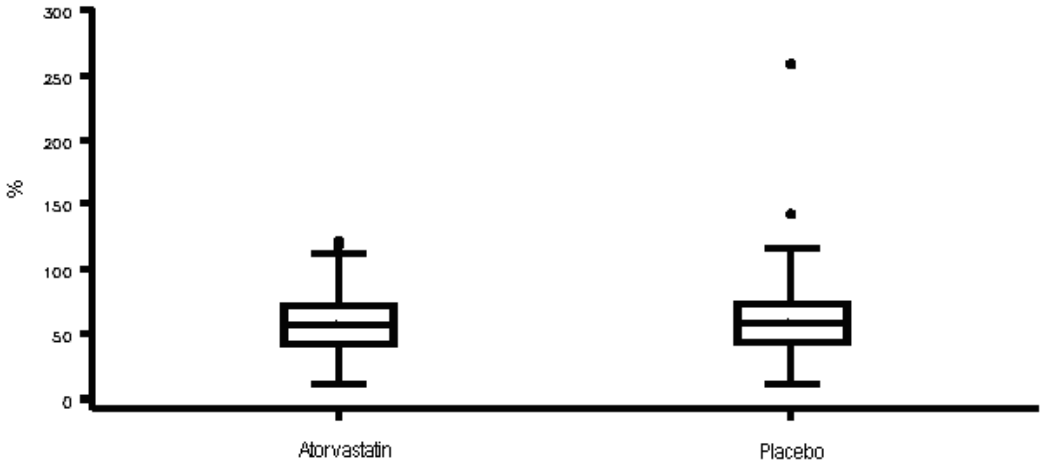


Figure 5.20 Box and whisker plot for percentage predicted FEF_{25-75} pre-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.521).



5.7.3.5 FEV_1/FVC ratio

Finally, no effect was seen on the mean FEV_1/FVC ratio, either pre-salbutamol [treatment difference -28.6% (95% CI -71.1 to 13.8) p=0.181] (table 5.32 and figure 5.21) or post-salbutamol [treatment difference -20.9% (-65.1 to 23.3) p=0.346] (table 5.32 and figure 5.22).

Table 5.32 FEV_1/FVC ratio results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Mean	SD	n	Mean	SD	difference		
FEV_1/FVC pre-salbutamol, %	48	75.4	10.7	50	102.1	139.1	-28.6	-71.1, 13.8	0.181
FEV_1/FVC post-salbutamol, %	49	85.8	44.9	50	106.7	140.1	-20.9	-65.1, 23.3	0.346

Figure 5.21 Box and whisker plot for FEV_1/FVC ratio pre-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.181).

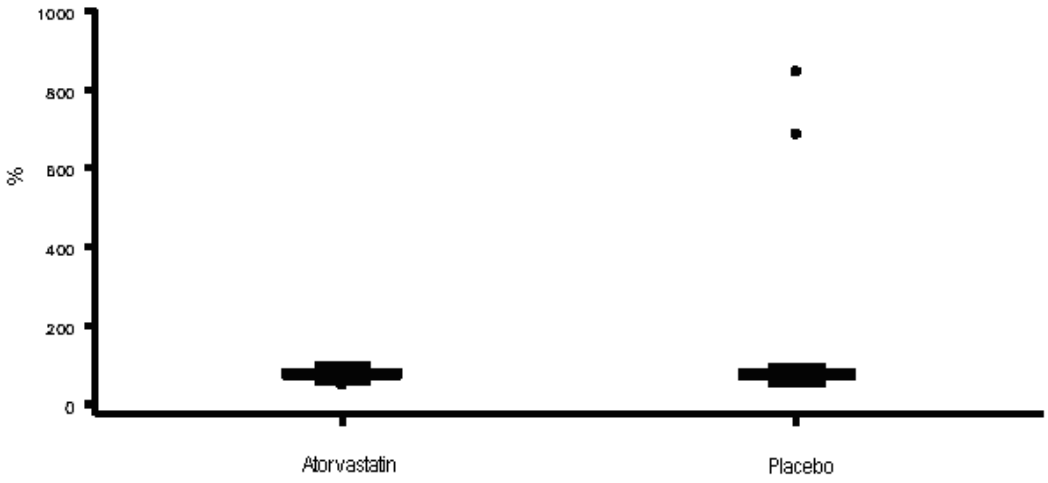
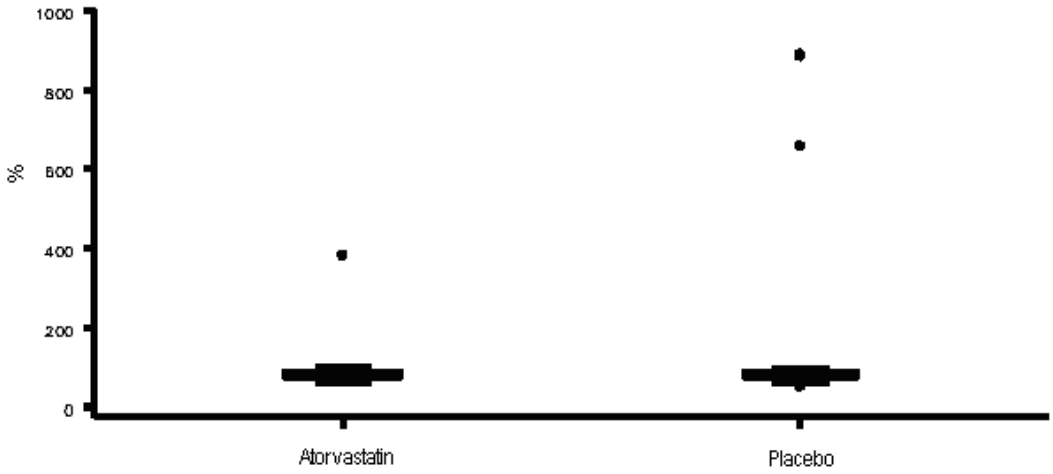


Figure 5.22 Box and whisker plot for FEV_1/FVC ratio post-salbutamol after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.346).



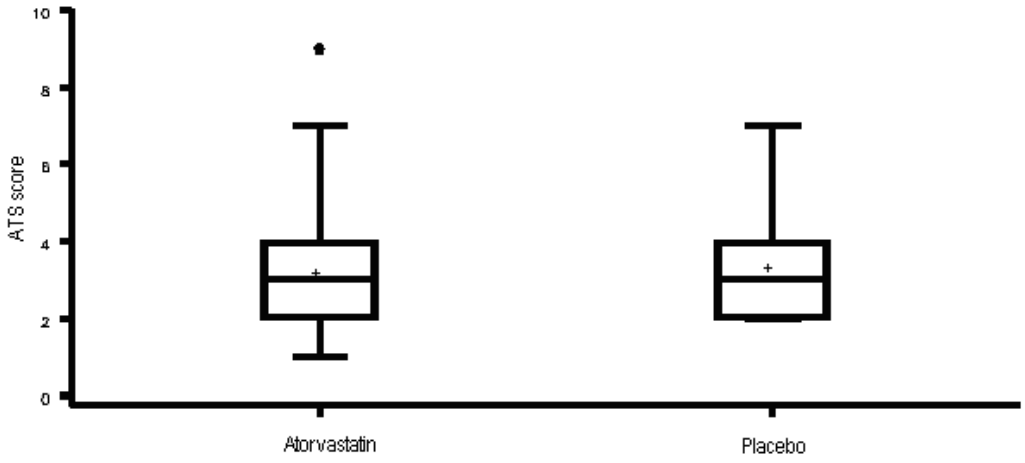
5.7.4 Asthma severity

No effect was seen on mean ATS score [treatment difference -0.16 (95% CI –0.49 to 0.17) p=0.323] (table 5.33 and figure 5.23).

Table 5.33 ATS score results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Mean	SD	n	Mean	SD	difference		
ATS score	48	3.2	1.6	50	3.4	1.4	-0.16	-0.49, 0.17	0.323

Figure 5.23 Box and whisker plot for ATS score after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.323).



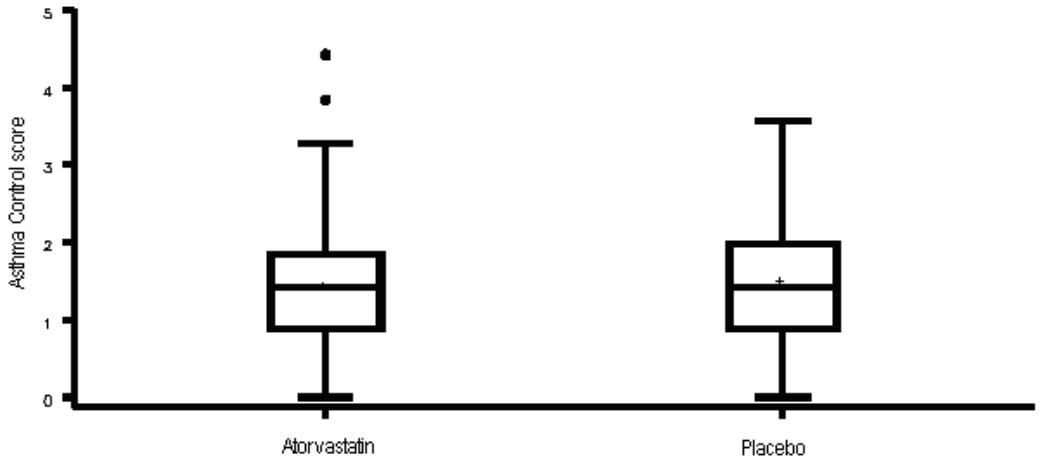
5.7.5 Asthma control questionnaire (ACQ) score

Statins showed no effect on ACQ score [treatment difference -0.03, (-0.28 to 0.21), p=0.783] (table 5.34, figure 5.24).

Table 5.34 ACQ score results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P
	n	Mean	SD	n	Mean	SD	difference		value
ACQ	49	1.4	0.9	50	1.5	0.8	-0.03	-0.28, 0.21	0.783

Figure 5.24 Box and whisker plot for ACQ score after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.783).



5.7.6 Asthma quality of life (AQLQ)

Total AQLQ score was not significantly different between atorvastatin and placebo [treatment difference 0.1 (-0.1 to 0.3) p=0.284] (table 5.35, figure 5.25). Subdivision into symptoms [0.1 (-0.1 to 0.4) p=0.316], activities [0.1 (-0.1 to 0.3) p=0.226], emotional well-being [0.1 (-0.2 to 0.4) p=0.540] and environment [0.1 (-0.2 to 0.3) p=0.590] did not show any difference between the categories (table 5.35, figure 5.26).

Table 5.35 AQLQ results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P
	n	Median	IQR	n	Median	IQR	difference		value
AQLQ - total	48	5.9	5.4, 6.6	49	5.9	5.4, 6.4	0.1	-0.1, 0.3	0.284
AQLQ – symptoms	48	6.0	5.2, 6.4	49	5.8	5.1, 6.3	0.1	-0.1, 0.4	0.316
AQLQ – activity	48	6.3	5.5, 6.9	49	6.3	5.6, 6.6	0.1	-0.1, 0.3	0.226
AQLQ – emotional well-being	48	6.0	4.9, 6.8	49	5.8	5.0, 6.4	0.1	-0.2, 0.4	0.540
AQLQ – environment	48	5.9	5.0, 6.5	49	5.8	5.0, 6.5	0.1	-0.2, 0.3	0.590

Figure 5.25 Box and whisker plot for AQLQ after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.284).

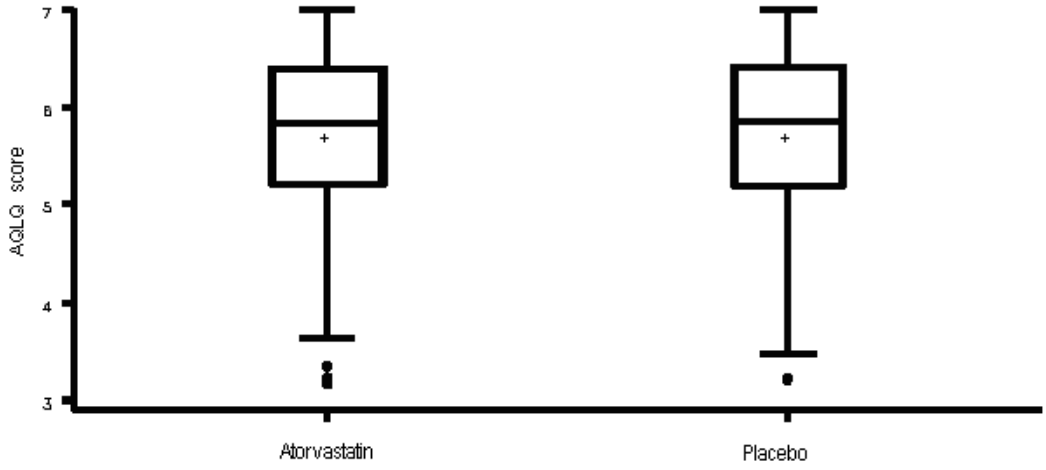
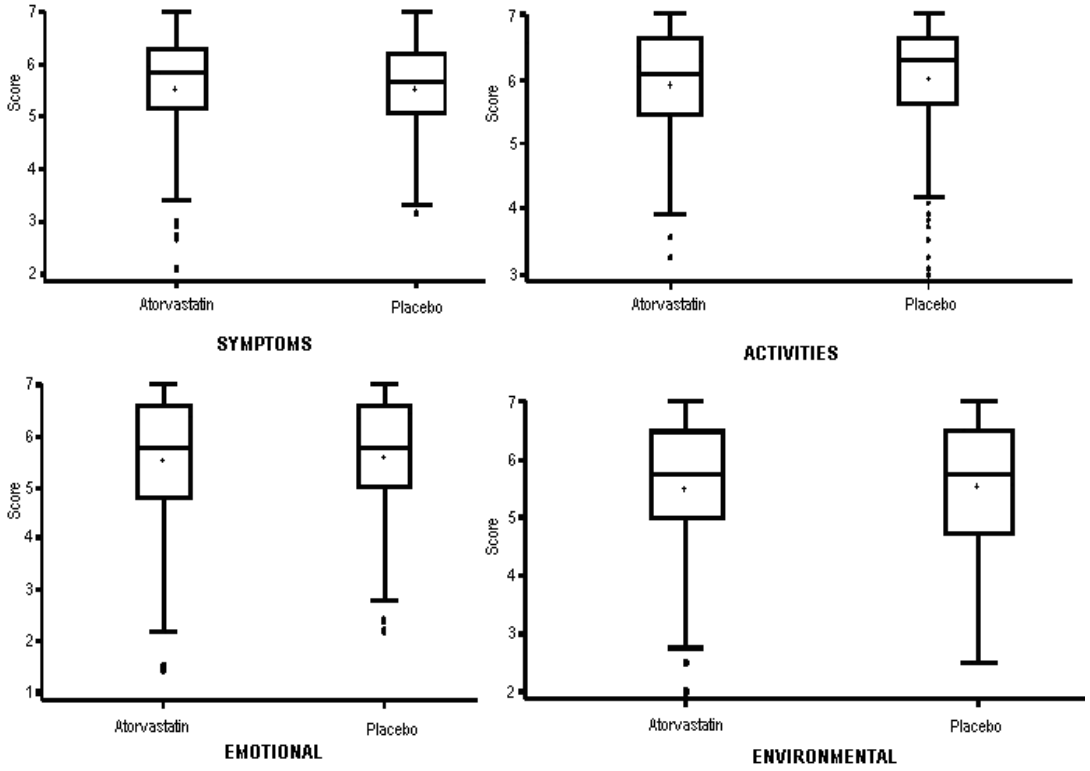


Figure 5.26 Box and whisker plots for AQLQ components after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (symptoms p=0.316, activity p=0.226, emotional well-being p=0.540, environment p=0.590).



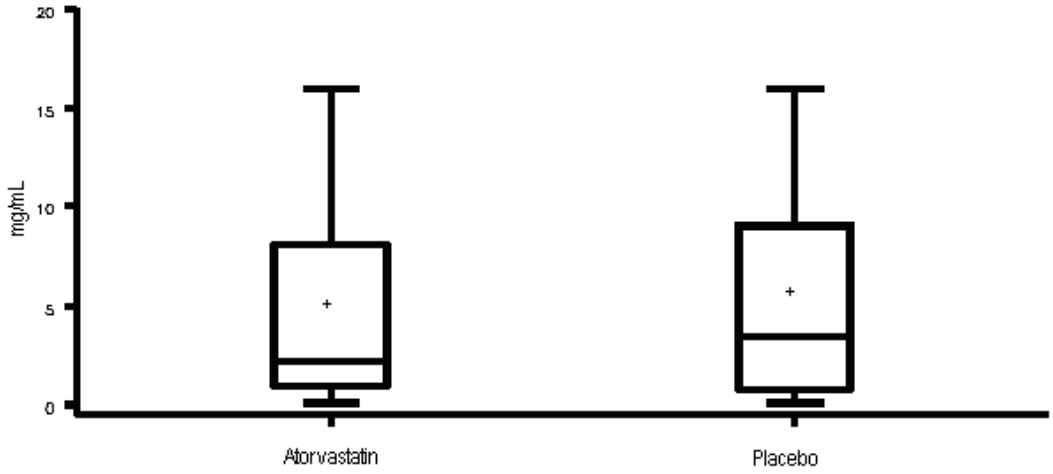
5.7.7 Methacholine responsiveness

No difference was seen in methacholine responsiveness (PC_{20}) between patients on atorvastatin and placebo [treatment difference 0.05 mg/mL (95% CI -1.60 to 1.70), p=0.949] (table 5.36 and figure 5.27).

Table 5.36 Methacholine responsiveness results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Geometric mean	Std dev	n	Geometric mean	Std dev			
Methacholine responsiveness (PC20) mg/mL	38	2.64	4.68	39	2.21	5.81	0.05	-1.60, 1.70	0.949

Figure 5.27 Box and whisker plot for methacholine responsiveness after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.949).



5.8 Biochemical outcomes

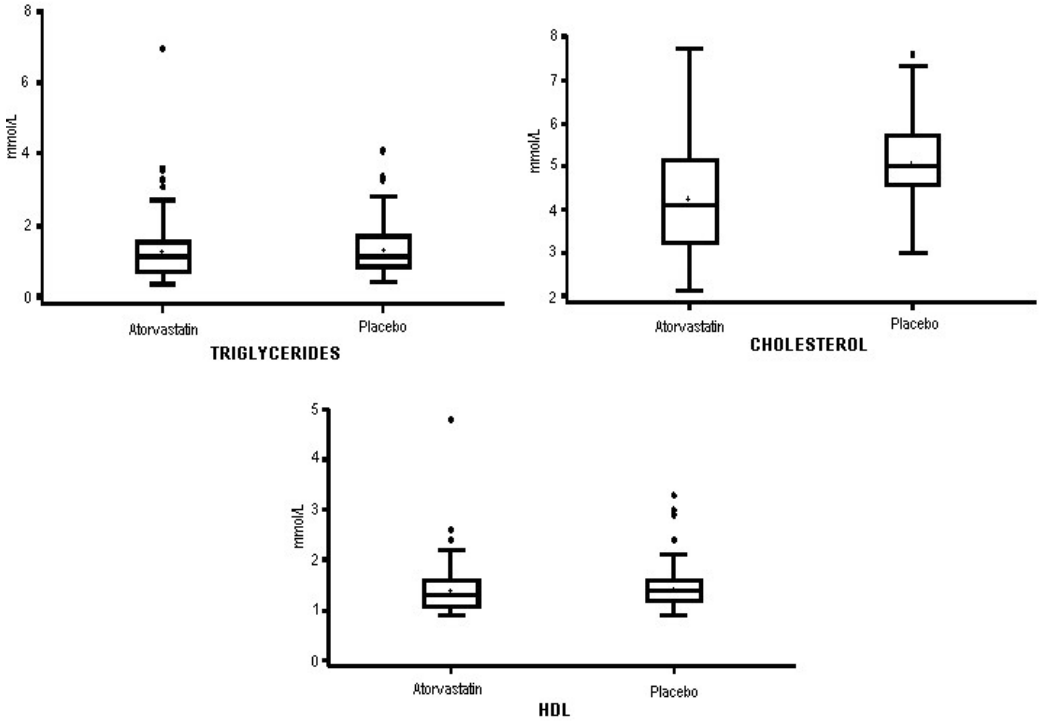
5.8.1 Lipids

No difference was seen between atorvastatin and placebo for triglycerides [treatment difference -0.2 mmol/L (95% CI -0.5 to 0.1), p=0.106] on serum lipid testing (table 5.37, figure 5.21). However, a significant change was seen in cholesterol [-1.7 mmol/L (-1.9 to -1.5) p<0.0001] and HDL [-0.1 mmol/L (-0.3 to -0.0) p=0.026] (table 5.37, figure 5.28). This suggests good compliance with medication and a clear effect on lipid profile within the timescale of the trial.

Table 5.37 Serum lipid results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
Triglycerides (mmol/L)	47	0.9	0.6, 1.3	47	1.1	0.8, 1.6	-0.2	-0.5, 0.1	0.106
Cholesterol (mmol/L)	47	3.3	2.9, 3.9	47	5.1	4.5, 5.7	-1.7	-1.9, -1.5	<0.001
HDL (mmol/L)	47	1.2	1.1, 1.5	47	1.4	1.1, 1.7	-0.1	-0.3, -0.0	0.026

Figure 5.28 Box and whisker plots for serum lipids after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. Significant differences are present for Cholesterol and HDL but not triglycerides (triglycerides p=0.106, cholesterol p<0.001, HDL p=0.026).



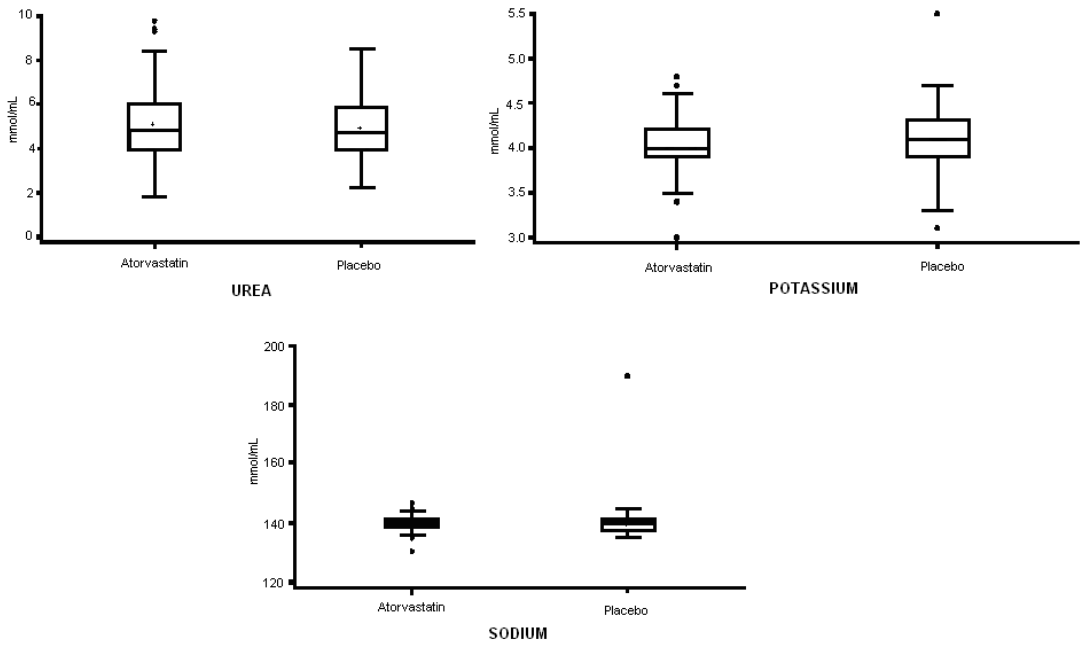
5.8.2 Urea and electrolytes

No effect was seen on urea [treatment difference 0.1 mmol/L (95% CI -0.2 to 0.4) p=0.586], potassium [-0.1 mmol/L (-0.3 to 0.0) p=0.104] or sodium [0.4 mmol/L (-0.5 to 1.3) p=0.340] (table 5.38, figure 5.29) during the trial.

Table 5.38 Urea and electrolyte results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
Urea (mmol/L)	46	5.1	4.0, 6.0	48	4.7	4.1, 5.8	0.1	-0.2, 0.4	0.586
Potassium (mmol/L)	46	4.0	3.8, 4.1	47	4.1	3.8, 4.3	-0.1	-0.3, 0.0	0.104
Sodium (mmol/L)	46	140.0	139.0, 141.0	48	139.0	138, 141	0.4	-0.5, 1.3	0.340

Figure 5.29 Box and whisker plot for urea and electrolytes after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant differences are seen (urea p=0.586, potassium p=0.104, sodium p=0.340).



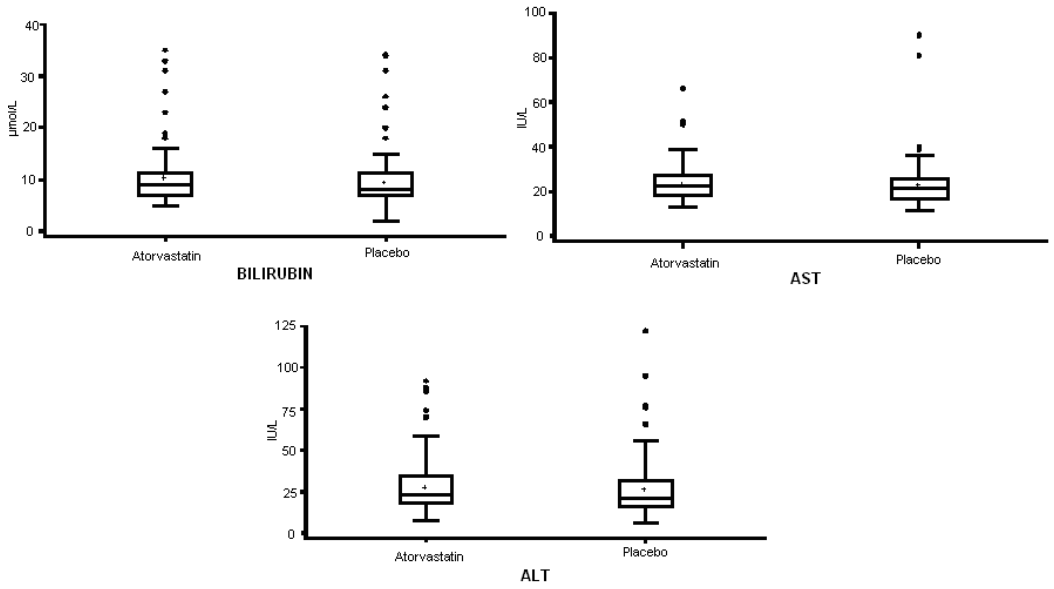
5.8.3 Liver function tests

Differences were statistically significant in serum bilirubin [treatment difference 1.8 μmol/L (95% CI 0.7 to 2.9) p=0.002], AST [2.9 IU/L (0.8 to 4.9) p=0.007] and ALT [5.6 IU/L (2.7 to 8.5) p<0.0001] (table 5.39, figure 5.30). This again suggests compliance with medication. These differences are clinically small and do not indicate any adverse events.

Table 5.39 Liver function test results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Median	IQR	n	Median	IQR	difference		
Bilirubin (μmol/L)	47	10	8, 13	47	8	6, 10	1.8	0.7, 2.9	0.002
AST (IU/l)	47	23	18, 27	47	20	16, 24	2.9	0.8, 4.9	0.007
ALT (IU/l)	47	26	18, 35	47	20	15, 33	5.6	2.7, 8.5	<0.001

Figure 5.30 Box and whisker plot for liver function tests after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. Significant differences are present in all cases (bilirubin p=0.002, AST p=0.007, ALT p<0.001).



5.9 Patients who did not complete the trial

5.9.1 Loss to follow up

Three patients were lost to follow up. A further two patients did not complete their final diary, so their PEF data could not be used for analysis of the primary end point.

5.9.2 Patient withdrawals

Three patients were withdrawn prematurely from the trial. One patient had medication-related side-effects (muscle cramps). One patient had a severe exacerbation of asthma

very early in the trial and elected to withdraw from the trial and commence long-acting beta agonists. The patient was followed-up clinically until symptoms had improved.

Unfortunately, one patient also died during this study. Following post mortem his death was attributed to alcohol excess and aspiration of gastric contents. There was no evidence to suggest that asthma played any part in his death.

5.10 Adverse events

5.10.1 Exacerbation rates

The rate of exacerbation is intended to measure the probability that a patient who is currently exacerbation-free will experience an exacerbation within a small time interval [2, 40]. In a cross-over study, the important comparison is between exacerbation rates in the two separate arms of the study. Numbers of both mild and severe exacerbations were similar between the two groups (table 5.40).

Table 5.40 Mild and severe exacerbations for atorvastatin and placebo

Treatment	Number of mild exacerbations (%)	Number of severe excerbations (%)
Atorvastatin (n=52)	33 (63.5)	1 (1.9)
Placebo (n=54)	38 (70.4)	1 (1.9)

5.10.2 Asthma related adverse events

In addition to exacerbation rates, treatment with oral steroids, unscheduled GP visits, GP home visits, Accident & Emergency visits and hospital admissions for asthma or respiratory symptoms were measured. Frequency of these events was similar between groups (table 5.41).

Table 5.41 Asthma related adverse events

Event	Total	Atorvastatin	Placebo
Oral steroid treatment	1	1	0
Unscheduled GP appointment	3	1	2
GP home visit	0	0	0
A&E visit	3	2	1
Hospital admission	0	0	0
Total asthma related adverse events	7	4	3

5.10.3 Possible drug related adverse events

Possible drug-related adverse events occurred on 38 occasions for patients taking atorvastatin and on 46 occasions for placebo. There were no striking differences between groups, including episodes of muscle cramps. All adverse events are listed in table 5.42.

Table 5.42 Adverse events in trial, comparing atorvastatin with placebo. The different denominators reflect the fact that two patients left the trial before receiving atorvastatin.

Event name	Atorvastatin Number of events (% total) n=52	Placebo Number of events (% total) n=54
Any adverse event	38 (73.1)	46 (85.2)
Muscle cramp	3 (5.8)	5 (9.3)
GI upset	7 (13.5)	7 (13.0)
Hayfever	0 (0.0)	2 (3.7)
Headache	7 (13.5)	7 (13.0)
Insomnia	0 (0.0)	1 (1.9)
Joint pain	7 (13.5)	8 (14.8)
Pins and needles	1 (1.9)	2 (3.7)
Tiredness	1 (1.9)	4 (7.4)
UTI	1 (1.9)	2 (3.7)
Ankle swelling	1 (1.9)	0 (0.0)
Breast pain	0 (0.0)	1 (1.9)
Chest tightness	1 (1.9)	0 (0.0)
Cough	0 (0.0)	1 (1.9)
Death	0 (0.0)	1 (1.9)
Depression	1 (1.9)	0 (0.0)
Dermatitis	2 (3.8)	1 (1.9)
Dizziness	0 (0.0)	2 (3.7)
Conjunctivitis	1 (1.9)	1 (1.9)
Hernia	0 (0.0)	1 (1.9)
Mouth swelling	1 (1.9)	0 (0.0)

Event name	Atorvastatin Number of events (% total) n=52	Placebo Number of events (% total) n=54
Nocturia	2 (3.8)	0 (0.0)
Nosebleed	0 (0.0)	2 (3.7)
Palpitations	1 (1.9)	0 (0.0)
Rectal bleeding	0 (0.0)	1 (1.9)
Rash	0 (0.0)	1 (1.9)
Sinusitis	1 (1.9)	1 (1.9)
Sore throat	2 (3.8)	2 (3.7)
Weak legs	0 (0.0)	1 (1.9)

5.11 Clinical results - Summary

A statistically significant difference was seen in post-salbutamol FVC, which was lower in the atorvastatin group. No other statistical or clinical difference can be seen between patients treated with eight weeks atorvastatin and placebo, although there was a non-significant trend towards lower post-salbutamol PEF and lower reversibility in the atorvastatin-treated group. These findings are clinically insignificant but may indicate a subtle effect on lung function, or may simply be artefact. Serum biochemistry indicates compliance with medication.

5.12 Results – inflammatory markers

5.12.1 Sputum cell differential

5.12.1.1 Sputum quality indicators

Results are summarised in table 5.44. Total sputum cell count was unchanged between the two groups [treatment difference -0.1 $x10^6$ cells (95% CI -0.1 to 0.0), p=0.090]. There was no difference in cell viability [treatment difference 1.6% (-5.8 to 8.9), p=0.669], nor in total number of cells recovered [treatment difference -2.4 $x10^6$ cells (-6.7 to 1.9) p=0.257] or total viable cells recovered [treatment difference -2.0 $x10^6$ (-5.2 to 1.2) p=0.209] (table 5.43). 4 samples in the atorvastatin group and 6 samples in the placebo group were of insufficient quality for further analysis (as defined by the criteria in section 4.3.1, above).

Table 5.43 Sputum quality indicators for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P
	n	Median	IQR	n	Median	IQR	difference		value
Total cell count per ml sputum filtrate ($x10^6$/mL)	40	0.1	0.1, 0.2	38	0.1	0.1, 0.3	-0.1	-0.1, 0.0	0.090
Viability (%)	40	71.0	66.0, 81.0	38	70.0	60.0, 80.0	1.6	-5.8, 8.9	0.669
Total number of cells recovered from sample ($x10^6$)	40	2.3	1.3, 4.2	38	2.5	1.1, 5.7	-2.4	-6.7, 1.9	0.257
Total viable cells recovered from sample ($x10^6$)	40	1.7	0.9, 2.8	38	1.6	0.6, 4.7	-2.0	-5.2, 1.2	0.209

5.12.1.2 Sputum cell count

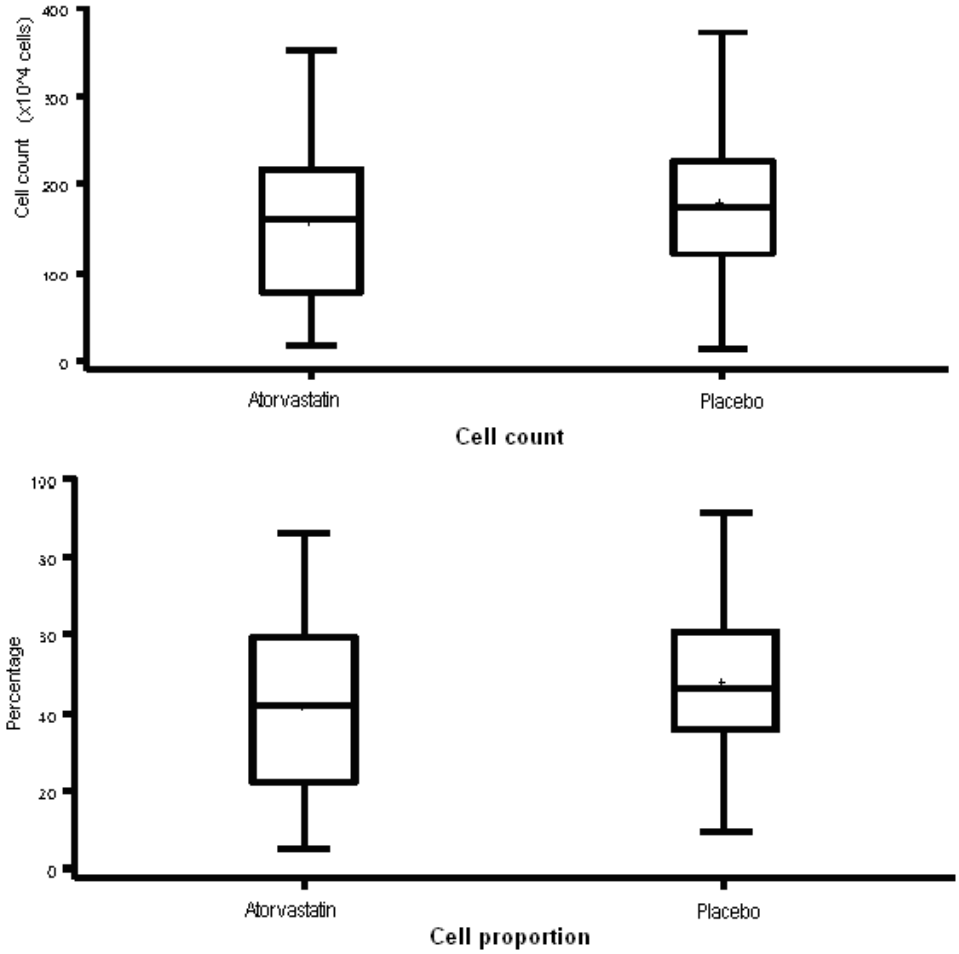
5.12.1.2.a Macrophages

There was a marked reduction in total macrophage count in the atorvastatin treated group [treatment difference -44.9 x10^4 cells (-80.1 to 9.7), p=0.015]. This corresponds to a reduction in the proportion of macrophages in the cell count [treatment difference -10.9% (-20.5 to -1.2), p=0.029] (table 5.44, figure 5.31).

Table 5.44 Sputum macrophage cell count and proportion for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
Macrophage cell count (x10^4 cells)	34	134.3	71.0, 201.0	32	171.25	135.0, 222.8	-44.9	-80.1, -9.7	0.015
Macrophage %	34	37.0	20.5, 52.5	32	45.3	36.5, 58.5	-10.9	-20.5, -1.2	0.029

Figure 5.31 Box and whisker plot for macrophage cell count and proportion after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. Significant differences are seen between groups (cell count p=0.015, percentage p=0.029).



5.12.1.2.b Neutrophils

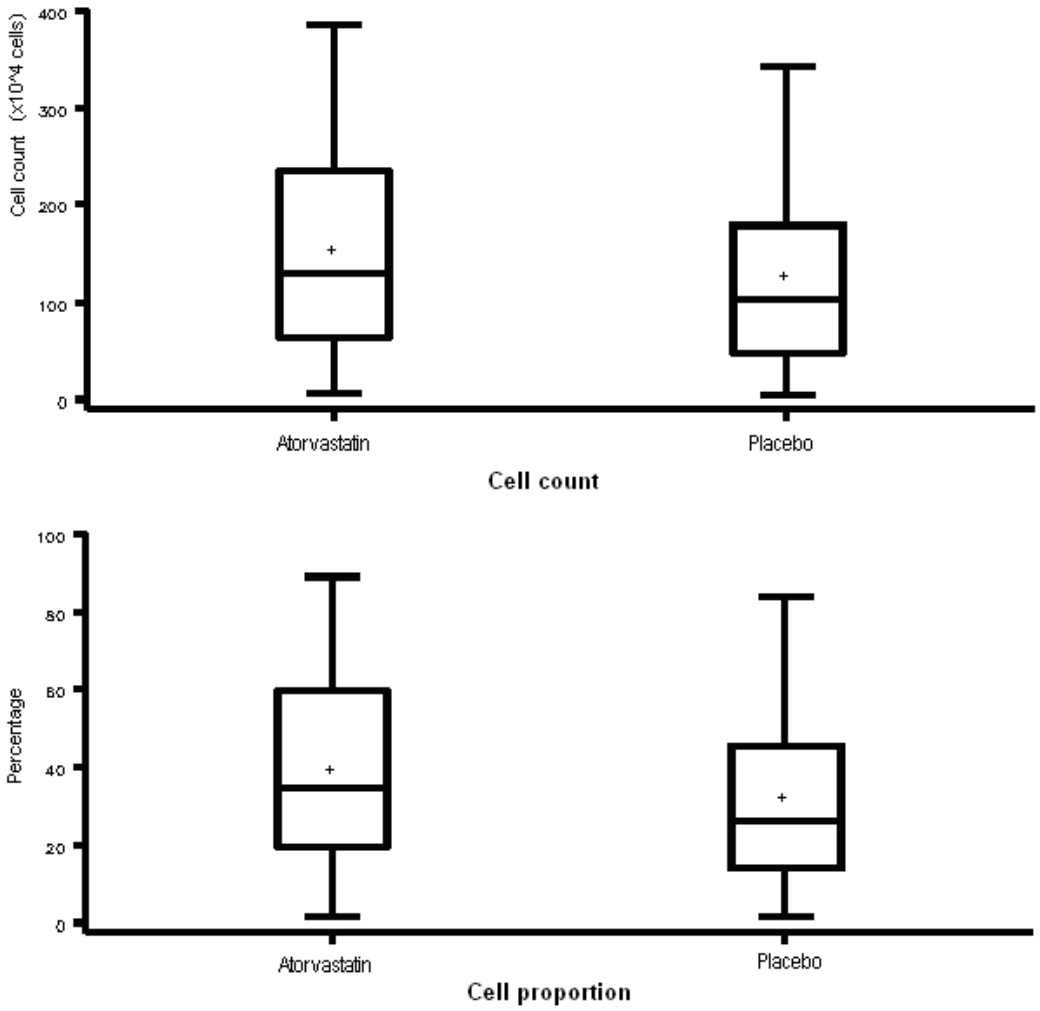
A small rise in neutrophil numbers were seen in the atorvastatin treated group [treatment difference 46.9 x10^4 cells (-2.1 to 95.9), p=0.060] (table 5.45, figure 5.32), with a

comparable shift in neutrophil percentage of total count [treatment difference 13.1% (1.8 to 24.3), p=0.025].

Table 5.45 Sputum neutrophil cell count and proportion for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
Neutrophil cell count ($x10^4$ cells)	34	133.3	81.5, 234.0	32	100	50.3, 167.8	46.9	-2.1, 95.9	0.060
Neutrophil %	34	34.8	22.0, 65.0	32	25.0	15.0, 41.9	13.1	1.8, 24.3	0.025

Figure 5.32 Box and whisker plot for neutrophil cell count and proportion after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. Cell count shows a trend towards significance (p=0.060), while percentage shows a significant difference between groups (p=0.025).



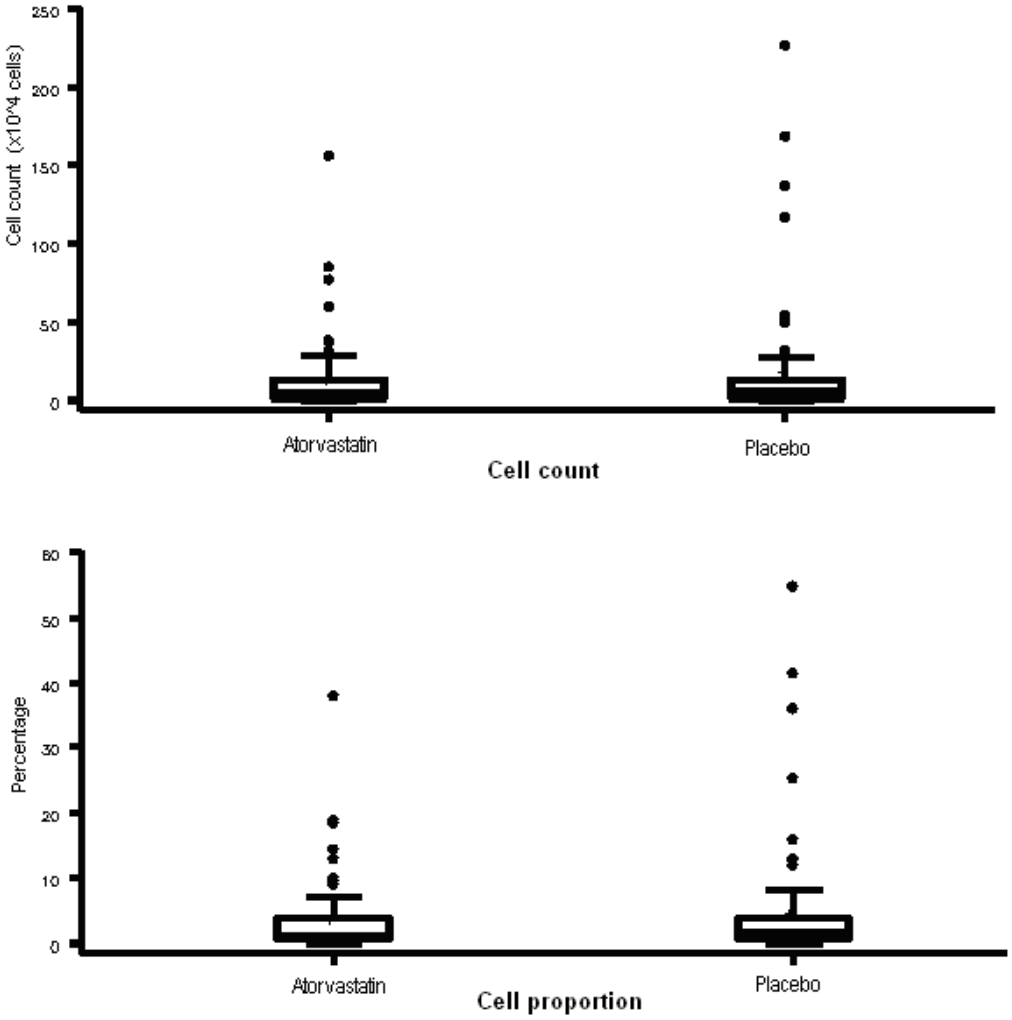
5.12.1.2.c Eosinophils

No difference was seen in sputum eosinophil count [treatment difference -12.1 x10^4 cells (-32.8 to 8.7), p=0.242] (table 5.46, figure 5.33), or eosinophil percentage of total count [treatment difference -2.7% (-7.0 to 1.7), p=0.219].

Table 5.46 Sputum eosinophil cell count and proportion for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
Eosinophil cell count (x10^4 cells)	34	3.8	2.0, 10.5	32	5	1.0, 10.5	-12.1	-32.8, 8.7	0.242
Eosinophil %	34	1.0	0.5, 3.0	32	1.5	0.4, 3.0	-2.7	-7.0, 1.7	0.219

Figure 5.33 Box and whisker plot for eosinophil cell count and proportion after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (cell count p=0.242, percentage p=0.219).



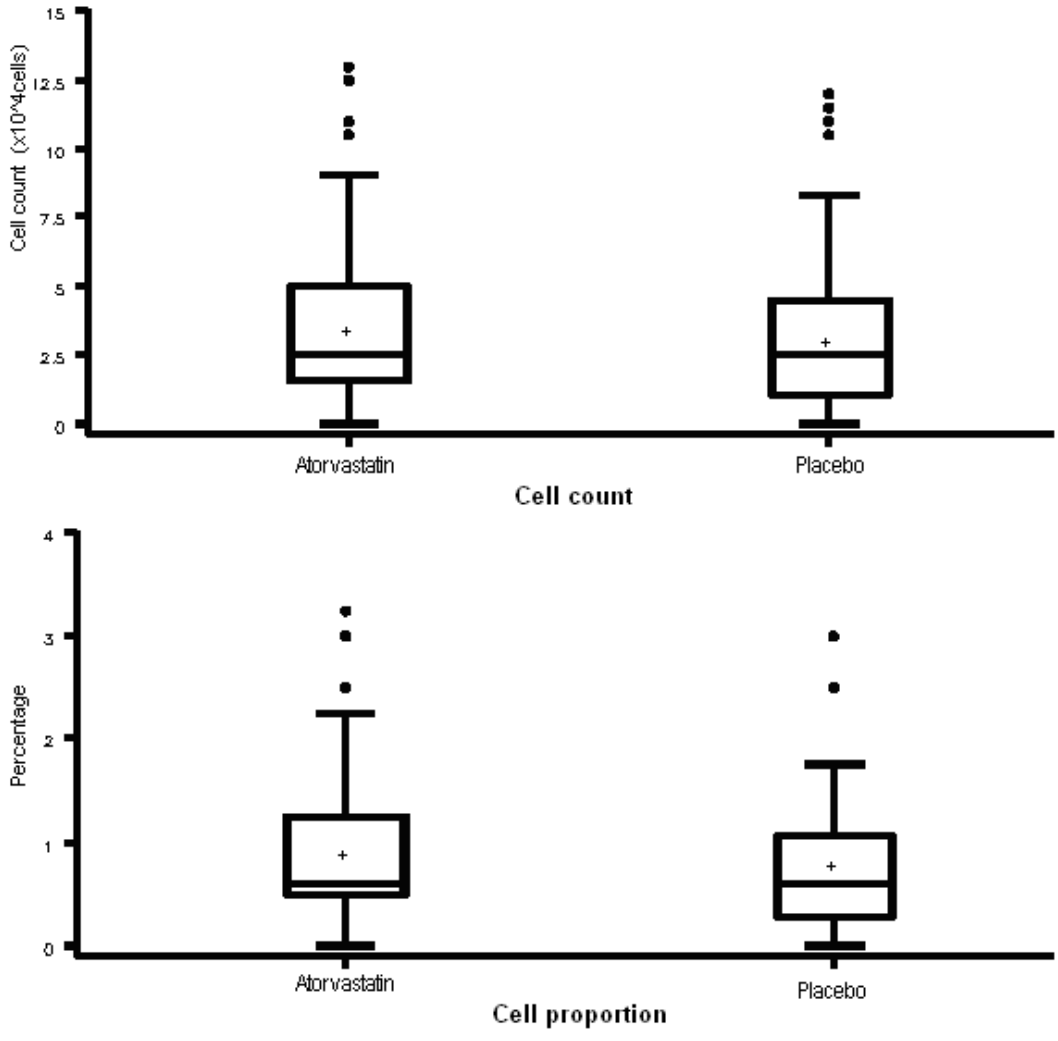
5.12.1.2.d Lymphocytes

No difference was seen in sputum lymphocyte count [treatment difference 0.5 x10^4 cells (-0.8 to 1.7), p=0.455] (table 5.47, figure 5.34), or lymphocyte percentage of total count [treatment difference 0.2% (-0.1 to 0.5), p=0.271].

Table 5.47 Sputum lymphocyte cell count and proportion for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Median	IQR	n	Median	IQR	difference		
Lymphocyte cell count x10^4 cells	34	2.0	1.0, 4.5	32	1.9	1.0, 5.0	0.5	-0.8, 1.7	0.455
Lymphocyte %	34	0.6	0.4, 1.0	32	0.5	0.3, 1.4	0.2	-0.1, 0.5	0.271

Figure 5.34 Box and whisker plot for lymphocyte cell count and proportion after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (cell count p=0.455, percentage p=0.271).



5.12.1.2.e Bronchial epithelial and squamous epithelial cells

No difference was seen in sputum bronchial epithelial cell count [treatment difference -3.4 x10^4 cells (-23.2 to 16.3), p=0.723], or percentage of total count [treatment difference 0.3% (-5.3 to 5.9), p=0.913] (table 5.48, figure 5.35). There was also no effect on squamous epithelial cell count [treatment difference -79.5 x10^4 cells (-201.6 to 42.6) p=0.191] or proportion [-5.5% (-17.0 to 6.0) p=0.333] (table 5.48, figure 5.36).

Table 5.48 Sputum bronchial epithelial and squamous epithelial cell counts and proportions for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
Bronchial epithelial cell count (x10^4 cells)	34	38.8	17.0, 59.0	32	33.5	18.8, 69.3	-3.4	-23.2, 16.3	0.723
Bronchial epithelial cell %	34	10.3	4.0, 24.0	32	11.9	5.0, 17.8	0.3	-5.3, 5.9	0.913
Squamous epithelial cell count (x10^4 cells)	34	156.0	101.0, 325.5	32	288.0	162.8, 352.5	-79.5	-201.6, 42.6	0.191
Squamous cells as % of absolute count	34	31.0	20.0, 46.5	32	41.8	25.3, 48.8	-5.5	-17.0, 6.0	0.333

Figure 5.35 Box and whisker plot for bronchial epithelial cell count and proportion after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (cell count p=0.723, percentage p=0.913).

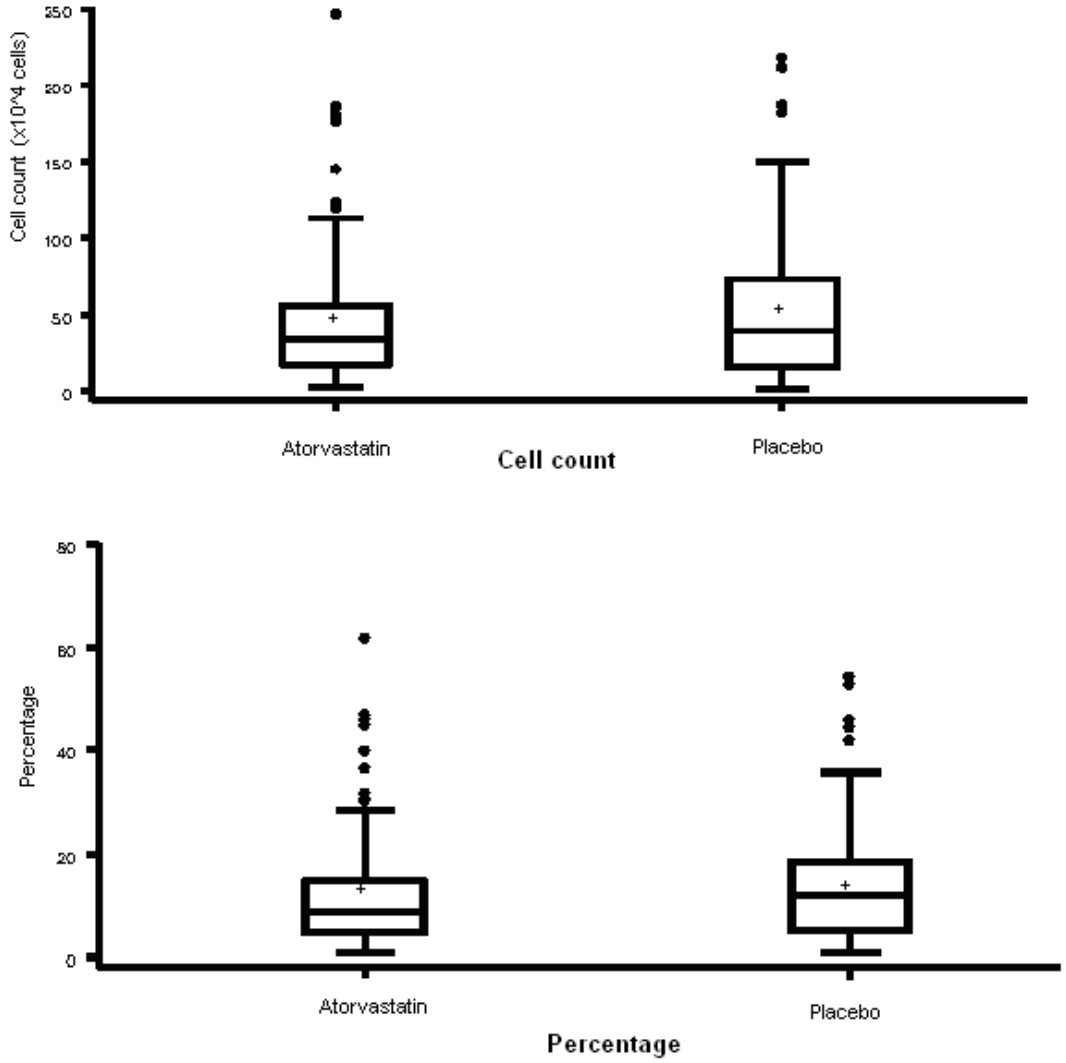
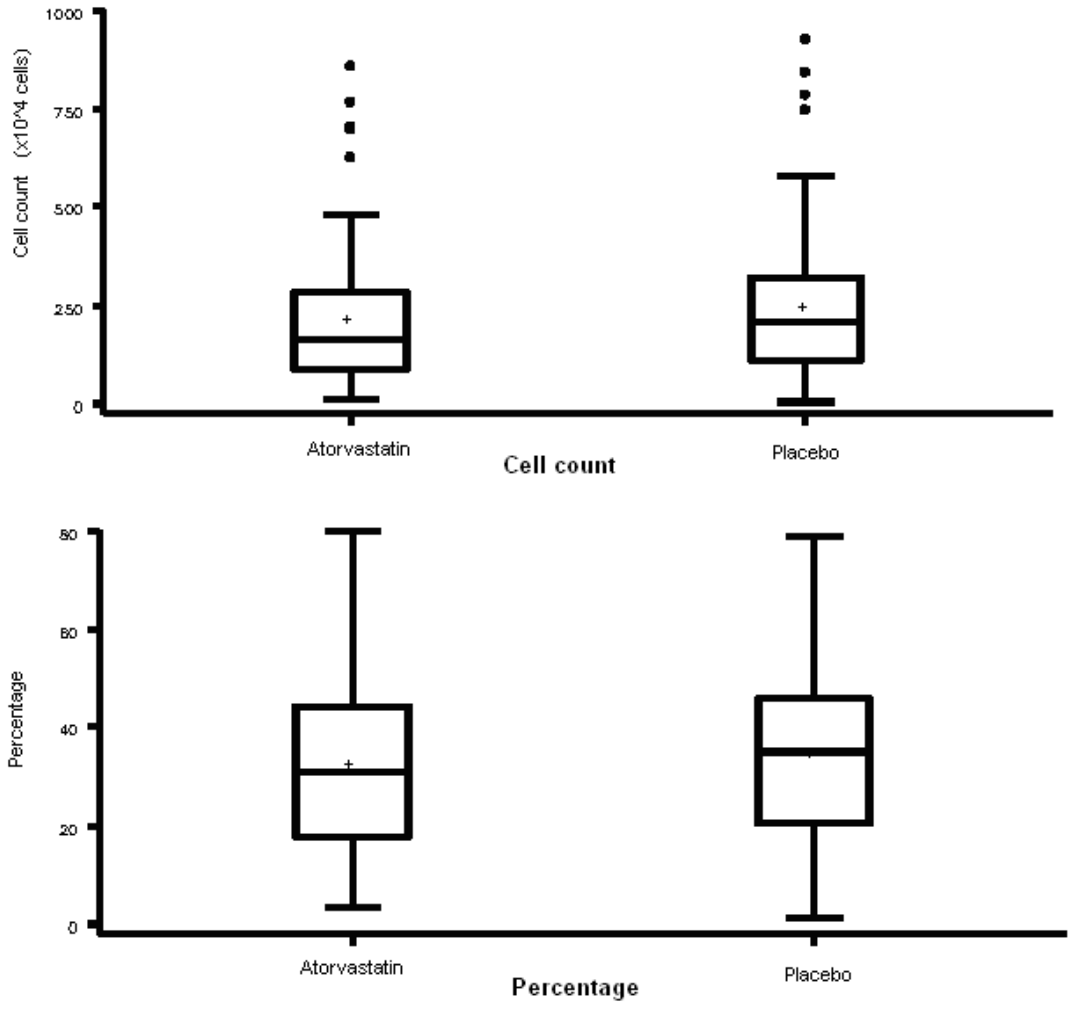


Figure 5.36 Box and whisker plot for squamous epithelial cell count and proportion after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (cell count p=0.191, percentage p=0.333).



5.12.2 Sputum cytokines

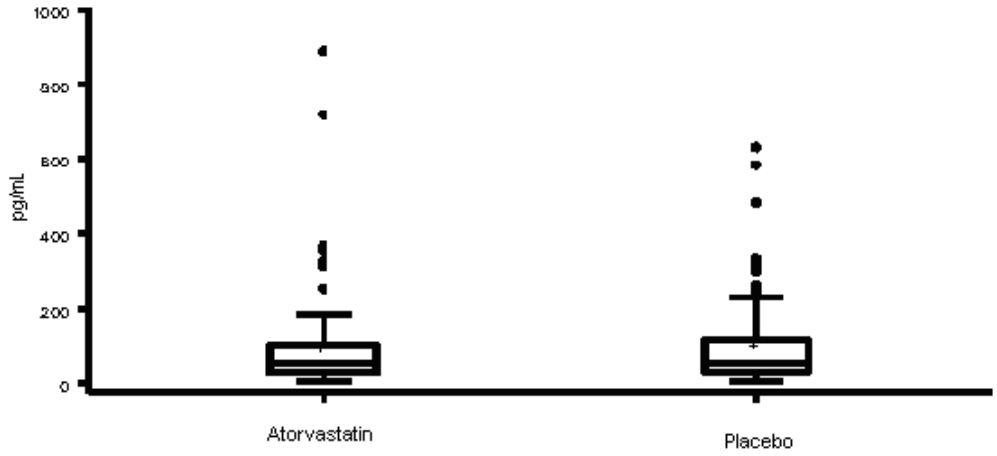
5.12.2.1 LTB_4

Sputum supernatant LTB_4 was significantly reduced in the atorvastatin treated group compared with placebo [treatment difference -88.1 pg/mL (95% CI -156.4 to -19.9) p=0.014] (table 5.49, figure 5.37).

Table 5.49 Sputum supernatant LTB_4 for atorvastatin compared with placebo after 8 weeks, with treatment difference. Lower sample numbers reflect the fact that the total number of assays that could be run was limited by the necessity of internal controls, as discussed in the methodology section.

Variable	Atorvastatin			Placebo			Treatment	95% CI	P
	n	Median	IQR	n	Median	IQR	difference		value
LTB_4 (pg/mL)	37	50.4	27.6, 79.8	33	68.2	28.6, 130.2	-88.1	-156.4, -19.9	0.014

Figure 5.37 Box and whisker plot for sputum supernatant LTB_4 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. A significant difference is seen between groups (p=0.014).



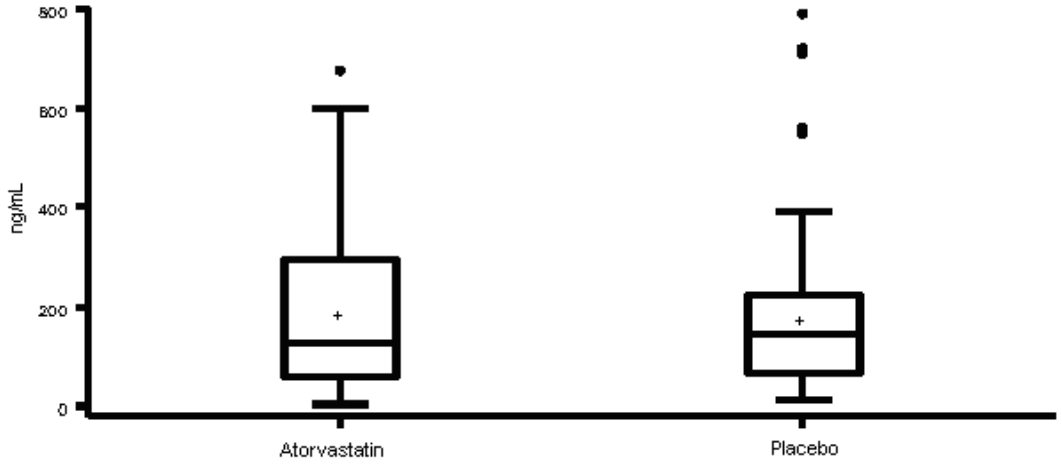
5.12.2.2 MPO

There was no significant difference in sputum supernatant MPO in the group treated with atorvastatin compared with placebo [treatment difference -32.6 ng/mL (95% CI -112.8 to 47.7) p=0.414] (table 5.50, figure 5.38).

Table 5.50 Sputum supernatant MPO for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Median	IQR	n	Median	IQR	difference		
MPO (ng/ml)	41	87.5	51.0, 193.0	38	112.3	56.5, 206.0	-32.6	-112.8, 47.7	0.414

Figure 5.38 Box and whisker plot for sputum supernatant MPO after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.414).



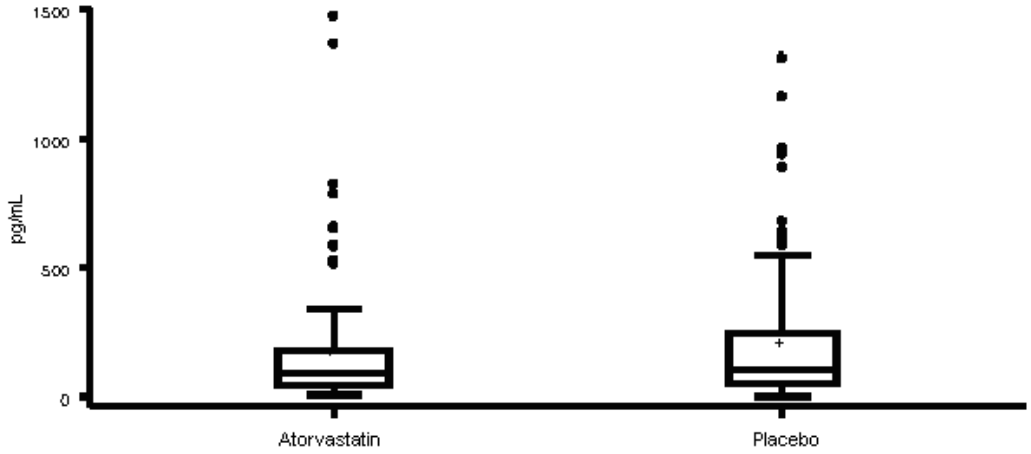
5.12.2.3 IL-1β

There was no significant difference in sputum supernatant IL-1β in the group treated with atorvastatin compared with placebo [treatment difference -35.8 ng/mL (95% CI -156.2 to 84.6) p=0.548] (table 5.51, figure 5.39).

Table 5.51 Sputum supernatant IL-1β for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P
	N	Median	IQR	n	Median	IQR	difference		value
IL-1β (pg/mL)	41	89.7	34.9, 178.14	38	92.6	34.7, 353.5	-35.8	-156.2, 84.6	0.548

Figure 5.39 Box and whisker plot for sputum supernatant IL-1β after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.548).



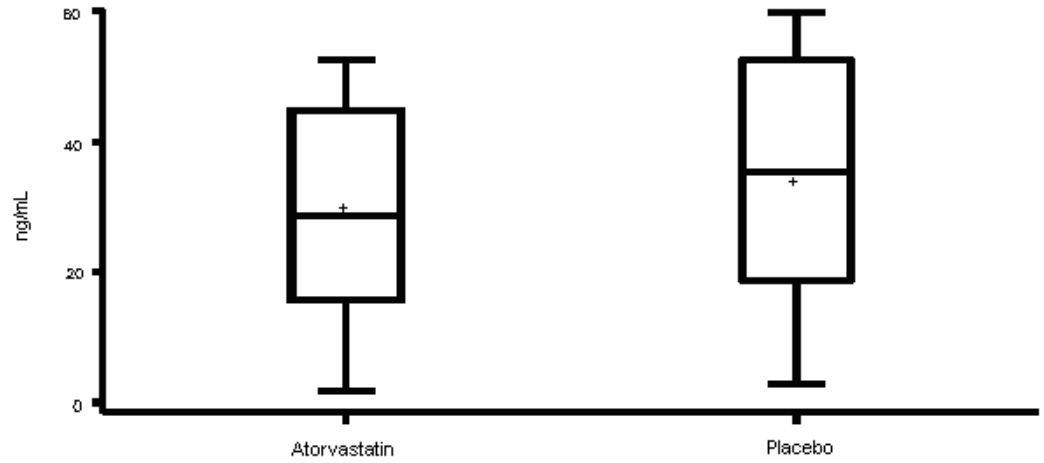
5.12.2.4 IL-1RA

There was no significant difference in sputum supernatant IL-1RA in the group treated with atorvastatin compared with placebo [treatment difference -3.4 ng/mL (95% CI -9.3 to 2.5) p=0.252] (table 5.52, figure 5.40).

Table 5.52 Sputum supernatant IL-1RA for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95%	P
	n	Median	IQR	n	Median	IQR	difference	CI	value
IL-1RA (ng/mL)	41	28.5	17.0, 46.0	38	35.4	19.3, 52.6	-3.4	-9.3, 2.5	0.252

Figure 5.40 Box and whisker plot for sputum supernatant IL-1RA after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.252).



5.12.2.5 IL-6, IL-8 and IL-17

There was no significant difference in sputum supernatant IL-6 in the group treated with atorvastatin compared with placebo [treatment difference -42.9 pg/mL (95% CI -118.7 to 33.0) p=0.258] (table 5.53, figure 5.41). IL-8 also showed no difference [-1607.3 pg/mL (-5678.1 to 2463.7) p=0.426] (table 5.53, figure 5.42). IL-17 was similarly unaffected [-30.5 pg/mL (-163.2 to 102.1) p=0.642] (table 5.53, figure 5.43).

Table 5.53 Sputum supernatant IL-6, IL-8 and IL-17 for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
IL-6 (pg/mL)	41	36.4	12.1, 75.6	38	48.1	12.9, 121.0	-42.9	-118.7, 33.0	0.258
IL-8 (pg/mL)	41	1474.2	544.0, 3615.7	38	1462.6	557.3, 5072.1	-1607.3	-5678.2, 2463.7	0.426
IL-17 (pg/mL)	41	41.8	3.6, 120.3	38	39.7	3.7, 342.3	-30.5	-163.2, 102.1	0.642

Figure 5.41 Box and whisker plot for sputum supernatant IL-6 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.258).

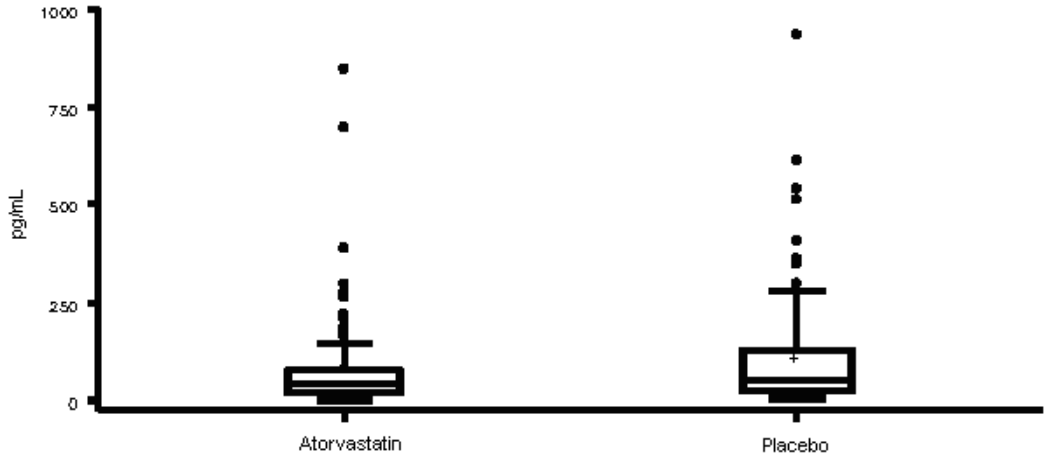


Figure 5.42 Box and whisker plot for sputum supernatant IL-8 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.426).

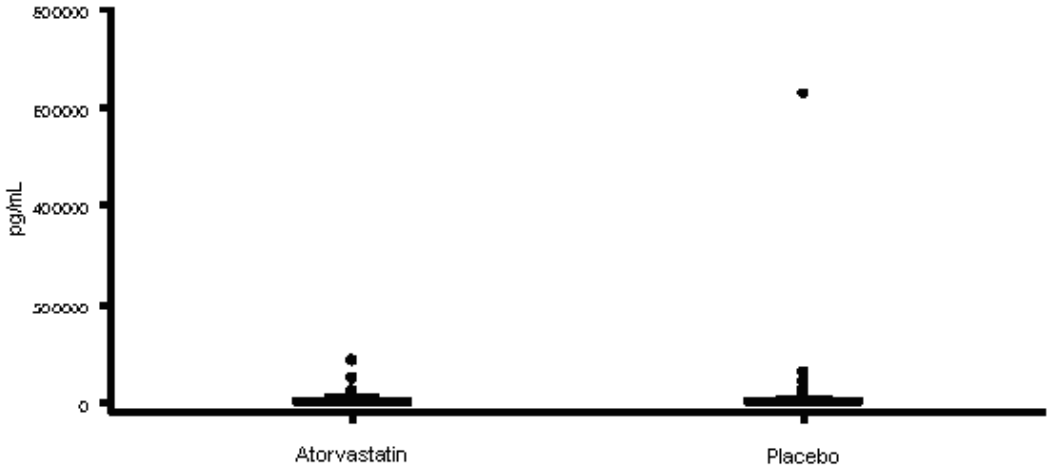
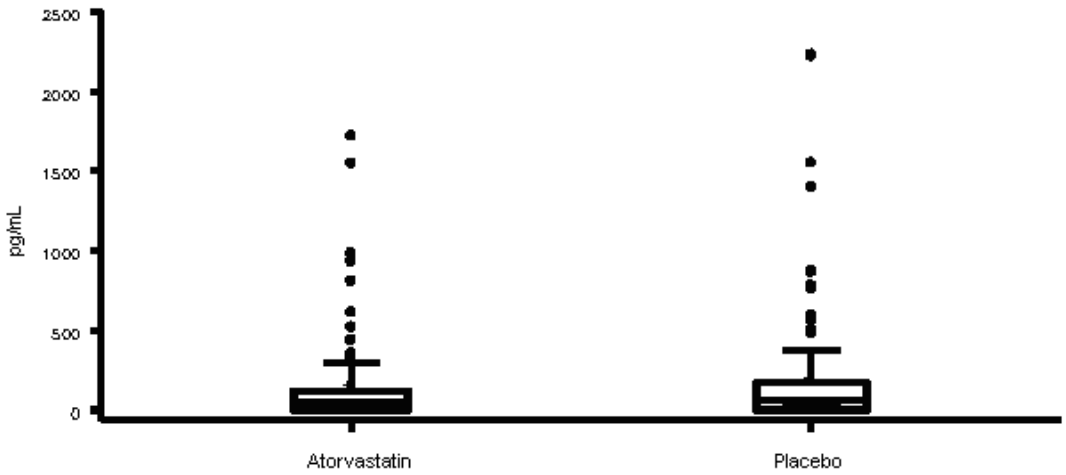


Figure 5.43 Box and whisker plot for sputum supernatant IL-17 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.642).



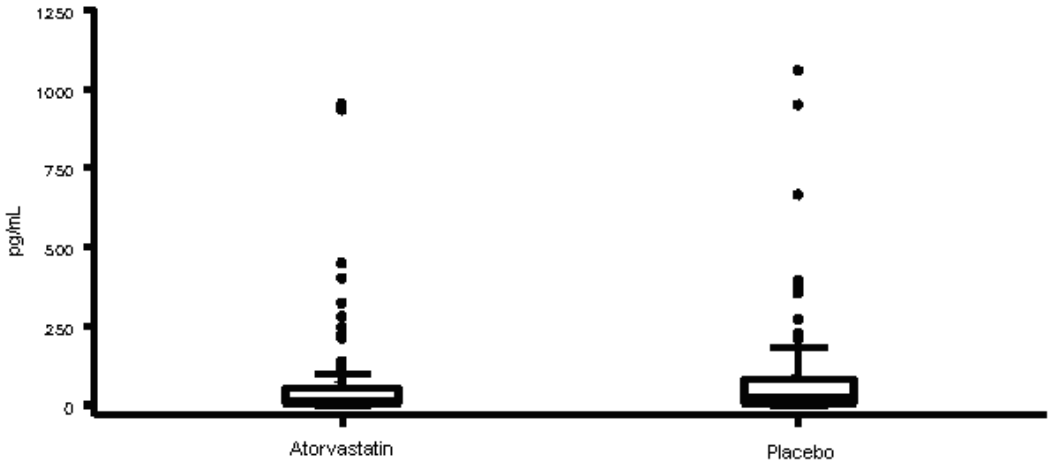
5.12.2.6 TNF-α

There was no significant difference in sputum supernatant TNF-α in the group treated with atorvastatin compared with placebo [treatment difference -30.3 pg/mL (95% CI -111.6 to 51.0) p=0.453] (table 5.54, figure 5.44).

Table 5.54 Sputum supernatant TNF-α for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P
	n	Median	IQR	n	Median	IQR	difference		value
TNFα (pg/mL)	41	12.5	4.7, 45.3	38	16.2	4.4, 123.0	-30.3	-111.6, 51.0	0.453

Figure 5.44 Box and whisker plot for sputum supernatant TNF-α after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.453).



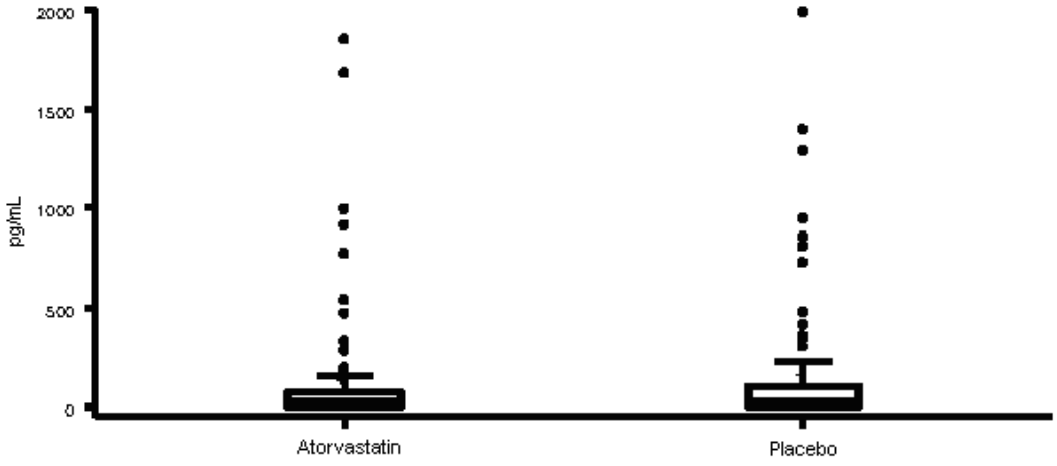
5.12.2.7 IFN-γ

There was no significant difference in sputum supernatant IFN-γ in the group treated with atorvastatin compared with placebo [treatment difference -38.4 pg/mL (95% CI -175.4 to 98.6) p=0.571] (table 5.55, figure 5.45).

Table 5.55 Sputum supernatant IFN-γ for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P
	n	Median	IQR	n	Median	IQR	difference		value
IFN-γ (pg/mL)	41	23.9	3.8, 678.0	38	21.5	3.1, 155.5	-38.4	-175.4, 98.6	0.571

Figure 5.45 Box and whisker plot for sputum supernatant IFN-γ after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.571).



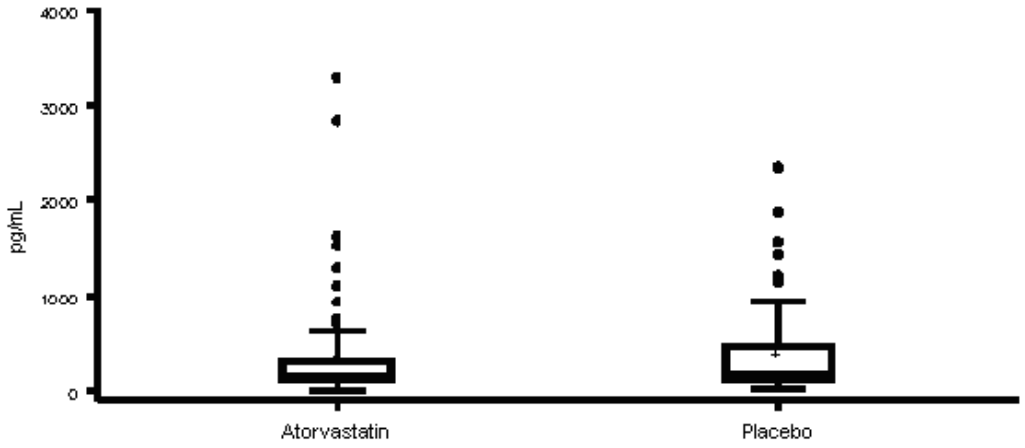
5.12.2.8 GM-CSF

There was no significant difference in sputum supernatant GM-CSF in the group treated with atorvastatin compared with placebo [treatment difference -44.2 pg/mL (95% CI -262.5 to 174.1) p=0.682] (table 5.56, figure 5.46).

Table 5.56 Sputum supernatant GM-CSF for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P
	n	Median	IQR	n	Median	IQR	difference		value
GM-CSF (pg/mL)	41	144.5	87.4, 287.5	38	159.4	99.7, 664.7	-44.2	-262.5, 174.1	0.682

Figure 5.46 Box and whisker plot for sputum supernatant GM-CSF after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.682).



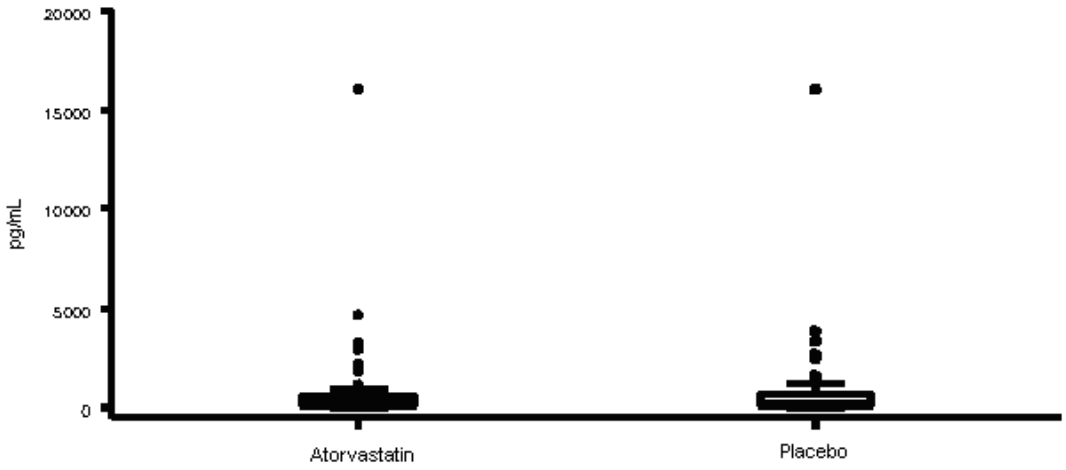
5.12.2.9 MIP-1α

There was no significant difference in sputum supernatant MIP-1α in the group treated with atorvastatin compared with placebo [treatment difference -513.2 pg/mL (95% CI -1588.4 to 562.0) p=0.337] (table 5.57, figure 5.47).

Table 5.57 Sputum supernatant MIP-1α for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
MIP-1α (pg/mL)	41	195.1	64.1, 466.8	38	176.4	63.0, 897.4	-513.2	-1588.4, 562.0	0.337

Figure 5.47 Box and whisker plot for sputum supernatant MIP-1α after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.337).



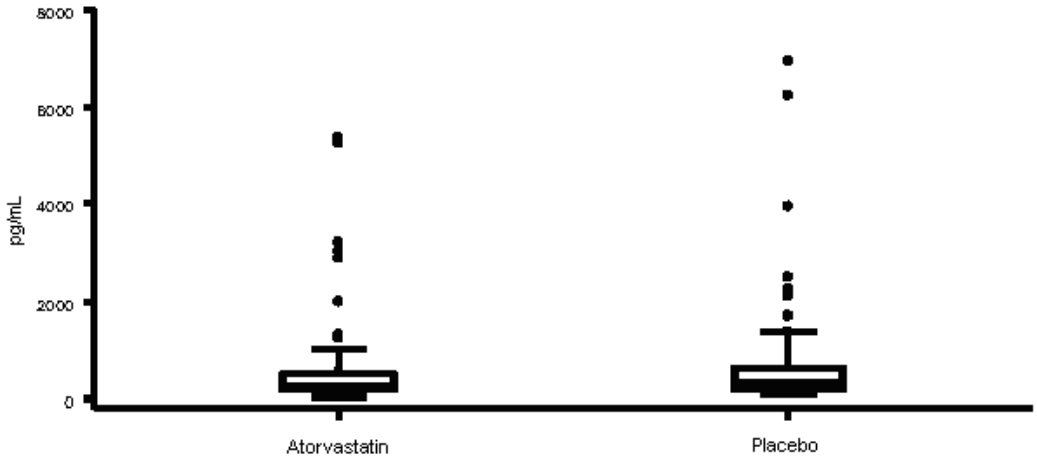
5.12.2.10 CCL2

There was no significant difference in sputum supernatant CCL2 in the group treated with atorvastatin compared with placebo [treatment difference -137.3 pg/mL (95% CI -612.7 to 338.2) p=0.560] (table 5.58, figure 5.48).

Table 5.58 Sputum supernatant CCL2 for atorvastatin compared with placebo after 8 weeks, with treatment difference.

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
CCL2 (pg/mL)	41	256.5	124.9, 495.6	38	300.9	124.9, 963.7	-137.3	-612.7, 338.2	0.560

Figure 5.48 Box and whisker plot for sputum supernatant CCL2 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.560).



5.12.3 Serum markers of inflammation

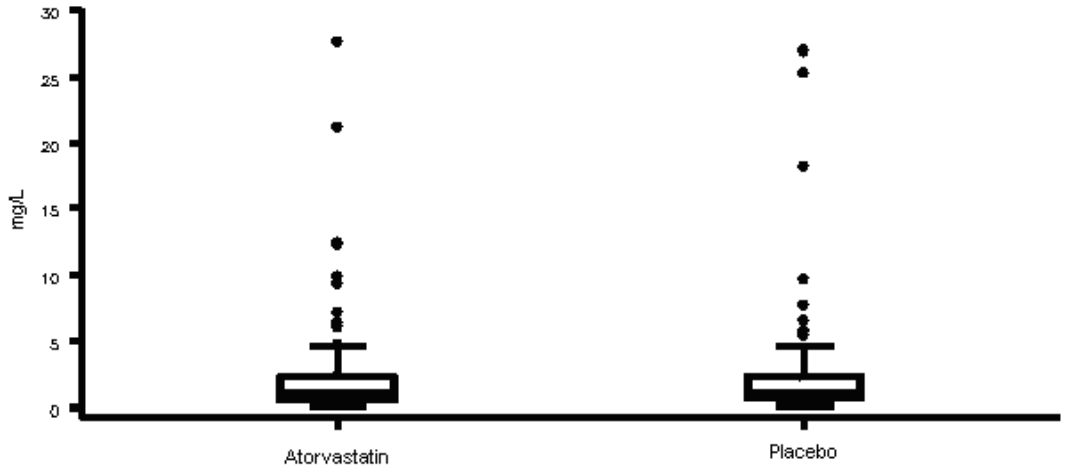
5.12.3.1 CRP

Serum CRP showed a trend towards decrease in the treatment group, although the result was not significantly different between atorvastatin and placebo after 8 weeks [treatment difference -0.7 mg/L (95% CI -1.4 to -0.1), p=0.082] (table 5.59, figure 5.49).

Table 5.59 Serum CRP for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Median	IQR	n	Median	IQR	difference		
CRP (mg/L)	47	0.6	0.5, 1.5	44	1.1	0.6, 2.5	-0.7	-1.4, 0.1	0.082

Figure 5.49 Box and whisker plot for serum CRP after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.086).



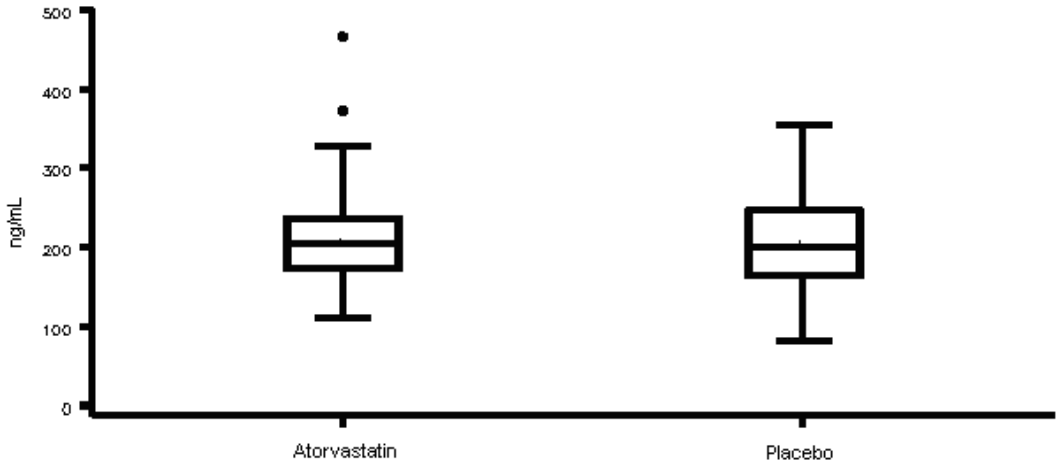
5.12.3.2 ICAM-1

No significant difference was seen in serum ICAM-1 between atorvastatin and placebo after 8 weeks [treatment difference -6.5 ng/mL (-21.4 to -8.4) p=0.382] (table 5.60, figure 5.50).

Table 5.60 Serum ICAM-1 for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Median	IQR	n	Median	IQR	difference		
ICAM-1 (ng/mL)	47	200.9	173.3, 221.8	46	204.6	164.1, 239.5	-6.5	-21.4, 8.4	0.382

Figure 5.50 Box and whisker plot for serum ICAM-1 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.382).



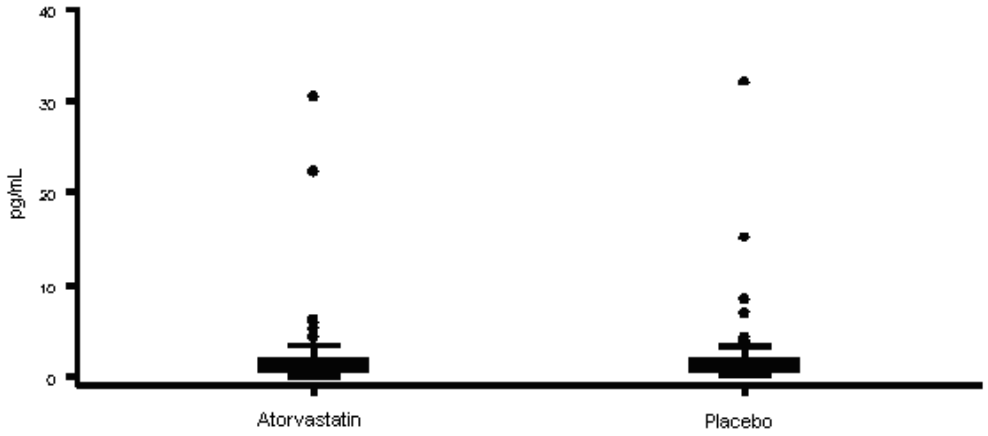
5.12.3.3 TNF-α

No significant difference was seen in serum TNF-α between atorvastatin and placebo after 8 weeks [treatment difference -0.5 pg/mL (-2.5 to -1.4), p=0.584] (table 5.61, figure 5.51).

Table 5.61 Serum TNF-α for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Median	IQR	n	Median	IQR	difference		
TNF-α (pg/mL)	47	1.0	0.6, 1.9	46	1.2	0.8, 1.9	-0.5	-2.5, 1.4	0.584

Figure 5.51 Box and whisker plot for serum TNF-α after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.584).



5.12.3.3 IL-1, IL-5, IL-6, IL-8 and IL-10

No significant difference was seen between atorvastatin and placebo after 8 weeks in serum IL-1 [treatment difference -10.2 pg/mL (-42.2 to 21.7), p=0.519] (table 5.62, figure 5.52), IL-5 [treatment difference -3.4 pg/mL (-11.3 to 4.5), p=0.378] (table 5.62, figure 5.53), IL-6 [treatment difference 0.0 pg/mL (-1.9 to 1.9), p=0.982] (table 5.62, figure 5.54), IL-8 [treatment difference -515.5 pg/mL (-1524.0 to 493.9), p=0.304] (table 5.62, figure 5.55), or IL-10 [treatment difference -5.8 pg/mL (-26.5 to 15.0), p=0.573] (table 5.62, figure 5.56).

Table 5.62 Serum IL-1, IL-5, IL-6, IL-8 and IL-10 for atorvastatin compared with placebo after 8 weeks, with treatment difference. Different samples sizes are due to beads sticking during analysis, as mentioned in the methodology section.

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Median	IQR	n	Median	IQR	difference		
IL-1 (pg/mL)	40	96.6	52.3, 142.5	42	97.1	56.1, 153.3	-10.2	-42.2, 21.7	0.519
IL-5 (pg/mL)	40	1.9	1.9, 21.6	31	1.9	1.9, 28.0	-3.4	-11.3, 4.5	0.378
IL-6 (pg/mL)	47	1.3	0.74, 3.12	46	1.2	0.7, 3.6	0.0	-1.9, 1.9	0.982
IL-8 (pg/mL)	40	0.7	0.6, 12.2	36	8.1	0.6, 17.5	-515.5	-1524.0, 493.9	0.304
IL-10 (pg/mL)	40	34.6	10.5, 70.9	39	38.5	10.5, 68.6	-5.8	-26.5, 15.0	0.573

Figure 5.52 Box and whisker plot for serum IL-1 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.519).

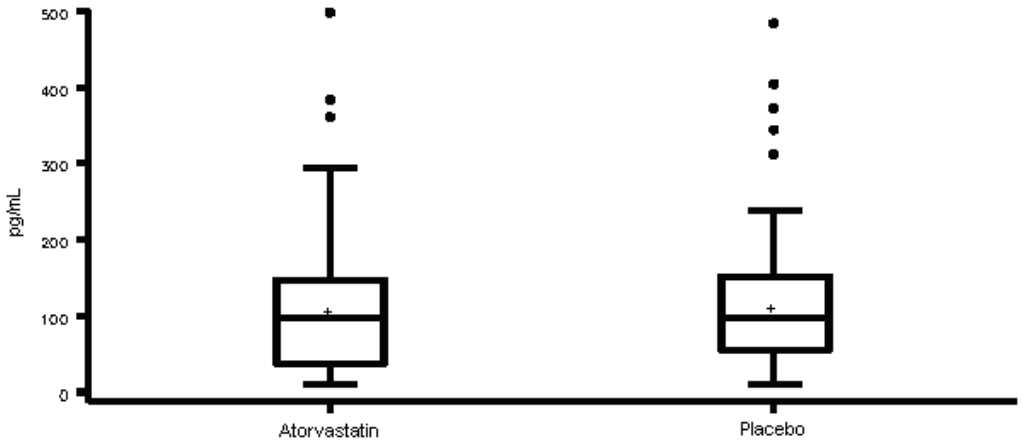


Figure 5.53 Box and whisker plot for serum IL-5 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.378).

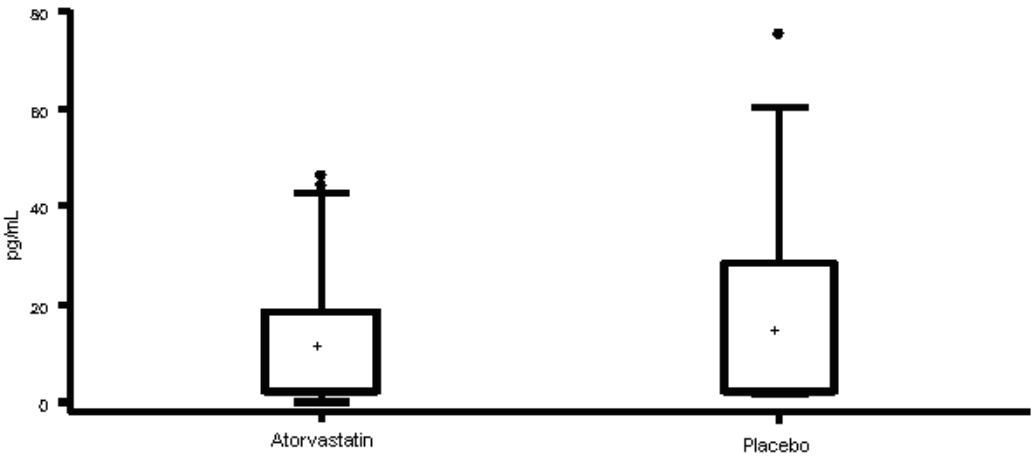


Figure 5.54 Box and whisker plot for serum IL-6 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.982).

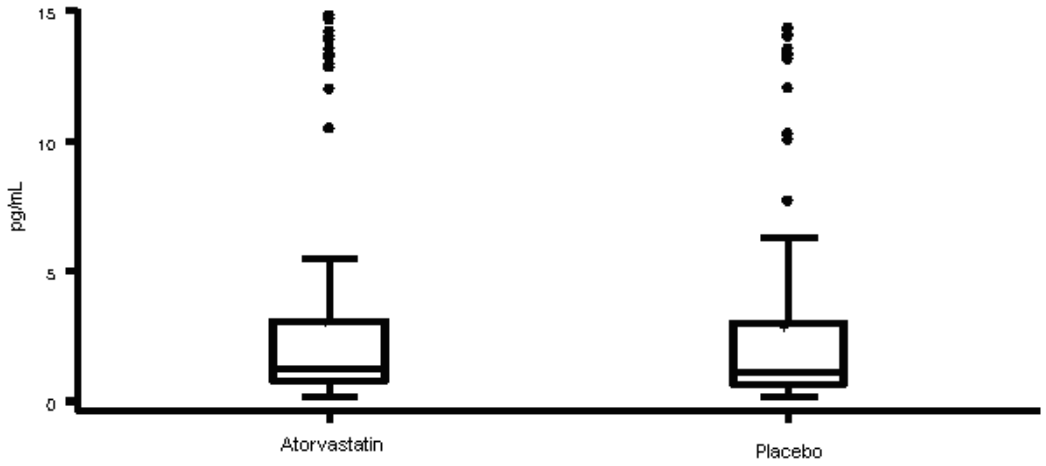


Figure 5.55 Box and whisker plot for serum IL-8 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.304).

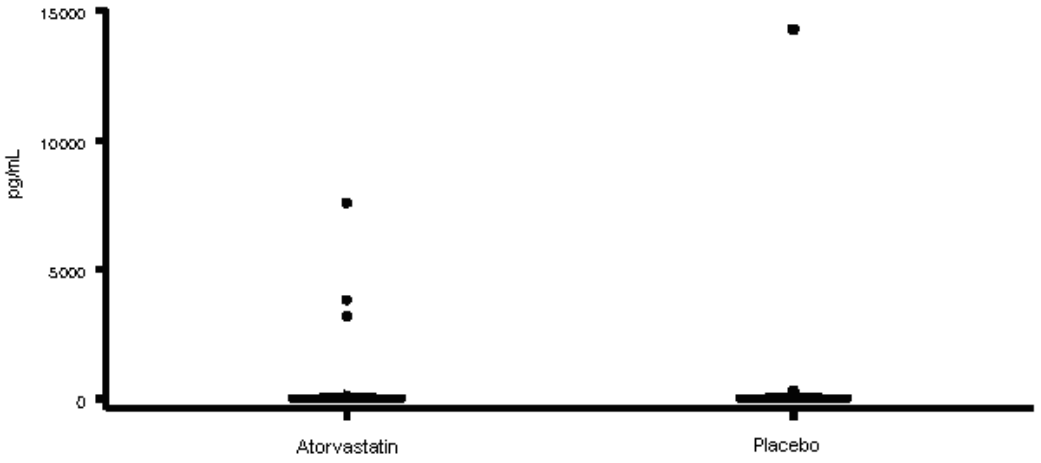
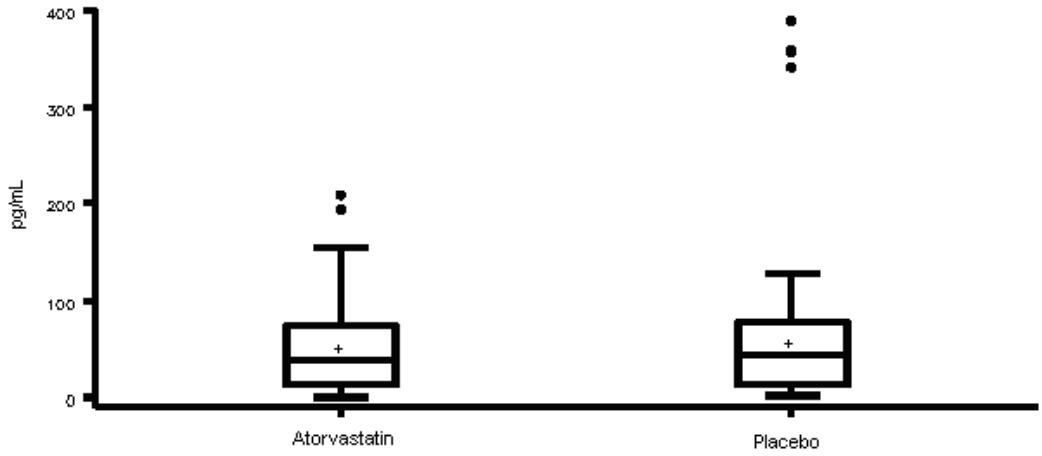


Figure 5.56 Box and whisker plot for serum IL-10 after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.573).



5.12.4 Lymphocyte proliferation

No significant difference was seen in lymphocyte proliferation between atorvastatin and placebo for control [treatment difference 107 counts/min/well (95% CI -89 to 303) p=0.276] (table 5.63, figure 5.57), PHA [treatment difference 4688 counts/min/well (-13351 to 22728) p=0.602] (table 5.63, figure 5.58) or anti-CD3/28 [treatment difference 3983 counts/min/well (-7681 to 15647) p=0.494] (table 5.63, figure 5.59).

Table 5.63 Lymphocyte proliferation results for atorvastatin compared with placebo after 8 weeks, with treatment difference

Variable	Atorvastatin			Placebo			Treatment difference	95% CI	P value
	n	Median	IQR	n	Median	IQR			
Control (counts/min/ well)	46	380	223, 691	46	329	186, 549	107	-89, 303	0.276
PHA (counts/min/ well)	46	5165	792, 43814	46	3422	375, 38951	4688	-13351, 22728	0.602
Anti-CD3/28 (counts/min/ well)	46	1544	494, 27139	46	1375	379,19088	3983	-7681, 15647	0.494

Figure 5.57 Box and whisker plot for lymphocyte proliferation (control) after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.276).

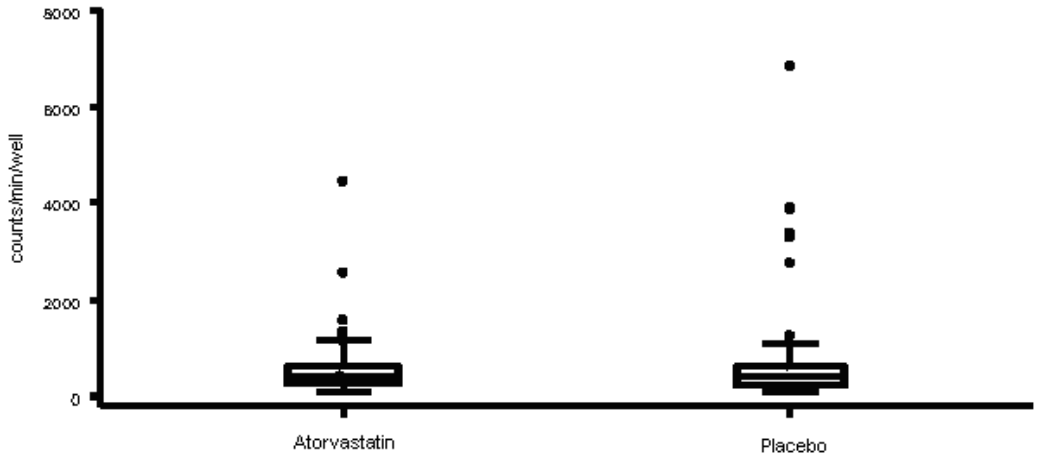


Figure 5.58 Box and whisker plot for lymphocyte proliferation (PHA) after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.602).

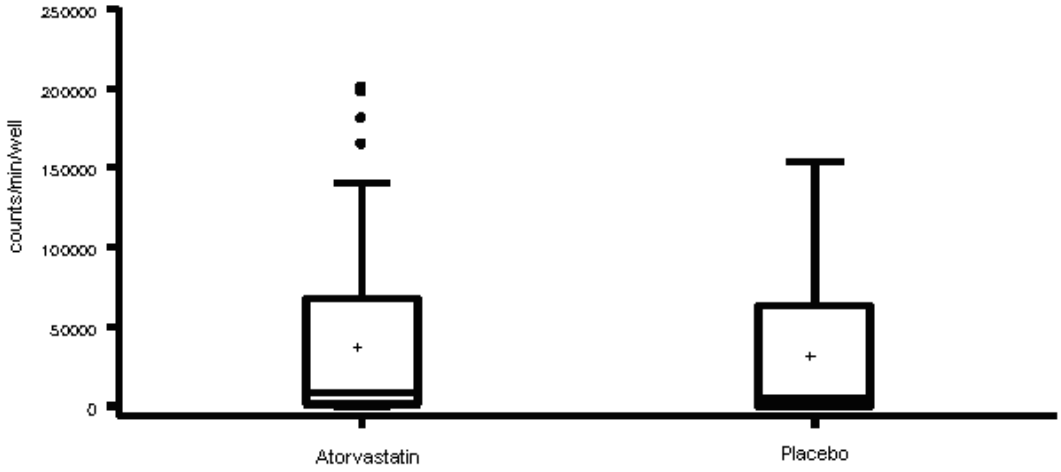
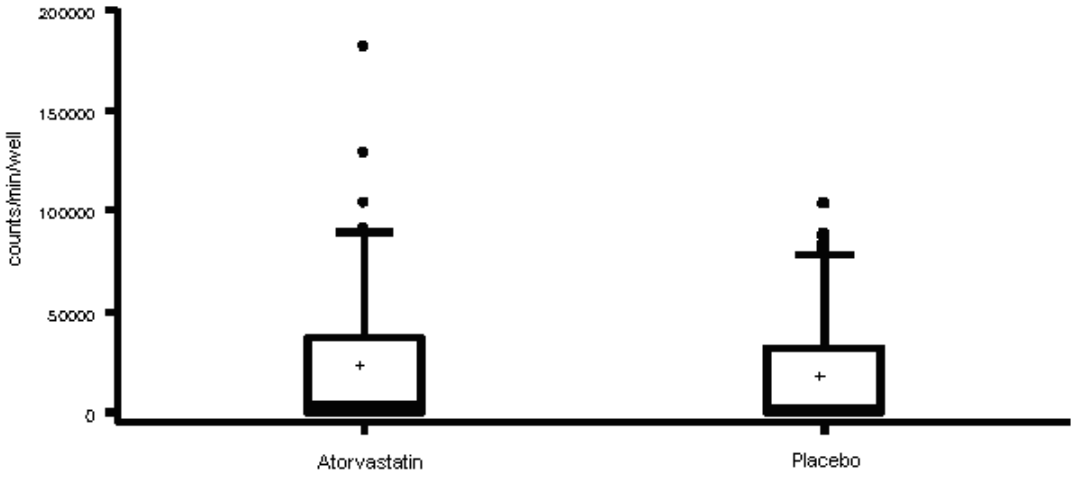


Figure 5.59 Box and whisker plot for lymphocyte proliferation (Anti-CD3/28) after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.494).



5.12.5 Exhaled gases

No difference was seen between atorvastatin and placebo for concentration of exhaled NO [treatment difference -1.6 ppb (95% CI -7.1 to 4.0), p=0.559] (table 5.64, figure 5.60), or CO [treatment difference -0.2 ppm (-0.6 to 0.3), p=0.438)] (table5.64, figure 5.61).

Table 5.64 Exhaled nitric oxide and carbon monoxide results for atorvastatin compared with placebo after 8 weeks, with treatment difference (due to machine dysfunction, one patient did not have an exhaled CO measurement at week 8 while on placebo).

Variable	Atorvastatin			Placebo			Treatment	95% CI	P value
	n	Median	IQR	n	Median	IQR	difference		
Exhaled NO (ppb)	44	16.2	9.0, 38.1	48	17.3	9.0, 42.7	-1.6	-7.1, 4.0	0.559
Exhaled CO (ppm)	44	4.2	3.7, 4.7	47	4.1	3.6, 5.1	-0.2	-0.6, 0.3	0.438

Figure 5.60 Box and whisker plot for exhaled nitric oxide after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.559).

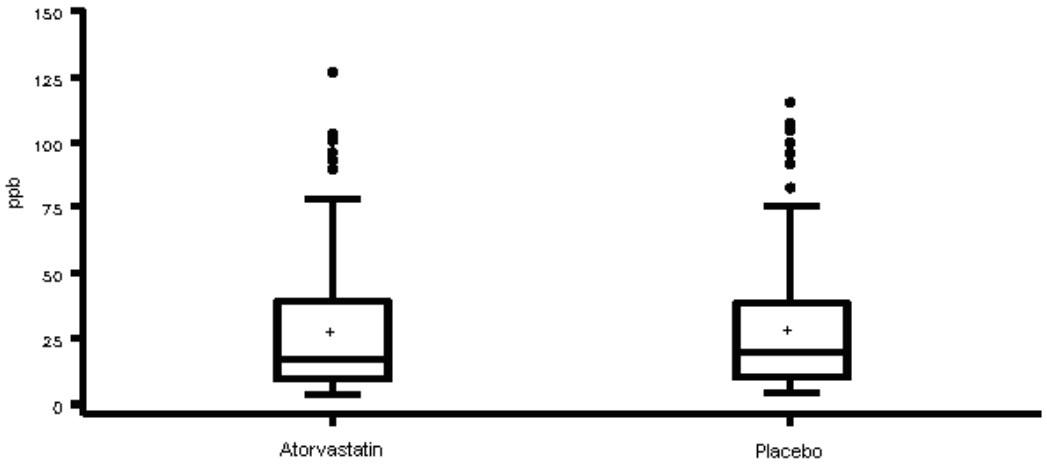
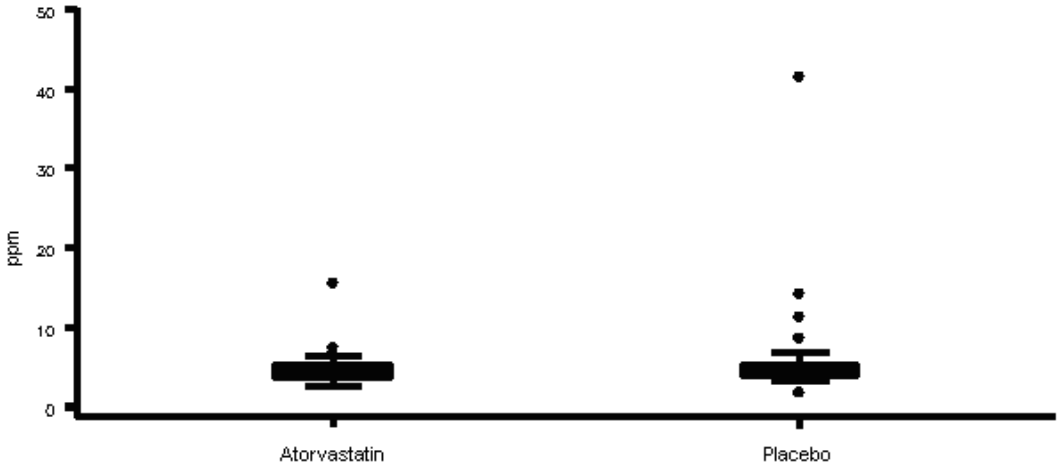


Figure 5.61 Box and whisker plot for exhaled carbon monoxide after 8 weeks for atorvastatin and placebo. The boxes represent the interquartile range, with the whiskers representing 1.5×IQR. Outliers are identified by *. Mean is indicated by +. No significant difference is seen between groups (p=0.438).



5.13 Inflammatory markers - summary

While no changes were seen in serum markers of inflammation or exhaled gases, changes were seen in sputum macrophage and neutrophil counts, with an associated fall in LTB_4 and a trend in reduction of CRP. This may suggest a localized reduction in inflammation, despite the lack of clinical effect. The implications of these changes are discussed in the following chapter.

5.14 Correlations

5.14.1 LTB_4

There was no correlation in week 8-week 0 delta between sputum LTB_4 and sputum IL-8, sputum macrophages, neutrophils or eosinophils, or with PEF (table 5.65).

Table 5.65 Pearson Correlation Coefficient in week 8-week 0 delta for LTB_4 with IL-8, sputum macrophage neutrophil and eosinophil count, and PEF

Variable	R value	R^2	P value	Number of observations
Sputum IL-8	0.220	0.048	0.114	53
Macrophage percentage	0.277	0.077	0.076	42
Macrophage cell count	0.114	0.013	0.471	42
Neutrophil cell count	-0.166	0.027	0.295	42
Neutrophil percentage	-0.166	0.027	0.294	42
Eosinophil cell count	-0.114	0.013	0.390	46
Eosinophil percentage	-0.090	0.008	0.500	46
Morning PEF	0.174	0.030	0.238	48
Evening PEF	-0.027	0.001	0.862	45

5.14.2 IL-8

There was no correlation at week 8-week 0 delta between sputum IL-8 and sputum macrophages, neutrophils or eosinophils, or with PEF (table 5.66).

Table 5.66 Pearson Correlation Coefficient in week 8-week 0 delta for IL-8 with sputum macrophage, neutrophil and eosinophil count, and PEF

Variable	R value	R^2	P value	Number of observations
Macrophage percentage	0.073	0.005	0.619	49
Macrophage cell count	0.072	0.005	0.623	49
Neutrophil cell count	-0.064	0.004	0.663	49
Neutrophil percentage	-0.073	0.005	0.618	49
Eosinophil cell count	0.147	0.021	0.240	46
Eosinophil percentage	0.143	0.021	0.251	46
Morning PEF	0.008	0.000	0.956	56
Evening PEF	-0.027	0.001	0.847	53

5.14.3 Sputum macrophages

There was a good correlation in week 8-week 0 delta between sputum macrophage cell count and neutrophils (r=-0.842, p<0.0001for cell counts). There was no correlation with eosinophils or PEF (table 5.67).

Table 5.67 Pearson Correlation Coefficient in week 8-week 0 delta for sputum macrophage count with neutrophil and eosinophil count and percentage, and PEF

Variable	R value	R^2	P value	Number of observations
Neutrophil cell count	-0.842	0.918	<0.0001	50
Neutrophil percentage	-0.848	0.921	<0.0001	50
Eosinophil cell count	-0.211	0.045	0.088	46
Eosinophil percentage	-0.161	0.026	0.200	46
Morning PEF	-0.195	0.442	0.193	46
Evening PEF	-0.011	0.103	0.946	44

5.14.4 Sputum neutrophils

There was no correlation in week 8-week 0 delta between sputum neutrophil cell count or percentage and eosinophils or PEF (table 5.68).

Table 5.68 Pearson Correlation Coefficient in week 8-week 0 delta for sputum neutrophil count with eosinophils and PEF

Variable	R value	R^2	P value	Number of observations
Eosinophil cell count	-0.092	0.009	0.458	46
Eosinophil percentage	-0.120	0.0.14	0.337	46
Morning PEF	0.246	0.496	0.099	46
Evening PEF	0.071	0.267	0.645	44

5.14.5 Sputum eosinophils

There was no correlation in week 8-week 0 delta between sputum eosinophil cell count or percentage and PEF (table 5.69).

Table 5.69 Pearson Correlation Coefficient in week 8-week 0 delta for sputum eosinophil count and PEF

Variable	R value	R^2	P value	Number of observations
Morning PEF	0.054	0.003	0.677	59
Evening PEF	0.132	0.017	0.310	48

5.15 Correlations summary

No correlation was seen between the inflammatory markers tested and macrophages, neutrophils, eosinophils or lung function. There was a significant correlation between sputum macrophages and neutrophils, and a trend towards a correlation between neutrophils and morning PEF.

6. DISCUSSION

6.1 Principal findings

This randomised controlled study examined the effects on asthma control and airway inflammation of oral atorvastatin 40 mg daily with that of a matched placebo in adults with allergic asthma. The hypothesis was that atorvastatin improves asthma control and airway inflammation in adults with asthma. We found that atorvastatin has no effect on a range of clinical indices of control of chronic allergic asthma after 2 months of treatment. Immunological investigations showed a change in both relative and absolute sputum macrophage and neutrophil counts, and an associated reduction in sputum LTB_4, but no other significant effects.

6.1.1 Clinical effects

The lack of any evidence of clinical benefit of atorvastatin in allergic asthma confirms and extends the findings of Menzies *et al* [243], who performed a crossover trial of simvastatin (2 weeks at 20mg, 2 weeks at 40mg vs. placebo with no washout) in 16 subjects with asthma. In our study the statin chosen, atorvastatin, was administered at a higher dose and for longer duration that the earlier study [243] and to larger group of participants (n=54), but we still showed no evidence of an improvement in asthma control.

6.1.2 Inflammatory outcomes

In addition to the cholesterol lowering effects of statins through the inhibition of HMG CoA reductase, statins have posited anti-inflammatory effects [244]. There is evidence that statins can influence the *in vitro* function of a range of inflammatory cells including T-lymphocytes [245], monocytes [246], macrophages [189], eosinophils [174] and neutrophils [161].

6.1.2.1 Eosinophils

Animal models suggest that statins decrease eosinophil survival [32] and increase eosinophil apoptosis [179]. This would suggest that patients treated with statins should show a decrease in sputum eosinophils. This was not the case in this study. In addition, to support the evidence of a lack of effect of statins on eosinophilic inflammation, there was no reduction in exhaled nitric oxide, which is closely linked with sputum eosinophilia [247]. Menzies at al [243] also failed to show any change in sputum eosinophils or eNO.

6.1.2.2 Macrophages

The reduction in sputum absolute alveolar macrophage count after atorvastatin treatment echoes similar findings in atherosclerosis [191]. Several mechanisms, either alone or in combination, might explain this decrease. Reduced macrophage growth, decreased recruitment, antioxidant effects, decreased cell adhesion and/or an altered cytokine profile may all go some way towards explaining the effect on macrophages seen in this study.

6.1.2.2.a Reduced macrophage growth

Statins reduce growth of both animal and human macrophages [191, 248] and possibly atorvastatin had a similar effect on sputum alveolar macrophage numbers.

6.1.2.2.b Decreased macrophage recruitment

LTB_4 is raised in induced sputum supernatant in asthma [249] and mediates the recruitment of alveolar macrophages through B leukotriene receptor 1 [250]. The decrease in sputum LTB_4 concentrations within the airways by atorvastatin therapy could contribute to the reduction found in sputum macrophage count. Atorvastatin has also been shown to reduce CCL2, involved in macrophage recruitment, in the arteries of hypercholesterolaemic rabbits [251], human vascular cells [252], and in *in vitro* models of sepsis [134].

6.1.2.2.c Antioxidant effects

Statins possess potent antioxidative properties [154, 155]. Simvastatin has been identified as an antioxidant in rat liver microsomes [157] and vascular smooth muscle [158], and human lipoprotein particles [159]. The reduced production of reactive oxygen species from human bronchial epithelial cells [253] and endothelial cells [124, 254] by atorvastatin might also influence alveolar macrophage function.

6.1.2.2.d Decreased cell adhesion

Statins could affect the chemokine and adhesion molecule-directed migration of inflammatory cells from blood into the airways [169, 175, 177]. Statins have specifically been shown to interfere with cell binding and macrophage recruitment to the lung [134, 255, 256]. Decreased monocyte to endothelial cell adhesion is due to attenuation of up-regulation of P-selectin on activated endothelial cells [133], and decreased expression of CCL2 receptor has also been seen on endothelial cells in rats [136, 137]. This reduction in binding could also relate to inhibition of LFA-1/ICAM-1 interaction [135], which could thus reduce macrophage function, although interestingly previous work suggests that expression of these molecules is increased by statins [256].

6.1.2.2.e Altered cytokine profile

Finally, statins reduce the *in vitro* release of cytokines and mediators including TNFα from monocytes [142], IL-6, IL-1β and endothelin-1 from endothelial cells, IL-5 and IFN-γ from human peripheral blood mononuclear cells (PBMC) [195], and *in vivo* circulating levels of TNFα, IL-6 [170], and CCL2, a chemokine responsible for the recruitment of monocytes to sites of inflammation [257, 258]. Similar effects on the release of these cytokines and mediators, including from cells within the airways could reduce the recruitment of alveolar macrophages to the airways. Against this latter mechanism, we found no reduction in sputum TNFα, or circulating IL-6, TNFα, and ICAM-1

concentrations following atorvastatin therapy. Future studies should examine these potential pathways.

In stable asthma alveolar macrophages are activated [259, 260] and show an increased capacity to release pro-inflammatory cytokines [261]. The absolute macrophage count in sputum is not elevated in asthma [262], and despite the reduction in absolute macrophage count by atorvastatin there was no improvement in clinical outcomes. It is not known whether the activation status of alveolar macrophages was impaired by atorvastatin in the study.

6.1.2.3 Neutrophils

In a mouse model, the neutrophilia associated with acute lung injury is markedly reduced with lovastatin treatment [180] and bronchoalveolar lavage neutrophil count is reduced in lung transplant recipients receiving statins [209]. Lovastatin has been shown to inhibit neutrophil chemotaxis [178]. Paradoxically we found a significant increase in the proportion of neutrophils in induced sputum with atorvastatin therapy. The absolute neutrophil counts were not significantly different between groups, suggesting that the increased proportion of neutrophils occurred because of the low macrophage proportion and the expression of the results as a percentage. In support of this explanation, the sputum LTB_4 concentration was reduced after atorvastatin therapy, which would be more likely to be associated with a reduced neutrophil count. LTB_4 is expressed mainly by activated macrophages and to a lesser extent neutrophils [250]. Decreased production of LTB_4 would

be consistent with a reduction in the numbers and activation status of sputum macrophage.

IL-8 is another important cytokine involved in the recruitment of neutrophils, and previous studies have suggested that lovastatin inhibits human alveolar epithelial production of IL-8 [178]. In this study however, the circulation and sputum concentrations of IL-8 were not altered by atorvastatin. There was no correlation between LTB_4 production and IL-8 production within patients in this study.

6.1.2.4 Inflammatory markers

Previous studies have shown that statins lower inflammatory markers such as CRP [91, 107 - 109] and IL-6 [112, 113] in ischaemic heart disease. Interestingly, this study showed very few anti-inflammatory effects of statin medication in patients with asthma. Most notably, there was no change in IL-6, and CRP reduction was only of borderline significance. Menzies *et al* also failed to show any change in CRP in their trial of simvastatin in asthma [243]. This contrasts with a recent study of atorvastatin in rheumatoid arthritis [164], where these were reduced by atorvastatin. This may be due to the fact that CRP levels were not high at baseline or due to lack of statistical power. Changes in CRP may also have been masked by concomitant use of inhaled corticosteroids.

However, a reduction in sputum LTB_4 was observed. LTB_4 is normally raised in asthma [263], and is expressed by macrophages and neutrophils. Decreased expression of

LTB_4 would be consistent with the alteration in sputum macrophage and neutrophil count, discussed above.

6.1.2.5 Summary

Despite the well-established anti-inflammatory actions of statins, it seems that they are not appropriate for the inflammatory phenotype associated with atopic asthma.

6.1.3 Duration of treatment

We estimated that 8 weeks duration of therapy was long enough to show a clinical effect, on the basis that a drop in cholesterol can already be seen within 6 weeks of statin treatment [99], and CRP has been shown to fall within four weeks [264], possibly even in the first two days [265]. Anti-inflammatory effects were seen in 28 days in animal models of allergic asthma [173]. It is however possible that a longer trial would have shown clinical effects. Alternatively, such changes may be more apparent in disease states with raised CRP levels, as a reduction of borderline significance was seen in the present study. It is possible that the administration of atorvastatin therapy for a longer duration might improve different clinical outcome measures of asthma such as indices of airway remodelling and statins have been shown to inhibit smooth muscle proliferation in both vascular [114] and airway smooth muscle [184, 203]and lower the expression of the profibrogenic cytokine transforming growth factor (TGF)-β1 [185]. Another possible line of investigation would be to measure rates of exacerbation, as previous studies have

suggested that statins decrease episodes of pneumonia [207] or may reduce the associated mortality [206].

6.1.4 Statin used

It is unlikely that using a different statin would have showed greater effects. An earlier trial in rheumatoid arthritis [164] used atorvastatin with success. However, the initial experimental work in inflammatory lung disease was using simvastatin [173] and pravastatin [174] with clear effect. Indeed, Kiener and colleagues [166] showed that lipophilic statins such as atorvastatin and simvastatin have a much greater effect on inflammatory responses in human and mouse models than the hydrophilic pravastatin. Additionally, Joukhadar *et al* showed no difference in effect on inflammatory parameters when comparing atorvastatin, simvastatin or pravastatin [266]. Furthermore no therapeutic effect was found in a previous trial of simvastatin in asthma [243], adding further weight to the idea that the lack of effect is not statin-specific.

6.1.5 Washout period

The six week washout period for this study was considered acceptable because evidence suggests that the anti-inflammatory effects do not last this long. Circulating levels of sVCAM-1 revert to normal in 2 days [267], CRP [268] and IL-6 begin to increase after at most 3 days and have returned to baseline after 7 days' withdrawal [187, 265, 269]. In contrast, a study examining CRP and MMPs showed that although there was a rapid restoration of CRP, MMPs remained unchanged 120 days after statin withdrawal [270]. In the event,

analysis of treatment-period interaction (Appendix 5) showed no significant results for any variable, indicating that the washout period selected was adequate.

6.1.6 Concomitant inhaled corticosteroid

One possible explanation for the apparent lack of efficacy in this study is that all patients were taking regular inhaled corticosteroid therapy. The anti-inflammatory action of this medication may be enough to overwhelm any modest effect from statins. This may in part explain why the observed reduction in CRP was of borderline significance, as corticosteroids have been shown to lower CRP in healthy individuals [271]. Interestingly, in the Menzies *et al* study, all patients were withdrawn from inhaled corticosteroids before treatment began, with patients receiving a long-acting $beta_2$-agonist instead [243]. This too could mask a subtle anti-inflammatory action [272]. They examined the effect of two weeks of 20mg and two weeks of 40mg simvastatin in a crossover trial of asthmatic patients taking LABA and SABA alone after withdrawal of corticosteroids and other anti-inflammatory medications. The study had no washout period. Their study also failed to show any effect on most clinical parameters.

An alternative would be to attempt to withdraw all long-acting medication from patients before starting statin therapy, but this is likely to create practical difficulties, as patients likely to benefit from this medication are unlikely to tolerate the withdrawal of all other long-acting treatments.

6.1.7 Compliance

Compliance with medication, as measured by tablet count, does not seem to have been problematic. This is borne out by the significant changes in serum lipids and liver function tests observed in the patients receiving atorvastatin.

6.2 Strengths and limitations of the study

6.2.1 The introduction of the EU Clinical trial directive.

The changes to regulation of clinical trials introduced in 2005 have had a wide-reaching impact on research in the UK [273 - 276]. In particular, the bureaucratic demands made of researchers have been significant. Every stage of the project has had additional delays due to the need to collect approval from sponsors, ethics and the MHRA. Many of these delays unfortunately had significant knock-on effects in terms of delays to recruitment. It has been argued that such cumbersome work, and particularly the demands placed on a sponsor, for a small-scale non-commercial trial may well hinder the implementation of hypothesis-testing work such as this in the future [277].

6.2.2 Recruitment issues and Generalisability

A Canadian cross-sectional study of asthma severity and prevalence [278] suggested that 78.9% of patients could be described as having mild to moderate asthma according to the Canadian Asthma Consensus Guidelines, broadly comparable with the patients included in the current study. Consequently, studying this group of patients is appropriate. If levels

of co-medication (e.g. with LABAs or leukotriene receptor antagonists) increase, then findings in milder patients may become less generalisable in future.

6.2.3 Severity of asthma

Baseline measures of asthma severity in this study indicate relatively mild asthma (all spirometric measures >75% predicted), with relatively low inflammatory indices (e.g. geometric mean methacholine responsiveness 2.23 mg/mL, median eosinophils 1.5%). This is essentially a feature of available patients for recruitment – more severe patients tend to be on additional medications or be more poorly controlled, which would have excluded them from this study. It is worth noting that mean baseline reliever use was 2.3 puffs of inhaler per day, mean ATS severity score was 3.3, and mean ACQ 1.5. There was thus some scope for improvement in asthma control.

Repeat analysis of the results, using only patients with baseline sputum eosinophils above 3% (to investigate the effect on more severe asthmatics) still showed no change in outcomes, and indeed the observed effects on sputum were no longer seen (Table 6.1). Although this subgroup analysis cannot be conclusive, particularly considering the small sample size (n=11), it adds weight to the argument that there is no clinical effect to be found.

Table 6.1 Reanalysis of primary end point and significant findings from main study, using only patients with baseline sputum eosinophils >3% (n=11). Only serum cholesterol shows a statistically significant effect

Variable	Treatment difference	95% CI	p value
Morning PEF , L/min	-0.42	-33.34, 32.51	0.975
Evening PEF, L/min	4.83	-20.78, 30.44	0.648
FEV1 pre-salbutamol, L	0.08	-0.2, 0.4	0.595
FEV1 post-salbutamol, L	0.03	-0.2, 0.3	0.797
Macrophage cell count, x106	-4.9	-69.2, 59.4	0.842
Neutrophil cell count, x106	41.7	-29.7, 113.2	0.180
Eosinophil cell count, x106	-27.9	-79.3, 23.4	0.206
Serum CRP, mg/L	-0.3	-1.3, 0.70	0.470
Serum cholesterol, mmol/L	-1.8	-2.7, -1.0	0.002
Sputum LTB4, ng/mL	-69.8	-196.1, 56.4	0.199

6.2.4 Power of the study

The original power calculations indicated that a sample size of 44 would give 90% power to detect a difference in means of 20L/min in peak expiratory flow (PEF) (primary endpoint), assuming a standard deviation of differences of 40L/min, using a paired t-test with a 0.05 two-sided significance level. We recruited a total of 54 patients to ensure that

44 patients completed the study. Fewer patients provided sputum samples, thus limiting the potential of the trial to find significant differences between the groups. Secondary end points must this be regarded as "hypothesis-generating", rather than definitive.

6.3 Implications for future research

6.3.1. Mechanism of macrophage reduction

Future studies could examine the potential pathways discussed above for reduction of sputum macrophage count.

6.3.1 Different statins

While atorvastatin has not been shown to have any clinical effect, there might be merit in examining the effect of other statins in the same setting.

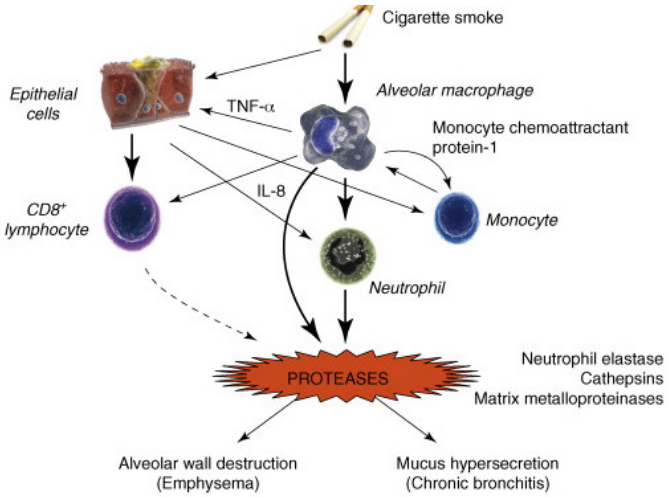
6.3.2 Smoking-related inflammatory lung disease

Smoking in asthma alters the clinical picture of the disease, with resistance to normal therapies, especially corticosteroids [194]. Most trials of asthma medications specifically exclude smokers, making this an area ripe for further investigation.

Although in the present study no improvement was seen in asthma control for non-smokers, a significant change in sputum macrophages and neutrophils was shown. This may have relevance to the treatment of other chronic lung diseases. Alveolar macrophages have been implicated in the pathogenesis of number of chronic lung

diseases, including COPD [279, 280]. The pathogenesis of COPD seems to involve both increased apoptosis and decreased clearance of apoptotic cells by macrophages (a process known as efferocytosis) [283 - 285], as well as an imbalance of macrophage and neutrophil-related inflammation in the lung parenchyma [286, 287] (figure 6.1). Lovastatin enhances the clearance of apoptotic cells by human and mouse macrophages, a statin-specific effect reversible with mevalonate, through modulation of Rho-GTPases [147, 148, 279, 280]. Lovastatin and simvastatin have also been shown to block Fc receptor-mediated phagocytosis by cultured human monocytes [149]. Macrophage expression and production of MMP-9 is also reduced by statins [191], which could have an impact on COPD. LTB_4 is also increased in COPD [288], and this study suggests that statins decrease expression. Recent evidence may also show that statins may inhibit lung parenchymal destruction, and peribronchial and perivascular infiltration of inflammatory cells in a rat model of smoking-induced emphysema [186]. Furthermore, a recent nested case-control study in humans has also suggested that statins may decrease respiratory mortality in patients with COPD [197].Taken together with the reduction in the sputum macrophage count with atorvastatin found in this study, these findings imply that the efficacy of statins should be investigated in COPD.

Figure 6.1 Chronic inflammation in COPD is driven initially by cigarette smoking and other inhaled irritants, which induce a specific pattern of inflammation that predominantly involves the peripheral airways and lung parenchyma. This pattern of inflammation is characterized by an increase in neutrophils, macrophages and CD8+ T lymphocytes in small and large airways and in lung parenchyma and pulmonary vasculature. Alveolar macrophages have a crucial part in orchestrating this inflammation through the release of proteases, such as MMP-9, inflammatory cytokines, such as TNF-α, and other cytokines, such as IL-8, that attract neutrophils into the airways. Reprinted from Cazzola *et al* [286], with permission from Elsevier



6.4 Conclusion

In conclusion, we have demonstrated that atorvastatin administered for 8 weeks to adults with mild to moderate atopic asthma resulted in no improvement in asthma control. The reduction in the sputum macrophage count suggests potential areas for investigation of atorvastatin in chronic lung disease in which activated alveolar macrophages have been implicated in the pathogenesis, such as COPD.

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Appendix 1: Asthma Diary

(from Santanello NC, Barber BL, Reiss TF, Friedman BS, Juniper EF, Zhang J. Measurement characteristics of two asthma symptom diary scales for use in clinical trials. *European Respiratory Journal* 1997;10:646-651.)

Circle year 2005/ 2006 **Effect of statins on control of chronic asthma**

Subject number		**MON**	**TUES**	**WED**	**THU**	**FRI**	**SAT**	**SUN**
	Day/Month DD/MM							
MORNING	**Peak flow (best of 3)**							
	Was blue (reliever) inhaler used in the 4 hours before peak flow testing?	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □
	1. How often did you experience asthma symptoms today? **0 → 6** **0= none of the time** **6 = all of the time**	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□
	2. How much did your asthma symptoms bother you today? **0 → 6** **0= not at all bothered** **6 = severely bothered.**	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□
	3. How much activity could you do today? **0 → 6** **0= more than usual activity** **6= less than usual activity.**	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□
	4. How often did your asthma affect your activities today? **0 → 6** **0=none of the time** **6=all of the time.**	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□	0□ 1□ 2□ 3□ 4□ 5□ 6□
EVENING	**Night peak flow (best of 3)**							
	Was blue inhaler taken within 4 hours of night peak flow ?	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □
	5. Did you wake up with asthma symptoms? **No = 0** **Once = 1** **More than once = 2** **Awake "all night" =3**	0□ 1□ 2□ 3□	0□ 1□ 2□ 3□	0□ 1□ 2□ 3□	0□ 1□ 2□ 3□	0□ 1□ 2□ 3□	0□ 1□ 2□ 3□	0□ 1□ 2□ 3□
	6. How many puffs of the blue inhaler did you use today?							
	Have you taken your study tablets today?	Yes □ No □ NA □	Yes □ No □ NA □	Yes □ No □ NA □	Yes □ No □ NA □	Yes □ No □ NA □	Yes □ No □ NA □	Yes □ No □ NA □

Appendix 2: ASTHMA QUALITY OF LIFE QUESTIONNAIRE (AQLQ)

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

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

	Totally Limited	Extremely Limited	Very Limited	Moderate Limitation	Some Limitation	A little Limitation	Not at all Limited
1. STRENUOUS ACTIVITIES (such as hurrying, exercising, running up stairs, sports)	1	2	3	4	5	6	7
2. MODERATE ACTIVITIES (such as walking, housework, gardening, shopping, climbing stairs)	1	2	3	4	5	6	7
3. SOCIAL ACTIVITIES (such as talking, playing with pets/children, visiting friends/relatives)	1	2	3	4	5	6	7
4. WORK-RELATED ACTIVITIES (tasks you have to do at work*) **If you are not employed or self-employed, these should be tasks you have to do most days.*	1	2	3	4	5	6	7
5. SLEEPING	1	2	3	4	5	6	7

HOW MUCH DISCOMFORT OR DISTRESS HAVE YOU FELT DURING THE LAST 2 WEEKS?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate Amount	Some Little	Very	None
6. How much discomfort or distress have you felt over the last 2 weeks as a result of CHEST TIGHTNESS?	1	2	3	4	5	6	7

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

	All of the time	Most of the time	A Good bit of the time	Some of the time	A Little of the time	Hardly any of the time	None of the time
7. Feel CONCERNED ABOUT HAVING ASTHMA?	1	2	3	4	5	6	7
8. Feel SHORT OF BREATH as a result of your asthma?	1	2	3	4	5	6	7
9. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO CIGARETTE SMOKE?	1	2	3	4	5	6	7
10. Experience a WHEEZE in your chest?	1	2	3	4	5	6	7
11. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF CIGARETTE SMOKE?	1	2	3	4	5	6	7

Appendix 2: ASTHMA QUALITY OF LIFE QUESTIONNAIRE (AQLQ)

HOW MUCH DISCOMFORT OR DISTRESS HAVE YOU FELT DURING THE LAST 2 WEEKS?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate Amount	Some	Very Little	None
12. How much discomfort or distress have you felt over the last 2 weeks as a result of COUGHING?	1	2	3	4	5	6	7

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

	All of the time	Most of the time	A good bit of the time	Some of time	A little the time	Hardly any of the time	None of of the time
13. Feel FRUSTRATED as a result of your asthma?	1	2	3	4	5	6	7
14. Experience a feeling of CHEST HEAVINESS?	1	2	3	4	5	6	7
15. Feel CONCERNED ABOUT THE NEED TO USE MEDICATION for your asthma?	1	2	3	4	5	6	7
16. Feel the need to CLEAR YOUR THROAT?	1	2	3	4	5	6	7
17. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO DUST?	1	2	3	4	5	6	7
18. Experience DIFFICULTY BREATHING OUT as a result of your asthma?	1	2	3	4	5	6	7
19. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF DUST?	1	2	3	4	5	6	7
20. WAKE UP IN THE MORNING WITH ASTHMA SYMPTOMS?	1	2	3	4	5	6	7
21. Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?	1	2	3	4	5	6	7
22. Feel bothered by HEAVY BREATHING?	1	2	3	4	5	6	7
23. Experience asthma symptoms as a RESULT OF THE WEATHER OR AIR POLLUTION OUTSIDE?	1	2	3	4	5	6	7

Appendix 2: ASTHMA QUALITY OF LIFE QUESTIONNAIRE (AQLQ)

24. Were you WOKEN AT NIGHT by your asthma?	1	2	3	4	5	6	7
25. AVOID OR LIMIT GOING OUTSIDE BECAUSE OF THE WEATHER OR AIR POLLUTION?	1	2	3	4	5	6	7
26. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO STRONG SMELLS OR PERFUME?	1	2	3	4	5	6	7
27. Feel AFRAID OF GETTING OUT OF BREATH?	1	2	3	4	5	6	7
28. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF STRONG SMELLS OR PERFUME?	1	2	3	4	5	6	7
29. Has your asthma INTERFERED WITH GETTING A GOOD NIGHT'S SLEEP?	1	2	3	4	5	6	7
30. Have a feeling of FIGHTING FOR AIR?	1	2	3	4	5	6	7

HOW LIMITED HAVE YOU BEEN **DURING THE LAST 2 WEEKS**?

	Most Not Done		Several Not Done		Very Few Not Done		No Limitation
31. Think of the OVERALL RANGE OF ACTIVITIES that you would have liked to have done during the last 2 weeks. How much has your range of activities been limited by your asthma?	1	2	3	4	5	6	7

HOW LIMITED HAVE YOU BEEN **DURING THE LAST 2 WEEKS?**

	Totally Limited	**Extremely Limited**	**Very Limited**	**Moderate Limitation**	**Some Limitation**	**A Little Limitation**	**Not at all Limited**
Overall, among ALL THE ACTIVITIES that you have done during the last 2 weeks, how limited have you been by your asthma?	1	2	3	4	5	6	7

Appendix 2: ASTHMA QUALITY OF LIFE QUESTIONNAIRE (AQLQ)

DOMAIN CODE:
SYMPTOMS: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 29, 30
ACTIVITY LIMITATION: 1, 2, 3, 4, 5, 11, 19, 25, 28, 31, 32
EMOTIONAL FUNCTION: 7, 13, 15, 21, 27
ENVIRONMENTAL STIMULI: 9, 17, 23, 26

Appendix 3: ASTHMA CONTROL QUESTIONNAIRE Score
Circle the number that best describes how your asthma has been during the night and this morning.

1 On average, during the past week, how often were you **woken by your asthma** during the night?

Never	0
Hardly ever	1
A few times	2
Several times	3
Many times	4
A great many times	5
Unable to sleep because of asthma	6

2 On average, during the past week, how **bad were your asthma symptoms when you woke** up in the morning?

No symptoms	0
Very mild symptoms	1
Mild symptoms	2
Moderate symptoms	3
Quite severe symptoms	4
Severe symptoms	5
Very severe symptoms	6

3 In general, during the past week, how **limited were you in your activities** because of your asthma?

Not limited at all	0
Very slightly limited	1
Slightly limited	2
Moderately limited	3
Very limited	4
Extremely limited	5
Totally limited	6

4 In general, during the past week, how much **shortness of breath** did you experience because of your asthma?

None	0
A very little	1
A little	2
A moderate amount	3
Quite a lot	4
A great deal	5
A very great deal	6

5 In general, during the past week, how much of the time did you **wheeze?**

Not at all	0
Hardly any time at all	1
A little of the time	2
A moderate amount of the time	3
A lot of the time	4
Most of the time	5
All the time	6

6 On average, during the past week, how many **puffs of short-acting bronchodilator** (eg Ventolin) have you used each day?

None	0
1-2 puffs most days	1
3-4 puffs most days	2
5-8 puffs most days	3
9-12 puffs most days	4
13-16 puffs most days	5
More than 16 puffs most days	6

To be completed by a member of the Research Team
[Record actual values in the shaded cells and score the FEV_1 % predicted in the last column]

FEV_1 prebronchodilator		> 95% predicted	0
		95-90%	1
FEV_1 predicted		89-80%	2
		79-70%	3
FEV_1 % predicted		69-60%	4
		59-50%	5
		<50% predicted	6

TOTAL SCORE	
SCORE / 7	

APPENDIX 4: MEASUREMENT OF NEBULISER OUTPUT - Wright's nebuliser

A4.1 Apparatus

Rotameter	3 ml syringe
Flow meter	Needle
O2 or air supply (50 psi)	Stop watch
Phosphate buffered saline	Digital balance
Wright nebuliser	

A4.2 Method

1. Add 3ml saline to vial
2. Attach vial to nebuliser and weigh on balance
3. Attach flow meter to rotameter
4. Adjust the flow meter until the rotameter indicates 6 l/min
5. Record the flow meter reading. (True flow rate is measured by the rotameter)
6. Disconnect the rotameter keeping the flow meter at exactly the same setting.
7. Attach the nebuliser to the flow meter for *exactly* 2 minutes.
8. Disconnect the flow meter and re-weight the nebuliser and vial. The difference in weight from Step 2 above is the nebuliser output for 2 minutes (assuming specific gravity of saline to be 1.0)
9. Repeat this procedure 5 times at true flow rates of 6 l/min, 7 l/min, and 8 l/min, 9 l/min and 10 l/min.
10. Mean the 5 measurements of nebuliser output.

Plot true flow rate against nebuliser output (figure A4.1) and read off the true flow rate that will give an output of 0.13 ml/min.

Plot flow meter reading against true flow rate (e.g. figure A4.2) to determine the flow meter setting that will produce an output of 0.13 ml/min.

Use this flow rate to operate the nebuliser during methacholine inhalation tests.

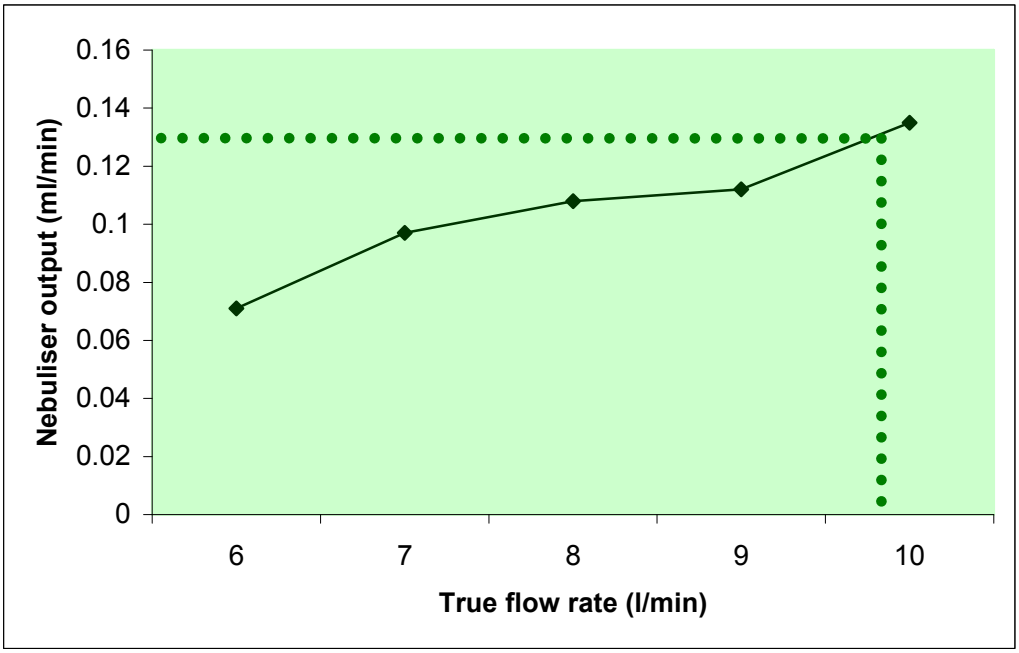

Figure A4.1 Calibration of nebuliser output: Nebuliser output versus true flow rate

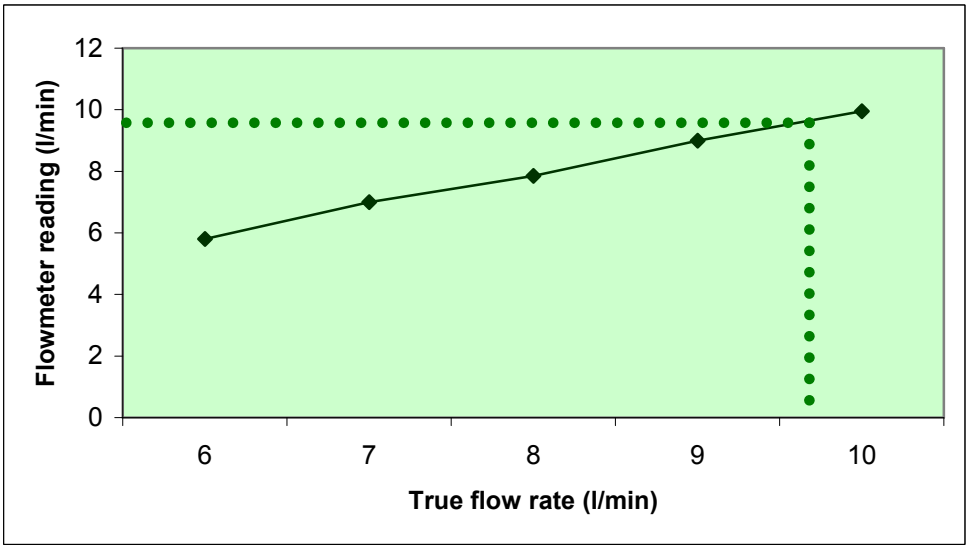

Figure A4.2 Calibration of nebuliser output: Flow meter reading versus true flow rate

Table A4.1 Calibration of nebuliser output: Data for calibration

Flow meter rate	Rotameter rate	Weight before	Weight after 2 minutes	Difference	Mean	Mean output/minute	Mean flow rate
5.75	6	107.33	107.21	0.12			
5.75	6	107.2	107.05	0.15			
6	6	107.05	106.91	0.14			
5.75	6	107.47	107.33	0.14			
5.75	6	107.58	107.42	0.16	0.142	**0.071**	**5.8**
7	7	108.03	107.83	0.2			
7	7	107.7	107.5	0.2			
7	7	107.75	107.56	0.19			
7	7	107.56	107.37	0.19			
7	7	107.8	107.61	0.19	0.194	**0.097**	**7**
8	8	107.77	107.54	0.23			
8	8	107.73	107.52	0.21			
7.5	8	107.52	107.32	0.2			
7.75	8	107.82	107.59	0.23			
8	8	107.91	107.69	0.21	0.216	**0.108**	**7.85**
9	9	107.88	1076.7	0.21			
9	9	107.39	107.17	0.22			
9	9	107.79	107.56	0.23			
9	9	108.07	107.84	0.23			
9	9	107.89	107.66	0.23	0.224	**0.112**	**9**
9.75	10	108.36	108.1	0.26			
10	10	107.99	107.71	0.28			
10	10	107.85	107.57	0.28			
10	10	107.85	107.57	0.28			
10	10	108.01	107.74	0.25	0.27	**0.135**	**9.95**

Appendix 5: Treatment-period interaction analysis.

Treatment-period interaction analysis for all outcomes in the trial. The p value represents the probability of no difference between groups, i.e. that the null hypothesis is true and both groups are the same. No values are statistically significant.

Variable	P value
Morning PEF	0.680
Evening PEF	0.666
Reliever inhaler use	0.887
ATS score	0.033
Asthma control Score	0.975
FEV_1 pre-salbutamol	0.081
FEV_1 post-salbutamol	0.167
% predicted FEV_1	0.360
FEV_1 reversibility	0.096
PEF (spirometry) pre-salbutamol	0.467
PEF (spirometry) post-sabutamol	0.610
% predicted PEF	0.846
FVC pre-salbutamol	0.110
FVC post-salbutamol	0.289
% predicted FVC	0.281
FEF_{25-75} pre-salbutamol	0.206
FEF_{25-75} post-salbutamol	0.407
% predicted FEF_{25-75}	0.383
FEV_1/FVC ratio pre-salbutamol	0.900
FEV_1/FVC ratio post-salbutamol	0.862
Sputum total cell count	0.471
Viability	0.610
Volume of filtrate	0.243
Total number of cells recovered from sample	0.303
Total viable cells recovered from sample	0.318
Macrophages	0.606
Neutrophils	0.198
Eosinophils	0.695
Lymphocytes	0.489

Variable	P value
Bronchial epithelial cells	0.358
Total non-squamous cells	0.674
Squamous epithelial cells	0.349
Absolute number of cells counted per slide	0.484
% macrophages	0.327
% neutrophils	0.204
% eosinophils	0.843
% lymphocytes	0.317
% bronchial epithelial cells	0.381
% squamous cells	0.567
Serum CRP	0.178
Serum ICAM	0.661
Serum TNFα	0.635
Serum IL-6	0.577
Serum IL-1	0.546
Serum IL-5	0.260
Serum IL-8	0.245
Serum IL-10	0.249
Serum Triglycerides	0.362
Serum Cholesterol	0.803
Serum HDL	0.595
Serum LDL	0.245
Serum Urea	0.576
Serum Potassium	0.250
Serum Sodium	0.975
Serum Bilirubin	0.395
Serum AST	0.716
Serum ALT	0.919
Exhaled NO	0.220
Exhaled CO	0.258
Quality of Life score	0.442
QOL Symptoms	0.326
QOL Activities	0.764
QOL Emotional well-being	0.213
QOL Environmental	0.850

Appendix 5

Variable	P value
Methacholine (PC_{20})	0.880
Sputum supernatant MPO	0.347
Sputum supernatant LTB_4	0.156
Sputum supernatant IL-1b	0.517
Sputum supernatant IL-1RA	0.584
Sputum supernatant IL-6	0.501
Sputum supernatant IL-8	0.460
Sputum supernatant IL-17	0.673
Sputum supernatant TNFα	0.387
Sputum supernatant IFNγ	0.437
Sputum supernatant GM-CSF	0.694
Sputum supernatant MIP1α	0.240
Sputum supernatant MCP1	0.596
Lymphocyte proliferation Control	0.071
Lymphocyte proliferation PHA	0.969
Lymphocyte proliferation Anti CD3/28	0.773

Appendix 6

Effects of atorvastatin added to inhaled corticosteroids on lung function and sputum cell counts in atopic asthma

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ABSTRACT

Background
Statins have anti-inflammatory properties that may be beneficial in the treatment of asthma. We tested the hypothesis that atorvastatin added to inhaled corticosteroids treatment improves lung function and airway inflammation in atopic adults with asthma

Methods
Fifty four adults with atopic asthma were recruited to a double-blind, randomised controlled crossover trial comparing the effect of oral atorvastatin 40 mg daily with that of a matched placebo on asthma control and airway inflammation. Each treatment was administered for 8 weeks separated by a 6-week washout period. The primary outcome was morning peak expiratory flow (PEF). Secondary outcomes included FEV_1, asthma control questionnaire score, airway hyperresponsiveness to methacholine, induced sputum cytology and inflammatory biomarkers.

Results
At 8 weeks, the change in mean morning PEF, as compared with baseline, did not differ substantially between the atorvastatin and placebo treatment periods [mean difference -0.5 L/min, 95% CI -10.6 to 9.6, p=0.921]. Values for other clinical outcomes were similar between the atorvastatin and placebo treatment periods. The absolute sputum macrophage count was reduced after atorvastatin compared to placebo [mean difference -45.0×10^4 cells, 95% CI -80.1 to -9.7, p=0.029], as was the sputum fluid leukotriene B_4 [mean difference -88.1pg/ml, 95% CI -156.4 to -19.9, p=0.014].

Conclusion
The addition of atorvastatin to inhaled corticosteroids results in no short-term improvement in asthma control, but reduces sputum macrophage counts in mild to moderate atopic asthma. We speculate that the change in sputum macrophage count suggests potential areas for investigation of statins in other chronic lung diseases.

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Key words
Asthma; atorvastatin; statins; inhaled corticosteroids; induced sputum; alveolar macrophages, leukotriene B_4

Dedication
This paper is dedicated to the memory of Dr Stuart Wood, Senior Lecturer in General Practice at the University of Glasgow, who died in March 2006.

INTRODUCTION

Statins are inhibitors of 3-Hydroxymethyl-3-glutaryl Coenzyme A reductase (HMG CoA reductase), a rate-limiting step in cholesterol synthesis. In addition to clinically important cholesterol lowering properties [1], statins also have diverse anti-inflammatory effects [2-5]. Statins prevent the isoprenylation of signalling molecules such as Ras and Rho, which are involved in driving many inflammatory processes including lymphocyte activation. Statin treatment therefore has the potential to modify immune-driven diseases , and this has been tested in experimental models of auto-immune diseases [6]. A recent trial of atorvastatin in rheumatoid arthritis showed improvement in clinical outcome measures [7] associated with reduction in blood inflammatory biomarkers such as erythrocyte sedimentation rate, C-reactive protein (CRP) and interleukin-6 (IL-6).

Atopic asthma is an immune-mediated airway disease associated with eosinophilic airway inflammation and Th_2 cytokine functional profile [8]. Recent studies have shown that simvastatin and pravastatin can effectively reduce these acute changes in murine models of allergic lung inflammation [3 9 10]. It has been postulated that the anti-inflammatory effects of statins may have relevance for the treatment of asthma and other respiratory disease [5].

The aim of this study was to test the hypothesis that atorvastatin added to inhaled corticosteroids treatment improves lung function and sputum cell counts in atopic adults with asthma. We designed a randomised double blind crossover placebo controlled trial to investigate the effect of oral atorvastatin 40 mg daily for 8 weeks on measures of asthma control and airway inflammation in adults with allergic asthma receiving inhaled corticosteroids alone. The dose and duration of statin therapy with atorvastatin was greater than that used in a previous placebo-controlled crossover trial of short-term, low dose simvastatin in 16 subjects with steroid-naïve asthma, which showed no clinical beneficial effects on asthma control [11]. Atorvastatin was chosen because of its favourable *in vitro* and *in vivo* anti-inflammatory properties [7 12 13] and evidence of clinical benefit at the dose of 40 mg daily in rheumatoid arthritis [7].

METHODS

Subjects

Chronic symptomatic atopic asthma subjects aged 18–70 years were recruited from general practices and hospital clinics. Subjects were taking ≤ 1000 mcg beclometasone equivalent daily, no other medication for asthma other than a short-acting $beta_2$-agonist and had been on stable medication for at least four weeks before randomisation. Atopy to common allergens was established by skin prick test or specific serum IgE antibody measurement. Subjects were excluded if they were pregnant or lactating, current smokers, ex-smokers with > 5 pack-year smoking history, receiving statins or had a known allergy to them, had a

respiratory tract infection or needed oral corticosteroids in the 6 weeks preceding inclusion. All participants gave written informed consent. The study was approved by the West Glasgow Ethics Committee.

Study design and assignment

The study was a 24-week randomised double blind crossover study comparing the effect of oral atorvastatin therapy (40 mg daily) with that of a matched placebo, on asthma control and airway inflammation. After a 2-week run-in period, each treatment was administered to randomised patients for 8 weeks, separated by a 6-week washout period. Randomisation was performed by a centralised telephone service in sequential blocks of 4. Researchers and participants were blinded to allocation and had no access to the randomisation code held by the Data Centre until completion of the study.

During the two-week run-in period, subjects continued their usual asthma medication and recorded peak expiratory flow (PEF) and symptoms twice daily, in their diary. Further visits were undertaken at randomisation, and after 2, 4, and 8 weeks (Phase 1). After a washout period of 6 weeks, Phase 2 of the crossover was started, with visits after 2, 4, and 8 weeks. At each visit, spirometry was performed. Patients recorded morning and evening PEF measurements and symptoms throughout the study. Before and after each treatment period, airway responsiveness to methacholine, a validated asthma control questionnaire (ACQ) score, asthma quality of life questionnaire (AQLQ) score, induced sputum and exhaled nitric oxide (Fe_{NO}) were recorded and blood samples were taken for immunological tests, lipids and liver functions. Tablets were counted at the end of each treatment period as a measure of compliance.

Measurements

Patients maintained a validated diary card[14], recording morning and night PEF (Mini-Wright; Clement Clarke, Harlow, UK), daytime symptoms, night awakenings, use of inhaled rescue medication, and study tablet consumption. Spirometry was measured with a dry spirometer (Vitalograph Ltd., Buckingham, UK)[15]. ACQ was recorded[16]. Methacholine airway responsiveness was measured in subjects with a baseline FEV_1 of > 60% predicted using Cockcroft's technique[17] with concentrations of methacholine from 0.03 to 16 mg/ml[18]. Skin prick tests for allergy to cat dander, house dust mite (*Dermatophagoides farinae*) and mixed grass pollen were performed (Soluprick, ALK, Horsholm, Denmark). Total IgE and specific IgE to house dust mite, grass pollen, and cat dander were assayed by the Unicap 100 system (Pharmacia UK Ltd, Milton Keynes, UK). Total IgE levels >120 IU/ml and specific IgE levels >0.35 AU/ml were considered raised. Atopy was defined when a patient had a positive skin prick test or elevated specific IgE level. Sputum was induced as previously described [19]. Differential cell count was performed and the sputum supernatant fluid analyzed for leukotriene (LT) B_4 and myeloperoxidase (MPO) using enzyme immunoassay

(EIA) (R&D Systems, Abingdon, UK), and IL-8, TNFα, IFN-γ using a Luminex microbead fluorescence kit (Biosource, Invitrogen, Paisley, UK). Fe_{NO} was measured using a chemiluminescence analyzer (Logan Research Ltd., Rochester, UK) at a flow rate of 50 ml/s according to consensus guidelines[20]. Serum was analysed for IL-5, high sensitivity (hs) IL-6, IL-8, hsTNF-α, sICAM-1 and hsCRP by EIA (R & D Systems). Peripheral blood concentrations of urea, electrolytes, lipids, liver function and full blood count were measured in the hospital accredited laboratories at the beginning and end of each treatment period.

Statistical analysis

Baseline characteristics were described by number and percentage of patients for categorical variables and mean (SD) for continuous variables. Response to atorvastatin on lung function, diary data, induced sputum, mediator levels and exhaled nitric oxide versus placebo was assessed by normal linear models that included parameters for patient, period and treatment.

A sample size of 44 was calculated to have 90% power to detect a difference in means of 20L/min in PEF (primary endpoint)[21], assuming a standard deviation of differences of 40L/min, using a paired t-test with a 5% two-sided significance level. A total of 54 patients were recruited to ensure that 44 patients completed the study. All data was analysed using SAS version 9 (SAS Institute, Cary, NC).

Response to atorvastatin on lung function, diary data, induced sputum, mediator levels and exhaled nitric oxide versus placebo was assessed by normal linear models that included parameters for patient, period and treatment. When variables were unsuitable for this, the within-patient treatment differences were calculated and then analysed by either t-test or Wilcoxon test, as appropriate. Significance at a level of 5% was accepted for the primary endpoint, the mean of the morning PEF measurements taken from the 7 days of diary recording before each study visit. The mean was calculated if there were at least three days of completed data within that period.

RESULTS

Recruitment and baseline characteristics

A total of 4303 asthmatic patients from 39 practices and hospital clinics were invited to participate (Figure 1). Of the 439 replies, screening visits were arranged for 137 volunteers and 54 were randomised. Baseline demographic and clinical characteristics of patients are listed in Table 1 and baseline inflammatory biomarkers in Table 2. Distributions of baseline characteristics were similar for patients starting with placebo and those starting with atorvastatin. All subjects were atopic.

Table 1: Demographics and clinical characteristics of subjects

Variable	All Patients (n=54)
Male sex, n (%)	23 (42.6%)
Ex-smoker, n (%)	6 (11.1%)
Age, years	42.5 (13.7)
Asthma duration, years	24.9 (16.6)
Positive IgE serology (%)	89%
Equivalent beclometasone daily dose, mcg, median (IQR)	400 (200-800)
Morning PEF, L/min	390.3 (103.5)
Evening PEF, L/min	403.3 (101.4)
FEV_1 pre-salbutamol, L	2.78 (0.86)
FEV_1 post-salbutamol, L	3.18 (0.84)
FEV_1 % predicted	85.7 (19.3)
FEV_1 %reversibility	14.9 (11.7)
Geometric mean (range) PC_{20} (mg/ml)	2.5 (0.9 - 6.2)
Asthma Control Questionnaire Score	1.5 (0.8)
AQLQ Score, median (IQR)	5.75 (5.03-6.19)

Definition of abbreviations: PEF, peak expiratory flow; FEV_1, Forced Expiratory Volume in one second; AQLQ, asthma quality of life questionnaire, PC_{20}, concentration of methacholine that reduces the FEV_1 by 20%.
Data represented as mean (SD) unless otherwise indicated.

Changes in clinical outcomes

Changes in clinical outcomes after atorvastatin treatment are listed on Table 3 and illustrated in Figure 2. At 8 weeks, the change in mean morning PEF, as compared with baseline, did not differ between the atorvastatin and placebo treatment periods [mean difference -0.5 L/min, 95% CI -10.6 to 9.6, p=0.921]. The estimated mean difference for the primary outcomes (20L/min) was much larger than that obtained in the study. No statistically significant effect of atorvastatin was seen in evening PEF, pre- and post- salbutamol FEV_1 or methacholine responsiveness (PC_{20}). Similarly, there was no significant difference in ACQ score or AQLQ score between atorvastatin and placebo treatment periods.

Table 2: Baseline induced cytology and inflammatory biomarker levels.

Variable	Median (IQR)
Induced sputum cell counts and proportion (%)	
Total cell count (x10^6)	1.4 (0.8- 4.3)
Macrophages (x10^4)	196.5 (101.3 - 235.0)
Macrophages %	49.5 (30.5 - 57.5)
Neutrophils (x10^4)	129.8 (60.0 - 186.5)
Neutrophils %	35.5 (14.5 - 48.3)
Eosinophils (x10^4)	6.4 (2.0 - 16.3)
Eosinophils %	1.5 (0.6 - 5.0)
Lymphocytes (x10^4)	2.5 (1.0 - 5.5)
Lymphocytes %	0.7 (0.3 - 1.1)
Bronchial epithelial cells (x10^4)	44.0 (12.0 - 60.3)
Bronchial epithelial cells %	10.0 (8.0 - 316.0)
Mediators	
MPO (ng/mL)	179.0 (73.5 - 276.0)
LTB_4 (pg/mL)	47.8 (32.6 - 80.4)
IL-8 (ng/mL)	1.9 (0.8 - 5.3)
TNFα (pg/mL)	11.4 (4.6 - 46.5)
IFN-γ (pg/mL)	19.8 (4.9 - 71.6)
Exhaled nitric oxide	
Fe_{NO} ppb	18.3 (10.1 - 33.1)
Serum biomarkers	
hs-CRP (mg/L)	1.68. (0.5 – 3.8).
sICAM (ng/mL)	209.4 (185.7 - 245.4)
IL-5 (pg/mL)	7.3 (1.9 - 26.4)
IL-8 (pg/mL)	14.3 (0.6 - 24.0)
TNF-α (pg/mL)	1.1 (0.7 - 1.8)
IL-6 (pg/mL)	1.1 (0.7 - 3.1)

Abbreviations: CRP, C-reactive protein; HDL, high density lipoprotein; NO, nitric oxide; ppb, parts per billion; sICAM, soluble inter-cellular adhesion molecule; TNFα, tumour necrosis factor-α; IL, interleukin; MPO, Myeloperoxidase; LT, Leukotriene; IFN, interferon.

Changes in inflammatory biomarkers

Induced sputum cytology

The total cell counts recovered from sputum were similar after atorvastatin compared to after placebo treatment (Table 3). After 8 weeks, the mean absolute and relative sputum macrophage count was significantly reduced after atorvastatin compared to placebo [mean absolute difference -44.9x10^4 cells, 95% CI -80.1 to -9.7, p=0.029] (Table 3, Figure 3). There was a reciprocal increase in the relative proportion of sputum neutrophils [mean proportion difference 13.1% (1.8 to 24.4), p=0.025] (Table 3, Figure 3), but there were no significant changes in the absolute count of these cells or the counts and proportions of the other sputum cell phenotypes under atorvastatin treatment.

Table 3: Treatment differences in outcome measures after 8-weeks atorvastatin compared to placebo.

Variable	Atorvastatin	Placebo	Treatment difference, atorvastatin minus placebo (95% CI)
Clinical outcomes [mean (SD)]			
Morning PEF L/min	387.0 (106.5)	393.9 (114.2)	-0.5 (-10.6, 9.6)
Evening PEF L/min	395.3 (101.0)	403.8 (116.3)	4.6 (-5.8, 15.0)
FEV_1 pre-salbutamol, L	2.7 (0.8)	2.7 (0.8)	0.01 (-0.01, 0.10)
FEV_1 post-salbutamol, L	3.1 (0.8)	3.1 (0.8)	-0.05 (-0.13, 0.03)
PC_{20} methacholine geometric mean (range), mg/ml	2.7 (0.9, 10.3)	3.0 (0.6, 9.6)	0.05 (-1.6, - 1.7)
ACQ	1.4 (0.9)	1.5 (0.8)	-0.03 (-0.28, 0.21)
AQLQ, median (IQR)	5.9 (5.4, 6.6)	5.9 (5.4, 6.4)	0.1 (-0.1, 0.3)
Sputum differential cell count and proportion (%)			
Total cell count ($x10^6$)	1.7 (0.9, 2.8)	1.6 (0.6, 4.7)	-0.07 (-0.14, 0.01)
Macrophage ($x10^4$)	134.3 (71.0, 201.0)	171.3 (135.0, 222.8)	-44.9 (-80.1, -9.7) *
Macrophage %	37.0 (20.5, 52.5)	45.3 (36.5, 58.5)	-10.9 (-20.5, -1.2) *
Neutrophil ($x10^4$)	133.3 (81.5, 234.0)	100.0 (50.3, 167.8)	47.1 (-2.0, 96.2)
Neutrophil %	34.8 (22.0, 65.0)	25.0 (15.0, 41.9)	13.1 (1.8, 24.4) *
Eosinophil ($x10^4$)	3.8 (2.0, 10.5)	5.0 (1.0, 10.5)	-12.1 (-32.9, 8.6)
Eosinophil %	1.0 (0.5, 3.0)	1.5 (0.4, 3.0)	-2.7 (-7.1, 1.7)
Lymphocyte ($x10^4$)	2.0 (1.0, 4.5)	1.9 (1.0, 5.0)	0.4 (-0.9, 1.7)
Lymphocyte %	0.6 (0.4, 1.0)	0.5 (0.3, 1.4)	0.15 (-0.18, 0.49)
Bronchial epithelial cell ($x10^4$)	38.8 (17.0, 59.0)	33.5 (18.8, 69.3)	-3.5 (-23.3, 16.3)
Br. epithelial cell %	10.3 (4.0, 24.0)	11.9 (5.0, 17.8)	0.28 (-5.32, 5.88)
Sputum mediators			
Leukotriene B_4	50.4 (27.6, 79.8)	68.2 (28.6, 130.2)	-88.1 (-156.4, -19.9) *
Interferon-γ	23.9 (3.8, 68.0)	21.5 (3.1, 155.5)	-38.4 (-175.4, 98.6)
MPO (ng/mL)	87.5 (51.0, 193.0)	112.3 (56.5, 206.0)	-32.6 (-112.8, 47.7)
TNFα	12.5 (4.7, 45.3)	16.2 (4.4, 123.0)	-30.3 (-111.6, 51.0)
Interleukin-8	1.5 (0.5, 3.6)	1.5 (0.6, 5.1)	-1.6 (-5.7, 2.5)
Exhaled NO (ppb)			
	16.17 (8.99, 38.1)	17.32 (9.0, 42.69)	-1.6 (-7.1, 3.9)
Serum biomarkers			
hs-CRP (mg/L)	0.64 (0.45, 1.46)	1.06 (0.58, 2.48)	-0.65 (-1.38, 0.09)
sICAM (ng/mL)	201 (173, 222)	204 (164, 239)	-6.5 (-21.4, 8.4)
TNFα	1.03 (0.60, 1.90)	1.17 (0.77, 1.87)	-0.5 (2.5, 1.4)
Interleukin-5	1.87 (1.87, 21.63)	1.87 (1.87, 27.96)	-3.4 (-11.3, 4.5)
Interleukin-6	1.28 (0.74, 3.12)	1.19 (0.69, 3.56)	-0.02 (-1.89, 1.85)
Interleukin-8	0.71 (0.56, 12.17)	8.06 (0.56, 17.54)	-0.5 (-1.5, 0.5)
Serum biochemical markers			
Triglycerides (mmol/l)	0.9 (0.6, 1.3)	1.1 (0.8, 1.6)	-0.24 (-0.54, 0.05)
Cholesterol (mmol/l)	3.3 (2.9, 3.9)	5.1 (4.5, 5.7)	-1.7 (-1.9, -1.5) ***
HDL-cholesterol (mmol/l)	1.2 (1.1, 1.5)	1.4 (1.1, 1.7)	-0.14 (-0.26, -0.02) *
Bilirubin (µmol/l)	10.0 (8.0, 13.0)	8.0 (6.0, 10.0)	1.8 (0.7, 2.9) **
AST (IU/l)	23.0 (18.0, 27.0)	20.0 (16.0, 24.0)	2.9 (0.8, 4.9) **
ALT (IU/l)	26 (18, 35)	20 (15, 33)	5.6 (2.7, 8.5) ***

Abbreviations: ACQ, Asthma Control Questionnaire; ALT, alanine aminotransferase; AQoL, Asthma Quality of Life Questionnaire; AST, aspartate aminotransferase; CRP, C-reactive protein; diff., differential; FEV_1, forced expiratory volume in one second; HDL, high density lipoprotein; MPO, myeloperoxisade; NO, nitric oxide; ppb, parts per billion; PEF, peak expiratory flow rate; sICAM, soluble inter-cellular adhesion molecule; TNFα, tumour necrosis factor-α. Mediator levels pg/ml unless otherwise indicated.
Data represented as median (IQR) unless otherwise indicated.
* = $p<0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Inflammatory biomarkers in sputum
The sputum concentrations of inflammatory cytokines and mediators were similar after atorvastatin compared to after placebo treatment (Table 3) other than leukotriene B_4 (LTB_4) which was significantly reduced [mean difference -88.1 pg/mL (95% CI -156.4 to -19.9) p=0.014].

Exhaled nitric oxide
There was no significant change in Fe_{NO} after atorvastatin compared to placebo, [mean difference -1.6 ppb, 95% CI -7.1 to 3.9, p=0.559] (Table 3).

Inflammatory biomarkers in serum
No significant difference was seen in the concentration of any serum marker of inflammation between atorvastatin and placebo treatment periods (Table 3). The change in hsCRP was of borderline significance [mean difference -0.65 mg/L, 95% CI -1.38 to 0.09, p=0.082], but there were no changes in sICAM-1, TNF-α, IL-5, IL-6 and IL-8.

Change in biochemical markers

The biochemical effects of atorvastatin therapy were reflected in significant reduction in concentration of serum lipids; cholesterol (mean difference -1.71 mmol/l, 95% CI -1.94 to -1.48 p<0.0001), HDL-cholesterol (mean difference -0.14 mmol/l, 95% CI -0.26 to -0.02 p=0.026), but not triglycerides [Table 3]. There were significant, albeit modest, increases in mean bilirubin, AST and ALT.

Adverse events

Adverse event rates were similar in patients taking atorvastatin compared with placebo. One patient receiving atorvastatin required oral steroid, while none in the placebo group did. 2 patients from each group made unscheduled visits to their general practitioner, and 2 patients from the atorvastatin group compared with one from the placebo group attended the emergency department in relation to their asthma during the study. Equal numbers of patients were lost to follow-up in both arms of the study. One patient died of unrelated causes while taking the placebo medication.

Compliance

There was no difference in compliance, assessed by number of tablets returned and by biochemical results.

DISCUSSION

This randomised controlled study tested the hypothesis that atorvastatin added to inhaled corticosteroids treatment could improve lung function and airway inflammation in adults with allergic asthma. We found that that there were no clinically important improvements in a range of clinical indices of asthma control after eight weeks of treatment with atorvastatin despite expected changes in serum lipids. There were, however changes in airway inflammation and in particular, a reduction in the absolute sputum macrophage count after atorvastatin compared to placebo and an associated reduction in sputum LTB_4.

The lack of any evidence of clinical benefit of atorvastatin in allergic asthma confirms and extends the findings of Menzies et al [11], who performed a randomised placebo controlled crossover trial of simvastatin in 16 steroid-naïve subjects with asthma. Simvastatin was administered for 2 weeks at 20 mg daily and 2 weeks at 40 mg daily, with no washout between active and placebo treatment periods [11]. In our study the statin chosen, atorvastatin, was administered at a higher dose and for longer duration and to a larger group of participants (n=54), but we found no evidence of improvement in lung function or airway hyperresponsiveness. We estimated that 8 weeks duration of therapy was long enough to show a clinical effect, on the basis that serum cholesterol levels fall within 6 weeks of statin treatment [22], and our own findings show a highly significant drop in cholesterol after 8 weeks' treatment. Plasma CRP concentration has been shown to fall within four weeks , and possibly even in the first two days of treatment[23]. However, CRP reduction was only of borderline significance in the present study possibly because the CRP levels were not high at baseline or due to lack of statistical power. Changes in CRP may be more apparent in lung diseases with raised CRP levels which could include COPD, or in exacerbations, because previous studies have suggested that statins decrease episodes of pneumonia [24]. Statins have an inhibitory effect on human airway smooth muscle cell proliferation [25], therefore it is possible that the administration of atorvastatin therapy for a longer duration of time might have improved different clinical outcome measures of asthma such as indices of airway remodelling.

It is unlikely that using a different statin would have shown any greater effects. A trial in rheumatoid arthritis [7] used atorvastatin with evidence of clinical benefit. Although the initial experimental work in inflammatory lung disease used simvastatin [3 10] and pravastatin [9], Joukhadar and colleagues showed no difference in effect on inflammatory parameters when comparing atorvastatin, simvastatin or pravastatin [12]. Furthermore no therapeutic effect was found with

simvastatin in asthma [11]. The six week washout period for the present study was considered acceptable because evidence suggests that the anti-inflammatory effects of statins do not last this long. Circulating levels of sVCAM-1 revert to normal in 2 days [26], and CRP [27] and IL-6 concentrations have returned to baseline after 7 days withdrawal [23 28]. No evidence of a carry-over effect on clinical outcome measures was seen in the present study.

One possible explanation for the apparent lack of clinical efficacy in this study is that all patients were taking regular inhaled corticosteroid therapy and had a low basal sputum eosinophil count. In the study by Menzies et al, all patients were withdrawn from inhaled corticosteroids before treatment began, with patients receiving a long-acting beta$_2$-agonist instead[11]. The lack of efficacy with atorvastatin is unlikely due to insufficient room for improvement in clinical outcome measures since the patients group had evidence of reversibility in FEV_1 of approximately 15% and has a raised mean ACQ score of 1.5. Compliance with medication was supported by tablet counting and by the significant changes in serum lipids and liver function tests observed in the patients receiving atorvastatin.

There was no effect of statin therapy on any of the inflammatory biomarkers other than decreases in sputum macrophage count and LTB_4 concentration. Several mechanisms, either alone or in combination, might explain these observations. Firstly, statins reduce growth of both animal and human macrophages [29 30] and possibly atorvastatin had a similar effect on sputum alveolar macrophage numbers. Secondly, LTB_4 is raised in induced sputum supernatant in asthma [31] and mediates the recruitment of alveolar macrophages through the B leukotriene receptor-1 [32]. The decrease in sputum LTB_4 concentrations observed in patients treated with atorvastatin could contribute to the reduced sputum macrophage count. Thirdly, statins interfere with cell binding by reducing monocyte to endothelial cell adhesion and the recruitment of macrophages to the lung [33 34]. Finally, statins reduce the *in vitro* release of cytokines and mediators including TNFα from monocytes [35] and endothelial cells [36 37]. Similar effects on the release of these cytokines and mediators, including from cells within the airways could reduce the recruitment of alveolar macrophages to the airways. Against this latter mechanism, we found no reduction in sputum TNFα, or circulating IL-6, TNFα, and ICAM-1 concentrations following atorvastatin therapy. Future studies should examine these potential pathways.

The reduction in alveolar macrophage count found in patients with allergic asthma may have relevance to the treatment of chronic lung diseases such as COPD in which alveolar macrophage function has been implicated in the pathogenesis [38]. Lovastatin has effects on human and mouse macrophage function by enhancing the clearance of apoptotic cells, a statin-specific effect reversible with mevalonate, through modulation of Rho-GTPases [39]. Production of MMP-9 is also reduced by statins [29], which could also have an impact on

COPD. Recent evidence suggests that statins may inhibit lung parenchymal destruction, and peribronchial and perivascular infiltration of inflammatory cells in a rat model of smoking-induced emphysema [40].

In a mouse model, the neutrophilia associated with acute lung injury is markedly reduced with lovastatin treatment [41]. Paradoxically we found a significant increase in the proportion of neutrophils in induced sputum with atorvastatin therapy. The absolute neutrophil counts were not significantly different between groups, suggesting that the increased proportion of neutrophils occurred because of the low macrophage proportion and the expression of the results as a percentage. In support of this explanation, the sputum LTB_4 concentration was reduced after atorvastatin therapy, which would be more likely to be associated with a reduced neutrophil count.

In conclusion, we have demonstrated that short-term therapy with statins does not improve lung function or other indices of asthma control of patients with mild to moderate atopic asthma who are already receiving treatment with inhaled corticosteroids. The reduction in the sputum macrophage count suggests potential areas for investigation of atorvastatin in chronic lung disease in which activated alveolar macrophages have been implicated in the pathogenesis, such as COPD.

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COMPETING INTERESTS

We declare that we have no conflicts of interest related to the publication of this article.

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FIGURE LEGENDS

Figure 1: Flow of subjects through the study

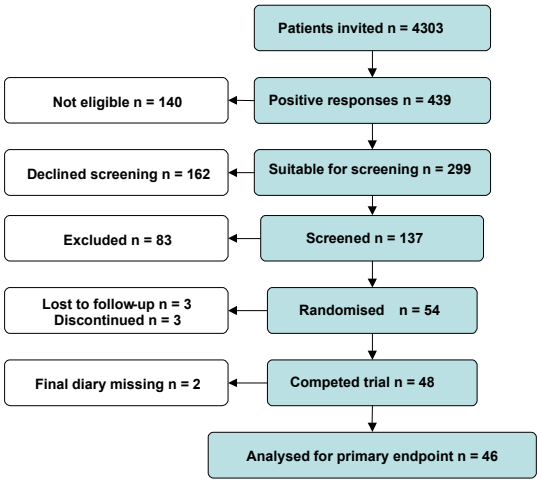


Figure 2: Mean (95% confidence interval) difference between atorvastatin and placebo treatment periods in morning PEF (L/min), FEV_1 (L), PC_{20} methacholine (mg/ml) and ACQ score (range 0-6).

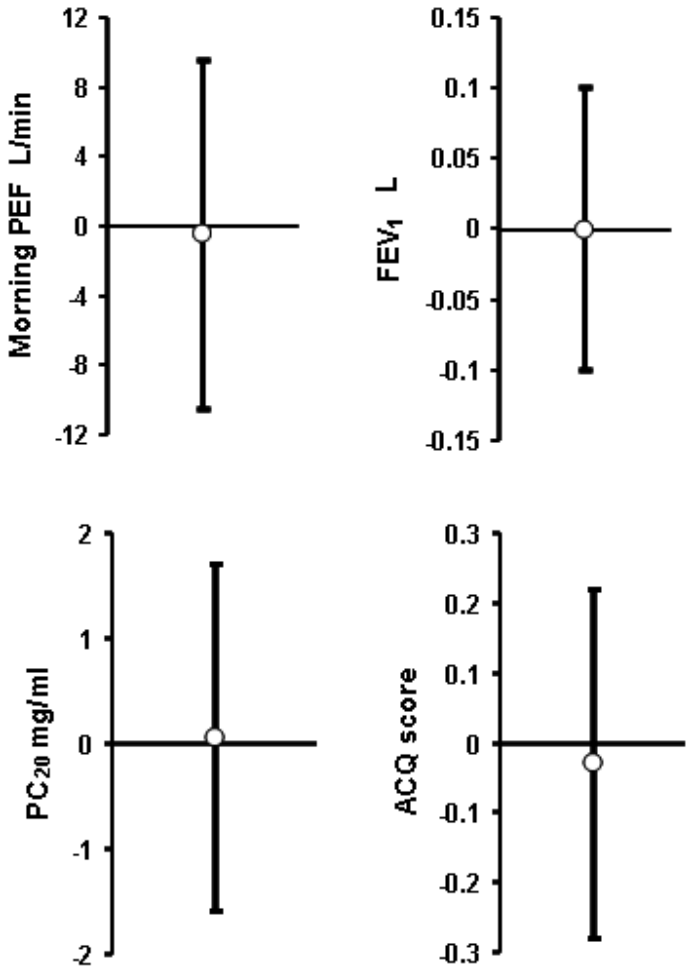
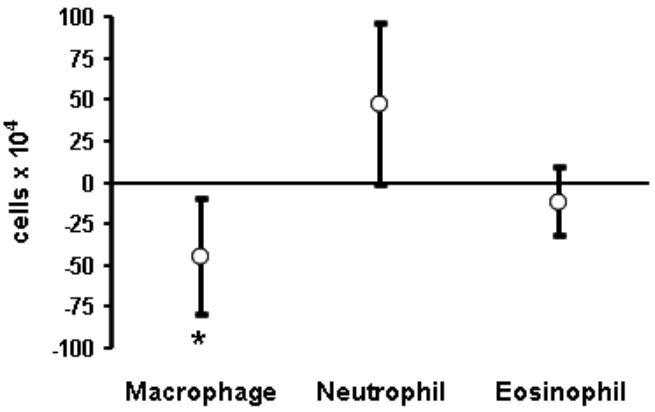


Figure 3: Mean (95% confidence interval) difference between atorvastatin and placebo treatment periods in change in absolute macrophage, neutrophil and eosinophil counts (x10^4 cells) compared with baseline. * = $p<0.05$



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