



SIRPA LEIVO-KORPELA

Adipokines in Inflammatory Lung Diseases



ACADEMIC DISSERTATION

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Adipokines in Inflammatory Lung Diseases

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by Roman numerals I–IV. The articles are included with the kind permission of the copyright owners.

- I Leivo-Korpela S, Lehtimäki L, Vuolteenaho K, Nieminen R, Kankaanranta H, Saarelainen S and Moilanen E (2011): Adipokine resistin predicts anti-inflammatory effect of glucocorticoids in asthma. *Journal of Inflammation* 2011 May 26;8:12. doi: 10.1186/1476-9255-8-12.
- II Leivo-Korpela S, Lehtimäki L, Nieminen R, Oksa P, Vierikko T, Järvenpää R, Uitti J and Moilanen E (2012): Adipokine adiponin is associated with the degree of lung fibrosis in asbestos-exposed workers. *Respiratory Medicine* 2012 Oct;106(10):1435-40. doi: 10.1016/j.rmed.2012.07.003. Epub 2012 Jul 24.
- III Leivo-Korpela S, Lehtimäki L, Vuolteenaho K, Nieminen R, Kööbi L, Järvenpää R, Kankaanranta H, Saarelainen S and Moilanen E (2014): Adiponectin is associated with dynamic hyperinflation and a favourable response to inhaled glucocorticoids in patients with COPD. *Respiratory Medicine* 2014 Jan;108(1):122-8. doi: 10.1016/j.rmed.2013.08.016. Epub 2013 Aug 30.
- IV Leivo-Korpela S, Lehtimäki L, Hämäläinen M, Vuolteenaho K, Kööbi L, Järvenpää R, Kankaanranta H, Saarelainen S and Moilanen E (2014): Adipokines NUCB2/nesfatin-1 and visfatin as novel inflammatory factors in chronic obstructive pulmonary disease. *Mediators of Inflammation* 2014;232167. doi: 10.1155/2014/232167. Epub 2014 May 6.

In addition, some unpublished data are presented.

ABBREVIATIONS

AEC	alveolar epithelial cells
BAL	bronchoalveolar lavage
B-eos	blood eosinophil count
BMI	body mass index
$C_A\text{NO}$	alveolar nitric oxide concentration
CO	carbon monoxide
COPD	chronic obstructive pulmonary disease
DAMP	damage-associated molecular pattern
$D_{L,CO}$	pulmonary diffusing capacity of carbon monoxide
ECP	eosinophil cationic protein
EIA	enzyme-immuno-assay
EPX	eosinophil protein X
ESR	erythrocyte sedimentation rate
$F_\epsilon\text{NO}$	fractional exhaled nitric oxide concentration
FEV ₁	forced expiratory volume in 1 second
FVC	forced vital capacity
FRC	functional residual capacity
GC	glucocorticoid
GM-CSF	granulocyte macrophage colony-stimulating factor
$\text{Hb-D}_{L,CO}/V_A$	pulmonary diffusing capacity of carbon monoxide per unit of alveolar volume standardized for haemoglobin concentration
HRCT	high-resolution computed tomography
IFN	interferon
Ig	immunoglobulin
IL	interleukin
iNOS	inducible nitric oxide synthase
$J_{aw}\text{NO}$	bronchial nitric oxide flux
LTB ₄	leukotriene B ₄
MMP	matrix metalloproteinase

MPO	myeloperoxidase
NF- κ B	nuclear factor kappa B
NE	neutrophil elastase
NO	nitric oxide
OA	osteoarthritis
PAMP	pathogen-associated molecular pattern
PDGF	platelet-derived growth factor
PEF	peak expiratory flow
Raw	airway resistance
RIA	radioimmunoassay
RNS	reactive nitrogen species
ROS	reactive oxygen species
SGRQ	St George's Respiratory Questionnaire
SEM	standard error of mean
Tc	cytotoxic T cell
TGF- β	transforming growth factor beta
Th	T helper cell
TNF- α	tumour necrosis factor alpha
VC	vital capacity
WAT	white adipose tissue

ABSTRACT

Chronic inflammation is present in many lung diseases, not only in airway diseases, like asthma and chronic obstructive pulmonary disease (COPD), but also in interstitial lung disorders. Different cell types and cytokines are known to be involved in the complex inflammatory processes encountered in these disorders, but still many pieces are lacking in our understanding on the pathogenesis of these diseases.

Asthma and COPD are heterogeneous syndromes with different inflammatory profiles, clinical phenotypes and treatment responses, and therefore the characterization and the management of these patients is challenging. The pathogenesis of asbestos-induced interstitial fibrosis i.e. asbestosis, is poorly understood, and furthermore there is no clinical tool which could monitor the current activity of the asbestos-induced immune response. Although inflammation is important in the pathogenesis of these diseases, their diagnosis and follow-up are not based on measurement of the inflammatory response itself but on revealing the secondary changes either by radiography or by measuring pulmonary function. Clinically useful biomarkers for the detection of the inflammatory process and its activity are therefore needed.

Adipokines (also known as adipocytokines) are a group of hormone-like mediators secreted by adipose tissue. They were first described as regulators of energy metabolism, but later also recognized as being produced by inflammatory cells and to be involved in many immune and inflammatory processes in the human body. Recently, adipokines have been found to mediate inflammation responses also in the human lung and associations between some adipokines and obstructive airway diseases have been described, but there is practically no data on whether adipokines are involved in interstitial lung diseases.

The aim of the present study was to investigate if plasma adipokines would be associated with the airway and systemic inflammation and disease severity in asthma (I), COPD (III–IV) and pulmonary fibrosis in asbestos-exposed subjects (II). Another major aim was to examine if adipokines would be related to glucocorticoid-responsiveness in asthma and/or COPD. Steroid-naïve, newly diagnosed patients with asthma (n = 35) and patients with COPD (n = 43) were recruited by the Department of Respiratory Medicine (I, III–IV) and subjects with moderate to heavy occupational exposure to asbestos (n = 85) by the Department of Occupational Medicine (II) at Tampere University Hospital.

It was found that the plasma leptin levels were associated with disease severity in non-obese, steroid-naïve asthmatics suggesting that the relationship between leptin and asthma is not restricted to obesity. In addition, high pre-treatment plasma resistin levels predicted a more favourable anti-inflammatory effect of inhaled glucocorticoids indicating that resistin may be a marker of the steroid-sensitive phenotype in asthma.

Plasma levels of adiponectin were associated with peripheral airway obstruction and dynamic hyperinflation in COPD and also with favourable relief of symptoms and hyperinflation during glucocorticoid treatment. These findings support the experimental data that adiponectin can act as a pro-inflammatory mediator able to induce tissue matrix degradation and to evoke smooth muscle contraction in COPD. In addition, the present study introduced adipokines nesfatin-1 and visfatin as novel factors associated with systemic inflammation in emphysematous COPD.

Plasma levels of adipisin were associated with the degree of interstitial fibrosis, with impairment of pulmonary diffusing capacity and with inflammatory activity in workers with a history of moderate to heavy exposure to asbestos. These findings suggest that adipisin may have a role in the pathogenesis of asbestos-induced lung injury.

This study provides new information on the role of adipokines in non-obese patients with asthma and COPD and presents as an original finding the fact that adipisin is associated with asbestos-induced interstitial lung disease. In the light of these results in the future it would be interesting to determine whether the levels of resistin or adiponectin can be used clinically to identify steroid-sensitive phenotypes of asthma and COPD, respectively, or if adipisin could be used as a biomarker of ongoing disease activity in asbestos-exposed subjects.

TIIVISTELMÄ

Krooninen tulehdus eli inflammaatio on keskeinen osa monien keuhkosairauksien patofysiologiaa. Useat solut ja välittäjäaineet ovat osallisena sekä keuhkoputkistoon että keuhkokuokseen kohdistuvissa tulehdusprosesseissa. Yleiset kansansairaudet astma ja keuhkoah-
taumatauti (COPD), sekä harvinaisempi asbestialtistuksen aiheuttama keuhkofibroosi eli asbestoosi luetaan osaksi tulehduksellisia keuhkosairauksia.

Viime vuosien tutkimus on tuonut uutta tietoa astman ja COPD:n patogeneesistä. On ymmärretty niiden olevan monimuotoisia sairauksia erilaisin tauti-ilmentymin, jotka poikkeavat toisistaan taudinkulultaan ja ennusteeltaan. Asbestialtistuksen aiheuttaman keuhkofibroosin immunologiset ja patofysiologiset mekanismit tunnetaan edelleen puutteellisesti. Näiden sairauksien diagnostisointi ja seuranta perustuu pääosin tulehduksen aiheuttamien seurannaismuutosten osoittamiseen keuhkofunktio- ja kuvantamistutkimuksilla, mutta taudin tulehduksellisen aktiiviteetin osoittamiseksi ei ole ollut käytännön työhön soveltuvia keinoja. Potilastyöhön tarvitaan merkkiaineita, joita mittaamalla obstruktiivisissa keuhkosairauksissa voitaisiin havaita taudin tulehduksellinen aktiivisuus ja tunnistaa erilaisia tautimuotoja. Vastaavasti asbestialtistuneilla henkilöillä merkkiaineita mittaamalla voitaisiin ennustaa tulehdus- ja fibroosiprosessin etenemistä keuhkoissa ja siten suunnata seuranta niihin potilaisiin, joilla on suurin etenevän taudin riski.

Adipokiinit (adiposytokiinit) ovat ryhmä rasvakudoksen ja makrofagien erittämiä välittäjäaineita. Ne on liitetty alun perin energia-aineenvaihduntaan, mutta sittemmin niiden on todettu säätelevän myös tulehdusvastetta ja immuunipuolustusta. Viime vuosina adipokiinien on todettu olevan osallisina obstruktiivisten keuhkosairauksien, kuten astman ja COPD:n, tulehdusprosesseissa. Samanaikaisesti on havaittu, että näihin sairauksiin liittyy hengitystietulehduksen lisäksi koko elimistöön kohdistuva systeeminen tulehdus. Adipokiinilöydökset obstruktiivisissa keuhkosairauksissa ovat yhä osin ristiriitaisia ja adipokiinien merkityksestä keuhkoparenkyymsairauksissa tiedetään vielä hyvin vähän. Lisää tietoa tarvitaan sairauksien solutasen tulehdusmekanismien ymmärtämiseen, jotta voisimme löytää merkkiaineita sairauksien luokittelun ja seurannan parantamiseksi sekä kehittää uusia, tulehdustyypin mukaisia hoitoja.

Tämän tutkimuksen tarkoituksena oli selvittää adipokiinien yhteyttä astmaan ja keuhkoah-
taumatautiin liittyvään hengitystietulehdukseen ja systeemiseen tulehdukseen. Tutkimuksessa selvitettiin adipokiinien suhdetta tulehdusvasteen voimakkuuteen, keuh-

kofunktioon ja oireisiin sekä mahdollisuutta käyttää adipokiinejä steroidihoitovasteen arviointiin. Lisäksi tutkittiin, ovatko adipokiinit yhteydessä asbestin aiheuttamaan tulehdusvasteeseen tai keuhkofibroosin vaikeusasteen. Näitä tavoitteita varten tutkimuksessa määritettiin adipokiinien (adipsiini, adiponektiini, leptiini, nesfatiini-1, resistiini ja visfatiini) plasmapitoisuuksia juuri diagnosoitujen, aiemmin steroidihoitoa käyttämättömien astmapotilaiden (n = 35), COPD-potilaiden (n = 43) sekä työssään kohtalaisesti tai voimakkaasti asbestille altistuneiden henkilöiden (n = 85) verinäytteistä. Osatyöt perustuivat kolmeen potilasaineistoon, jotka on kerätty Tampereen yliopistollisen sairaalan keuhkosairauksien (osatyö I: astma ja osatyöt III–IV: COPD) ja työlääketiiteen (osatyö II: asbestille altistuneet) poliklinikoilla.

Tutkimuksessa havaittiin, että adipokiinit ovat yhteydessä astmaan ja keuhkohtaumautiin lihavuudesta riippumatta. Astmassa ennen hoidon aloittamista mitattu suuri plasman resistiinipitoisuus liittyi hyvään steroidihoitovasteeseen ja suuri leptiinipitoisuus liittyi runsaampiin oireisiin ja huonompaan keuhkojen toimintaan. COPD:ssä suuri plasman adiponektiinipitoisuus ennusti hyvää vastetta inhalaatiosteroidihoidolle ja oli yhteydessä pienten hengitysteiden ahtautumiseen ja sen aiheuttamaan keuhkojen ilmatäyteisyyden lisääntymiseen, joka on keskeinen tekijä COPD:n aiheuttamassa rasisuhengenahdistuksessa. Tutkimuksessa osoitettiin, että nesfatiini-1 ja visfatiini ovat uusia tulehdustekijöitä COPD:n liittyvässä systeemisessä tulehduksessa. Lisäksi visfatiinin todettiin liittyvän keuhkokudoksen kaasujenvaihdunnan vaikeutumiseen stabiilissa, emfysemaattisessa taudissa.

Tässä tutkimuksessa osoitettiin ensimmäisen kerran adipokiinien yhteys asbestialtistuksen aiheuttamaan tulehdusprosessiin ja keuhkofibroosiin eli asbestoosiin. Plasman adipsiini oli yhteydessä systeemisiin tulehdustekijöihin, kuten veren interleukiini-6-pitoisuuteen ja laskoon, keuhkopussin paksuuntumien (pleuraplakkien) laajuuteen, keuhkofibroosin vaikeusasteeseen ja alentuneeseen keuhkojen kaasujenvaihduntaan eli pienentyneeseen diffuusiokapasiteettiin.

Tämä väitöskirjatutkimus lisäsi tietoa adipokiinien osallisuudesta astmassa ja COPD:ssä ja toi uutta tietoa vähemmän tutkittujen adipokiinien, kuten adipsiinin, resistiinin, nesfatiini-1:n ja visfatiinin merkityksestä näissä sairauksissa. Kokonaan uutta tietoa saatiin adipokiinien yhteydestä asbestialtistuksen aiheuttamaan keuhkofibroosiin ja adipokiinien yhteydestä steroidihoitovasteeseen astmassa ja keuhkohtaumataudissa. Näiden tulosten perusteella lisätutkimuksia adipokiinimääritysten käytettävyydestä steroidihoitovasteen arvioinnissa astma- ja keuhkohtaumatautipotilailla sekä adipsiinin soveltuvuudesta fibroosiriskin arviointiin asbestialtistuneilla henkilöillä kannattaisi tehdä. Lisätutkimuksia tarvitaan myös adipokiinien vaikutusmekanismeista ja käyttökelpoisuudesta mahdollisina lääkevaikutuskohteina.

INTRODUCTION

Asthma and COPD are the two most prevalent obstructive lung diseases affecting millions of people worldwide and their incidences are rising globally (GINA 2012; GOLD 2013). These diseases are characterized by chronic airway inflammation and airflow limitation, but systemic inflammation may also be present (Barnes & Celli, 2009). Both diseases are heterogeneous displaying a variety of inflammatory and clinical profiles, and furthermore there is an overlap syndrome of asthma and COPD (Carolan & Sutherland, 2013). This makes the diagnosis and treatment of these diseases challenging. Although different phenotypes are recognized in both asthma and COPD (Carolan & Sutherland, 2013), no phenotype-specific biomarkers are available at present.

Asbestos is a group of naturally occurring crystalline mineral fibers and exposure to asbestos has been related to both malignant and non-malignant diseases of the lungs and the pleura (Manning et al., 2002; American Thoracic Society, 2004). Asbestosis is a slowly progressing diffuse interstitial pulmonary fibrosis caused by moderate to severe exposure to asbestos which becomes manifested after a long latency period (American Thoracic Society, 2004). The detailed pathogenesis of asbestosis is poorly understood, but the persistent inflammation driven by macrophages with the generation of pro-inflammatory and pro-fibrotic mediators plays a significant role (Robledo & Mossman, 1999; G. Liu et al., 2013). It has been shown that pulmonary inflammation is a typical feature also in the early stages of the disease with only minimal interstitial changes (Lehtimäki et al., 2010). Since only a fraction of asbestos-exposed subjects develop asbestosis (Paris et al., 2004), there is a clinical need for prognostic tools to reveal the current activity of the asbestos-induced immune response in order to predict the individual risk for development of pulmonary fibrosis.

Adipokines are a new group of mediators which were first linked to energy metabolism and appetite. According to the more recent studies they are also recognized as being involved in the regulation of the immune response and inflammation (Tilg & Moschen, 2006; Ouchi et al., 2011). Adipokines, especially leptin and adiponectin, have recently been shown to be associated with pulmonary inflammation, particularly in asthma and COPD (Sood, 2010). Adipokines are secreted by adipocytes and by other cells, especially by macrophages (Fantuzzi, 2005), which play an important role in the inflammatory processes typical for asthma, COPD and asbestos-induced pulmonary fibrosis. Therefore, it is worthwhile investigating the possible association between adipokines and these inflammatory lung diseases. There is little previous knowledge on adipokines in asthma

and COPD with much of the data being conflicting. In addition, the role of adipokines in non-obese asthma or emphysematous COPD is not well defined. Finally, there is virtually nothing known of the involvement of adipokines in interstitial lung diseases like asbestosis.

Despite recent advances in the understanding of the pathogenesis of inflammatory lung diseases, more research is needed to determine whether adipokines could be used as biomarkers in these prevalent chronic diseases to predict the prognosis and/or treatment responses. It would also be interesting to clarify whether adipokines could help to phenotype the different inflammatory profiles in asthma and COPD. More information is also needed on the role of adipokines in the pathogenesis of inflammatory lung diseases and if they could represent new treatment targets in these currently incurable disorders.

The aim of the present study was to investigate if adipokines would be associated with inflammatory activity or disease severity in asthma, COPD and asbestos-induced interstitial lung disease. An additional aim was to study if adipokines could be useful in predicting the response to glucocorticoid treatment in asthma or COPD.

REVIEW OF THE LITERATURE

1 Inflammation and inflammatory lung diseases

1.1 Acute and chronic inflammation

Inflammation is a protective and repair mechanism against a wide range of exogenous damaging agents such as microbes or toxins. Acute inflammation is the immediate defensive reaction in a tissue to the presence of an infection or an injury which can trigger the recruitment of leukocytes and plasma proteins into the affected tissue or organ with the aim being to remove foreign agents (Kumar et al., 2010). Blood vessels near the site of injury become dilated and inflammatory mediators released leading to locally increased blood flow and oedema. Together these make up the four classical signs of acute inflammation: redness (rubor), swelling (tumor), heat (calor) and pain (dolor) (Kumar et al., 2010).

If this elimination process is prolonged or fails, or if the regulatory mechanisms are inappropriate, chronic inflammation may ensue. It is characterized by the presence of lymphocytes in addition to macrophages and mast cells, the proliferation of blood vessels, tissue destruction together with an attempt to heal the tissue injury by producing connective tissue and fibrosis (Kumar et al., 2010). Lysosomal enzymes, reactive oxygen and nitrogen species, proteases, cytokines and other mediators of inflammation are secreted by activated macrophages and are responsible for many of these changes present in chronic inflammation (Kumar et al., 2010). Persistent infections, immune-mediated inflammatory diseases such as allergies, asthma, lung fibrosis and rheumatoid arthritis and diseases caused by prolonged exposure to foreign bodies such as asbestosis represent different forms of chronic inflammatory processes (Kumar et al., 2010). It is also known that in addition to the chronic inflammatory processes in the affected organs, a persistent low-grade systemic inflammation exists in many common, often obesity-related, chronic diseases such as type II diabetes, atherosclerosis and chronic obstructive lung disease (Hotamisligil, 2006; Wouters et al., 2009).

1.2 Inflammatory lung diseases

Chronic inflammation is a key element in many pulmonary diseases. Ongoing inflammation may cause functional and structural changes such as airway hyperresponsiveness, airway wall thickening, alveolar wall destruction and parenchymal fibrosis (Mason et al., 2010).

Inflammation is a central feature, not only in the two most prevalent obstructive pulmonary diseases, i.e. asthma and COPD, but also in interstitial lung fibrosis with many different aetiologies including asbestosis. The present study focused first on asthma which affects mostly the airways, second on COPD with both airway and lung interstitial manifestations and third on the lung fibrosis caused by exposure to asbestos which has mainly a interstitial expression.

1.3 Asthma

Asthma is a chronic lung disease with two major pathological features, namely airway inflammation and bronchial hyperresponsiveness (GINA 2012). Asthmatic airway inflammation has usually been associated with IgE-mediated allergy and tissue eosinophilia, but also other types of asthma have been recognized (Wenzel, 2012; Pavord, 2012). Bronchial hyperresponsiveness, related to asthmatic airway inflammation causes the variable and reversible airways obstruction typical of asthma, and further leads to recurrent episodes of wheezing, breathlessness and chest tightness (GINA 2012).

Asthma is a common, worldwide disease with an estimated 300 million affected individuals and globally the prevalence of asthma ranges from 1% to 18% of the population in different countries (Masoli et al., 2004). In Finland, the prevalence of self-reported, physician diagnosed asthma in the adult population was 9.4% in 2007 as compared to a prevalence of 6.8% in 1996 (Pallasaho et al., 2011). Approximately 4.3% (238 716 individuals) of the total population were entitled to a special reimbursement of medicines due to asthma in 2011 (Haahtela et al., 2013).

1.3.1 Asthmatic inflammation and phenotypes of asthma

Asthma is a heterogeneous disease with several phenotypes which can be categorized according to the age of onset of the disease, symptoms, treatment responsiveness as well as other clinical characteristics (Wenzel, 2006). The differences between asthma phenotypes are related to different types of airway inflammation reflecting variations in genetic and environmental factors predisposing to asthma (Wenzel, 2006; Murphy & O'Byrne, 2010).

Asthmatic airway inflammation can be divided into Th2 (T helper 2 lymphocytes) and non-Th2 mediated disease (Wenzel, 2012). The best known phenotype of Th2 associated asthma is the early-onset allergic asthma characterized by high blood eosinophil count and high serum total and allergen specific IgE concentrations and by a favorable response to glucocorticoids (Wenzel, 2012). The other Th2 associated asthma phenotypes are late-onset, non-allergic, eosinophilic asthma and a part of exercise-induced asthma (Wenzel, 2012). Th2 cells produce cytokines that stimulate IgE synthesis (interleukin 4, IL-4), eosinophil and basophil activation (IL-5), mast cell proliferation (IL-9) and induce

airway hyperresponsiveness (IL-13) (Holgate, 2012). In addition, other cytokines like transforming growth factor- β (TGF- β), vascular endothelial growth factor (VEGF) and granulocyte macrophage colony-stimulating factor (GM-CSF) are known to be involved in Th2 type asthmatic inflammation (Holgate, 2012).

Only every second asthmatic suffers from the classical Th2-mediated airway inflammation, the other half have different kinds of non-Th2-driven mechanisms (Woodruff et al., 2009), which are still rather poorly understood. The non-Th2 asthma phenotypes include very late-onset asthma, obesity-associated, neutrophilic asthma (often associated with smoking) and smooth-muscle mediated, paucigranulocytic asthma. The onset of the non-Th2 types of asthma occurs usually in adulthood and the symptoms respond poorly to glucocorticoid treatment (Wenzel, 2012).

It has also been postulated that the more severe the asthma, the more complex will be the immunopathological mechanisms and structural changes that are involved (Wenzel, 2012). When asthmatic airway inflammation becomes prolonged it can cause structural changes in the bronchial wall, so-called airway remodelling, which aggravates the symptoms by thickening the airway wall and basement membrane, by increasing the number of bronchial smooth muscle cells, by inducing angiogenesis and by increasing the number of mucus producing goblet cells in airway epithelium (Holgate & Polosa, 2008). In this context it is easy to understand that effective suppression of asthmatic inflammation is the main goal of asthma treatment.

The diagnosis, treatment and follow-up of asthma are still largely based on symptoms and lung function measurements rather than on assessing the underlying inflammatory process. In most cases, asthma can be controlled with anti-inflammatory drug treatment, at present, there is no curative treatment, for example a drug which could switch off entirely the inflammatory process. However, a better diagnosis and clearer understanding of the different inflammatory profiles of asthma would be important not only in helping to phenotype asthma but also in the development of novel phenotype-specific therapeutic approaches.

1.4 Chronic obstructive pulmonary disease (COPD)

COPD is characterized by chronic airway inflammation, obstruction of small airways, destruction of lung parenchyma (emphysema) and systemic inflammation (GOLD 2013; Barnes, 2004; Sinden & Stockley, 2010). Smoking is the most common risk factor for COPD in the western world, but in many countries occupational exposure to noxious particles or gases and both outdoor and indoor air pollutants are also significant risk factors (GOLD 2013). Exposure to cigarette smoke or other triggering factors induces both pulmonary inflammation and low-grade systemic inflammation (Sinden & Stockley, 2010). The chronic pulmonary inflammation in COPD causes small airway injury and fibrosis leading

to irreversible airway obstruction (Barnes, 2004). Pulmonary inflammation also causes emphysema a condition that impairs gas diffusion in the lungs. The systemic inflammation in COPD is associated with extrapulmonary manifestations like cardiovascular diseases, cachexia, skeletal muscle dysfunction, osteoporosis and depression (Agusti et al., 2003; Barnes & Celli, 2009) that are typical co-morbidities in COPD. Thus, the term *chronic systemic inflammatory syndrome* has been proposed as being better for describing this disease (Fabbri & Rabe, 2007).

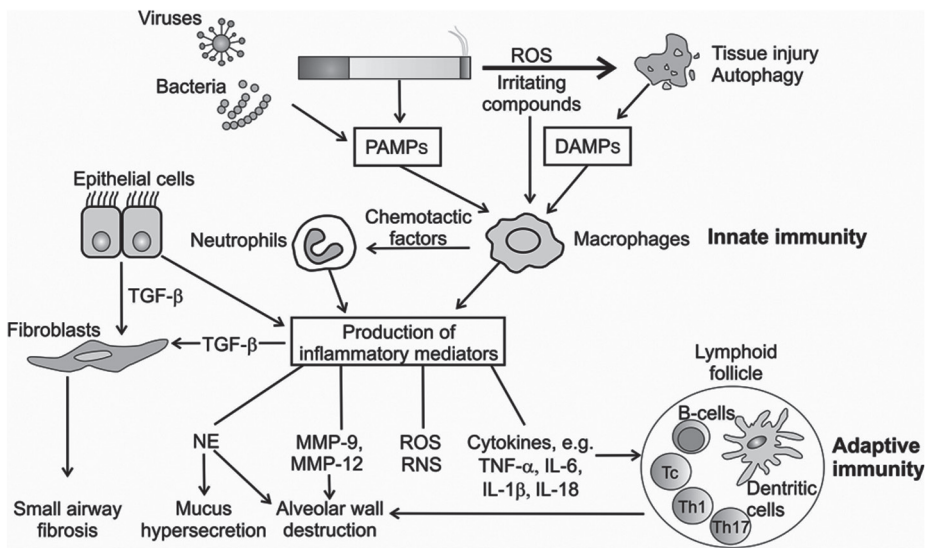
COPD is a major, but often undiagnosed, cause of morbidity and mortality affecting more than 200 million people worldwide (Lopez et al., 2006). At present, COPD is the fourth leading global cause of death, but WHO predicts that it will rise to third place by 2030 (WHO 2014). The COPD prevalence data reveal notable variations due to differences in diagnostic criteria, and survey techniques and analytic methods, but according to most national data, the prevalence of COPD is around 6% in the adult population (Halbert et al., 2006). Some recent data from Finland indicates that the prevalence of COPD according to GOLD criteria is 5.9% in the adult population living in Helsinki (Kainu et al., 2013). In addition to other common predisposing factors such as age, smoking history and prior history of asthma, the socioeconomic status based on occupation was significantly related to the incidence of COPD as industrial manual workers had a higher prevalence of COPD (Kainu et al., 2013). Despite the large economic and social burden of COPD, it is worthwhile remembering that it is a preventable and treatable disease (GOLD 2013) and much can be done to help the patients to cope with this multidimensional disease.

1.4.1 Inflammation in COPD

In COPD there is chronic inflammation in the airways, lung parenchyma and pulmonary vasculature (Hogg, 2004). In addition, there is systemic inflammation present, but it is unclear whether the systemic inflammation in COPD is a spillover of the inflammation present in the lungs or a primary feature of the COPD pathology (Fabbri & Rabe, 2007; Sinden & Stockley, 2010).

Both innate and adaptive immune systems play a role in the airway inflammation in COPD (Brusselle et al., 2011). Cigarette smoke or other irritants activate epithelial cells and innate immune cells like macrophages, neutrophils and natural killer cells by inducing oxidative stress and also by activating pattern recognition receptors through the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) (Brusselle et al., 2011). The activated inflammatory cells of innate immunity release pro-inflammatory mediators such as tumour necrosis factor α (TNF- α), IL-8 and interleukin 1 β (IL-1 β) that attract and activate the cells of the adaptive immune system like T helper 1 (Th1) and cytotoxic T cells (Barnes et al., 2003; Barnes, 2008; Brusselle et al., 2011). The inflammatory cells in conjunction with the epithelial

cells release matrix metalloproteinase 9 (MMP-9) and other proteases that degrade the extracellular matrix and in that way cause alveolar wall destruction and emphysema. In COPD, neutrophil elastase (NE) may also evoke mucus hypersecretion and the release of transforming growth factor- β (TGF- β) stimulates fibroblast proliferation (Barnes et al., 2003; Barnes, 2008). The inflammation of small airways leads to pathological changes in the bronchioles less than 2 mm in diameter, characterized by goblet cell metaplasia, inflammatory cell infiltration and thickening of the bronchiolar walls due to smooth muscle hypertrophy and peribronchial fibrosis (Kumar et al., 2010). The protease-antiprotease and oxidant-antioxidant imbalances have also central roles in the development of pulmonary emphysema (Kumar et al., 2010).



ROS, reactive oxygen species; PAMP, pathogen-associated molecular pattern; DAMP, damage-associated molecular pattern; TGF- β , transforming growth factor beta; NE, neutrophil elastase; MMP, matrix metalloproteinase; RNS, reactive nitrogen species; TNF- α , tumour necrosis factor alpha; IL, interleukin; Tc, cytotoxic T cell; Th, T helper cell.

Figure 1. Innate and adaptive immunity in the pathogenesis of COPD. (Modified from Brusselle et al. 2011, *Lancet* 378: 1015–1026 © Elsevier Ltd.)

1.4.2 COPD phenotypes

Already in the 1950's and 60's, the first descriptions of COPD phenotypes were proposed by Dornhorst, who introduced the clinically based descriptions of “pink puffers” and “blue bloaters” (Dornhorst, 1955) and later by Burrows et al who described the emphysematous and bronchial types of COPD (Burrows et al., 1966).

Today COPD is regarded as a heterogeneous, multisystem disorder with a variety of phenotypes and subgroups with different inflammatory profiles and treatment responses

(Parr, 2011; Miravittles, Soler-Cataluna, Calle, & Soriano, 2013). Multidimensional phenotyping taking into account respiratory symptoms, health status, physiology, structural changes, acute exacerbations, local and systemic biomarkers and comorbidities (Garcia-Aymerich et al., 2011) is informative for scientific purposes but too complicated to be of clinical value.

Clinical phenotyping is based on symptoms, exacerbations and the presence or absence of emphysema, chronic bronchitis and concurrent asthma (Hurst et al., 2010; Carolan & Sutherland, 2013; Miravittles et al., 2013). At least four clinically important phenotypes are recognized as 1) COPD with chronic bronchitis, 2) COPD with frequent exacerbations, 3) COPD with emphysema and 4) overlap of COPD and asthma (Mazur et al., 2013; Miravittles, Soler-Cataluna, Calle, Molina et al., 2013; Miravittles et al., 2013). It is important to develop robust and clinically meaningful methods with which to phenotype the patients in order to achieve earlier disease detection and to aid in the development of phenotype specific treatment strategies.

1.5 Asbestos-induced interstitial lung disease

Asbestos is the term for a heterogeneous group of naturally occurring, hydrated magnesium silicate minerals that have a tendency to separate into fibers (American Thoracic Society, 2004). Asbestos mineral fibers are involved in the development of malignant (lung cancer and mesothelioma) and non-malignant (pleural disorders, asbestosis, retroperitoneal fibrosis) diseases (Manning et al., 2002; Uibu et al., 2004; American Thoracic Society, 2004). The pathogenesis of asbestos-induced diseases is associated with a persistent inflammatory response to inhaled asbestos fibres causing cellular and immunological abnormalities (Manning et al., 2002; G. Liu et al., 2013). Asbestosis is a slowly progressing, diffuse interstitial pulmonary fibrosis, the development of which demands moderate or intense exposure to asbestos; the disease requires usually at least 15–20 years to become manifest (American Thoracic Society, 2004). Exposure to asbestos evokes an inflammatory response in the lungs driven by macrophages attempting to ingest and clear the fibres. The activated macrophages release many different cytokines e.g. IL-6, IL-1 β and TNF- α and growth factors e.g. TGF- β and platelet-derived growth factor (PDGF) that are known to stimulate fibroblast proliferation (G. Liu et al., 2013). In addition, the production of reactive oxygen species (ROS) in mitochondria promotes alveolar epithelial cell apoptosis (G. Liu et al., 2013). These changes lead to activation and proliferation of fibroblasts, myofibroblast differentiation, collagen deposition and ultimately to the appearance of pulmonary fibrosis (G. Liu et al., 2013).

The fibrosis distorts the architecture of the lung interstitium, creating enlarged airspaces surrounded by thickened fibrotic walls, until finally the affected areas become honeycombed (Robledo & Mossman, 1999). The degree of interstitial fibrosis can vary

extensively but the presence of many asbestos bodies is typical (Kumar et al., 2010). Asbestosis usually begins subpleurally in the lower pulmonary lobes (Kumar et al., 2010) and high-resolution computed tomography (HRCT) is a sensitive tool for detecting these asbestos-induced changes, even before any clinical signs are present (Oksa et al., 1994; Huuskonen et al., 2001). In fact, these early interstitial fibrotic changes that do not fulfil the diagnostic criteria of asbestosis are associated with enhanced pulmonary inflammation (Lehtimäki et al., 2010).

The widespread pulmonary fibrosis is responsible for an impairment in the pulmonary diffusing capacity and this may cause pulmonary hypertension and hence is a risk factor for chronic respiratory failure. In addition, asbestosis is also a major risk factor for lung cancer (Asbestos, asbestosis, and cancer: The Helsinki criteria for diagnosis and attribution, 1997). Fortunately, only some asbestos-exposed workers develop asbestosis, but no prognostic tools are currently available with which to estimate an individual's risk for developing asbestosis. In addition, there are no disease modifying or curative treatments available for asbestosis.

Asbestos was commonly used in Finland in the 1960's and 70's and it has been estimated that over 200 000 workers, 50 000 of them still alive, have a history of significant exposure to asbestos. Even though the use of asbestos products was forbidden in 1994, there are still many asbestos-exposed individuals and because of the long latency period (30–40 years), the incidence of asbestos-related diseases has not started to decline. Thus, there is a clinical need for biomarkers which would reveal the current activity of the asbestos-induced inflammatory response and the individual risk for further progression of asbestos related diseases.

2 Adipokines

Adipokines, also known as adipocytokines, are protein mediators produced mainly by adipocytes and macrophages as components of the adipose tissue (Fantuzzi, 2005; Tilg & Moschen, 2006). There is no agreement about which of the mediators should be considered as adipokines. Traditionally only the proteins which were first found to be produced mainly by adipocytes like adiponectin, adipisin, leptin, resistin and visfatin were classified as adipokines (Fantuzzi, 2005), but more recently also the cytokines secreted by adipose tissue, mainly by macrophages, such as IL-6 and IL-18, TNF- α and some chemokines have been incorporated into the adipokine classification (Ouchi et al., 2011). In humans, adipokines act as hormones by influencing energy balance and neuroendocrine functions, and as cytokines by affecting immune functions and inflammatory responses in either pro- or anti-inflammatory manners all over the body (Tilg & Moschen, 2006). This study has focused on the function of adipokines as factors in inflammatory lung diseases.

2.1 Sources of adipokines

Adipose tissue can be divided into white adipose tissue (WAT) and brown adipose tissue. WAT, which represent the major proportion of adipose tissue in humans, contains, in addition to adipocytes, also other cell types such as pre-adipocytes, macrophages, fibroblasts, lymphocytes, endothelial cells and vascular smooth muscle cells (Ouchi et al., 2011). Adipocytes are also found outside the adipose tissue, e.g. in the bone marrow, lungs and the adventitia of major blood vessels (Ouchi et al., 2011). Adipocytes and macrophages are the primary sources of adipokines, but also other cell types, such as endothelial cells, vascular smooth muscle cells, peripheral blood mononuclear cells and bronchial and alveolar epithelial cells can undertake adipokine secretion (Bruno et al., 2005; Ouchi et al., 2011).

2.2 Adipokines in inflammation and immunity

White adipose tissue is not only an energy storage site, but it also regulates many pathological processes e.g. by producing adipokines to influence immune functions and inflammatory responses (Tilg & Moschen, 2006). Disturbed adipokine levels have been observed in many inflammatory conditions such as obesity, cardiovascular and rheumatic diseases and more recently also in inflammatory lung diseases (Ouchi et al., 2011; Ali Assad & Sood, 2012; Scotece, Conde, Vuolteenaho et al., 2014) although their pathogenic role has not been conclusively defined.

Obesity is associated with a chronic low-grade systemic inflammation characterized by altered cytokine production, increased acute-phase reactants and the activation of pro-inflammatory signalling pathways (Wellen & Hotamisligil, 2005). The adipose tissue of obese individuals contains an increased number of macrophages and these produce significant amounts of inflammatory mediators such as TNF- α and IL-6 (Weisberg et al., 2003). These pro-inflammatory adipokines promote the obesity-linked metabolic diseases like diabetes and cardiovascular diseases (Ouchi et al., 2011). The activated macrophages, adipocytes and other cell types present in the adipose tissue contribute to the vicious cycle of macrophage recruitment and production of pro-inflammatory cytokines leading to both local and systemic inflammation (Wellen & Hotamisligil, 2005; Tilg & Moschen, 2006). It seems that macrophage accumulation within the adipose tissue is not only present in obesity, but it occurs in other inflammatory states as well (Wellen & Hotamisligil, 2005). The production of many adipokines is upregulated in obesity but there is increasing evidence that the best studied adipokines, adiponectin and leptin, are involved in many inflammatory diseases also independently of obesity (Fantuzzi, 2008; Ouchi et al., 2011).

Metabolism and immunity are closely linked i.e. the chronic disturbance of metabolic homeostasis in either malnutrition and overnutrition may lead to aberrant immune responses (Wellen & Hotamisligil, 2005). Both adipocytes and macrophages participate

in the innate immune response: adipocytes by releasing lipids that may modulate the inflammatory state or participate in the neutralization of pathogens and macrophages by killing pathogens and secreting inflammatory cytokines and chemokines (Wellen & Hotamisligil, 2005). Adiponectin and especially leptin exert many influences on adaptive immunity, e.g. leptin has been shown to induce lymphopoiesis (Howard et al., 1999) and to stimulate T-cell proliferation (Lord et al., 1998) but there is little data available on the effects of other adipokines on adaptive immunity.

The primary sources, main metabolic and inflammatory functions and the phenotypes of knockout mice of adipokines studied in the present study are presented in Table 1.

Table 1. The sources and the general functions of adipokines.

Adipokine*	Primary sources	Main metabolic effects	General role in inflammation	Knockout phenotype in mice
ADIPONECTIN (Acrp30) Mw: 30 kDa Chr. location: 3q27 Receptors: AdipoR1 and R2 Described in 1995	adipocytes, macrophages, lung epithelium	anti-diabetic, insulin sensitizer	anti-inflammatory and pro-inflammatory properties, acting on monocytes and endothelial cells	metabolic syndrome
ADIPSIN (complement factor D) Mw: 25.5 kDa Chr. location: 19p13.3 Receptors: unknown Described in 1986	adipocytes, monocytes, macrophages	rate limiting enzyme in the alternative complement cascade	pro-inflammatory	impaired complement activation
LEPTIN Mw: 16 kDa Chr. location: 7q31.3 Receptors: Ob-R Described in 1994	adipocytes, lymphocytes, monocytes, macrophages, lung epithelium, smooth muscle	regulates energy metabolism and appetite/reduces appetite	pro-inflammatory, immune-modulating, acting on monocytes	obesity
NESFATIN-1 (NUCB2) Mw: 9.8 kDa Chr. location: 11p15.1 Receptors: unknown Described in 2006	adipose tissue	reduces appetite and body weight	pro-inflammatory, cardioprotective	decreased heart rate, increased serum alkaline phosphatase
RESISTIN (FIZZ3) Mw: 12.5 kDa Chr. location: 19p13.2 Receptors: TLR4 Described in 2001	macrophages, adipocytes, lung epithelium, smooth muscle	pro-diabetic, promote insulin resistance	pro-inflammatory	impaired gluconeogenesis, insulin resistance (?)
VISFATIN (NAMPT) Mw: 52 kDa Chr. location: 7q22.3 Receptors: unknown Described in 1994	adipocytes, macrophages, granulocytes, monocytes	growth factor, glucose homeostasis, lipid metabolism	pro-inflammatory, inhibition of neutrophil apoptosis	homozygous: lethality heterozygous: impaired glucose tolerance

*Refers to human adipokines; Mw, molecular weight; Chr, chromosomal. For references see the text.

2.3 Adiponectin

2.3.1 Structure and general functions of adiponectin

Adiponectin was first isolated from the plasma of Siberian chipmunks as a novel protein, Acrp30 (adipocyte complement related protein of 30 kDa) in 1995 (Scherer et al., 1995). Human adiponectin is a 244-amino acid protein encoded by the apM1 gene located on chromosome 3q27 which was first identified in 1996 (Maeda et al., 1996). Adiponectin is composed of a collagen-like domain and a globular component, which is similar to complement factor C1q (Ouchi et al., 2003). The adiponectin monomers can trimerize through tight interactions in the collagenous domain and the trimers can then oligomerize to allow adiponectin to exist as a trimer (known as low-molecular-weight adiponectin), as a hexamer (middle-molecular weight adiponectin) and as a high-molecular weight 12- to 18-mer (Waki et al., 2003; Kadowaki & Yamauchi, 2005). Adiponectin acts through two known cellular receptors, one (AdipoR1) found predominantly in skeletal muscle and the other (AdipoR2) mainly in liver (Yamauchi et al., 2003). Adiponectin receptors have also been found to be expressed on human airway epithelial cells (Miller et al., 2009), human smooth muscle cells (Shin et al., 2008), human macrophages (Chinetti et al., 2004) and on chondrocytes (Lago et al., 2008).

Adiponectin is mainly produced by adipocytes and it circulates at relatively high concentrations (1–10 µg/ml) in the human bloodstream (Fantuzzi, 2005). Other cell types such as airway epithelial cells can also produce adiponectin (Miller et al., 2009). Both trimers and other oligomers of adiponectin are present in the circulation (Waki et al., 2003), but recent studies have suggested that the high-molecular-weight isoform is the most biologically active isoform of adiponectin in the metabolic syndrome (Hara et al., 2006), and may be also in inflammatory diseases (Daniele et al., 2012; Frommer et al., 2012).

Adiponectin is best known for its role in the regulation of insulin sensitivity and plasma adiponectin levels are decreased in obese individuals, especially in those subjects with the metabolic syndrome, type 2 diabetes and atherosclerosis (Arita et al., 1999). It has been shown that the production of adiponectin by adipocytes is inhibited by IL-6 and TNF- α (Ouchi et al., 2003) and by oxidative stress and hypoxia (Hosogai et al., 2007), all of which are typical features of obesity. Adiponectin decreases insulin resistance by stimulating glucose uptake, by increasing fatty acid oxidation and by reducing the synthesis of glucose in the liver and other tissues (Kadowaki & Yamauchi, 2005). Clinical studies have shown that low serum adiponectin is a risk factor for the development of obesity-linked heart diseases (Ouchi et al., 2011).

Adiponectin has been reported to act mainly as an anti-inflammatory adipokine (Tilg & Moschen, 2006; Ouchi & Walsh, 2007) by inducing the production of two

anti-inflammatory factors IL-10 and as well as the IL-1 receptor antagonist by human monocytes, macrophages and dendritic cells (Wolf et al., 2004) and by suppressing the nuclear factor kappa β (NF- κ β) dependent synthesis of two pro-inflammatory factors TNF- α (Ouchi et al., 2000) and interferon gamma (IFN- γ) in human macrophages (Wolf et al., 2004). Adiponectin also induces the apoptosis of monocytes and inhibits macrophage phagocytosis (Wolf et al., 2004).

The primary role of adiponectin as an anti-inflammatory adipocytokine has been challenged by recent findings. There is increasing evidence that adiponectin exerts a significant role in the pathogenesis of chronic inflammatory diseases like rheumatoid arthritis, SLE, inflammatory bowel disease and inflammatory lung diseases independently of obesity (Fantuzzi, 2008; Ouchi et al., 2011). It has been speculated that the increased adiponectin levels in present chronic inflammatory conditions may be a sign of its pro-inflammatory role or whether it may be a result of inflammation induced catabolic responses trying to extinguish the inflammatory process (Fantuzzi, 2008). In support of the former assumption, adiponectin has been shown to have pro-inflammatory properties under various circumstances. For instance, adiponectin has been reported to enhance the production of pro-inflammatory cytokine IL-8 in human airway epithelium (Miller et al., 2009) and to mediate pro-inflammatory and tissue matrix degrading effects in arthritis (Lago et al., 2008; Koskinen, Juslin et al., 2011). Higher adiponectin levels have been measured in patients with more severe osteoarthritis (OA) and adiponectin has been claimed to augment the production of MMP enzymes in OA cartilage (Koskinen et al., 2011).

2.3.2 Adiponectin in asthma

In mice, serum adiponectin levels decrease during allergic pulmonary reactions (Shore et al., 2006), but in human asthma inhalation of the allergen does not seem to affect serum adiponectin concentrations (Sood et al., 2009). Some human studies have revealed an association between asthma and adiponectin such that lower circulating adiponectin concentrations have been measured particularly in female asthmatics (Sood et al., 2008; Nagel et al., 2009; Sood et al., 2009). On the other hand, some other publications have detected no associations between asthma and adiponectin (Kim et al., 2008; Jartti et al., 2009).

High serum adiponectin levels seem to reduce the risk to develop asthma in women (Sood et al., 2008) and in obesity (Shore & Johnston, 2006), and a positive relationship has been reported between serum levels of adiponectin and improved asthma control (Kattan et al., 2010). This protective effect of adiponectin against asthma in humans is consistent with the findings in mice, in which treatment with adiponectin attenuated allergic airway inflammation and airway hyperresponsiveness (Shore et al., 2006). On the other hand,

adiponectin has also been related to more severe asthma in male patients (Sood et al., 2011), i.e. adiponectin may have both anti- and pro-asthmatic effects in different patient groups.

2.3.3 Adiponectin in COPD

Some human studies have detected higher circulating adiponectin levels in male patients with COPD in comparison to controls (Tomoda et al., 2007; Kirdar et al., 2009; Chan et al., 2010). In addition, unchanged adiponectin levels have been reported in a mixed population of female and male patients with COPD, and in this same study, adiponectin levels were higher in females than in males in both patients with COPD and healthy controls (Breyer et al., 2011). Tomoda et al showed that plasma adiponectin levels were elevated in both normal- and under-weight patients with COPD (Tomoda et al., 2007) and the levels further increased during an exacerbation of COPD (Kirdar et al., 2009).

In a mouse model, adiponectin has been reported to protect against the development of emphysema in animals not exposed to tobacco, and adiponectin deficiency led to increased secretion of pro-inflammatory mediators TNF- α and matrix metalloproteinase (MMP)-12 from alveolar macrophages and to an emphysema-like phenotype (Summer et al., 2008). Furthermore, Nakanishi et al reported that the adiponectin deficiency in adiponectin knockout mice was associated not only with an emphysema-like phenotype but also with systemic inflammation and extra-pulmonary effects such as weight loss, skeletal muscle atrophy and osteoporosis (Nakanishi et al., 2011) and they postulated that the endothelial apoptosis resulting from adiponectin deficiency could be an underlying mechanism linking COPD with the comorbidities. On the contrary, adiponectin knockout mice have been shown to be protected against tobacco-induced inflammation and increased emphysema, evidence that adiponectin plays a pro-inflammatory role in the lungs of tobacco exposed wild type mice (Miller et al., 2010).

Exposure to tobacco smoke in subjects without COPD has been reported to down-regulate adiponectin expression and this was proposed to be mediated via the increased production of reactive oxygen species (Miller et al., 2009). Furthermore, previous smoking has been found to decrease serum adiponectin levels in a dose-dependent manner (Takefuji et al., 2007). However, adiponectin is highly expressed in the lungs of patients with emphysematous COPD who have stopped smoking as compared to the levels in smokers or healthy controls (Miller et al., 2009). Recently, Carolan et al claimed that higher plasma adiponectin levels were associated with pulmonary emphysema, decreased body mass index, female sex, older age and lower bronchial reversibility in patients with COPD (Carolan et al., 2013).

These findings suggest that adiponectin is associated with COPD but virtually nothing is known about the associations of adiponectin with important clinical parameters like lung function, symptoms or treatment responsiveness.

2.3.4 Adiponectin in interstitial lung diseases

There are no previous studies which would have investigated the role of adiponectin in asbestos-induced lung diseases although there is one study examining silica-exposure. Sauni et al showed that plasma adiponectin levels were higher in silica exposed workers than in controls but the association did not remain significant after adjusting for current smoking and airway obstruction (Sauni et al., 2012). Arakawa et al proposed that adiponectin could be used as a biomarker for interstitial fibrosis as decreased levels of serum adiponectin were associated with a higher incidence of pulmonary fibrosis in patients with scleroderma (Arakawa et al., 2011).

2.4 Adipsin

2.4.1 Structure and functions of adipsin

Adipsin, also known as complement factor D, is a serine protease and its gene was isolated in 1986 (Min & Spiegelman, 1986). Adipsin was identified as an adipokine in 1987 (Cook et al., 1987) and two forms of adipsin have been found, proteins of 44 and 37 kDs, which are further converted to a 25.5 kD protein by enzymatic deglycosylation (Cook et al., 1987). Adipsin is expressed in both adipocytes and monocyte-macrophages in humans and it acts as the rate-limiting enzyme in the alternative complement cascade (White et al., 1992). This supports the role of adipsin as a pro-inflammatory factor together with the finding that adipsin expression is regulated by macrophage-derived factor TNF- α (Min & Spiegelman, 1986).

2.4.2 Adipsin in respiratory diseases

Adipsin has been associated with pulmonary hypertension in an experimental rat model (Zhu et al., 1994), but little is known about the role of adipsin in human lung diseases. There are only two publications which have investigated the concentrations of adipsin in human respiratory diseases, i.e. increased plasma adipsin levels have been found in males with seasonal allergic rhinitis (Ciprandi et al., 2009) or with heavy occupational exposure to silica (Sauni et al., 2012). So far there are no reports on adipsin in obstructive or other interstitial lung diseases.

2.5 Leptin

2.5.1 Structure and general functions of leptin

The first and the best characterized adipokine, leptin, is a 16 kDa protein found in 1994 and encoded by the obese gene (*ob*) located on chromosome 7q31.3 (Y. Zhang et al., 1994; Green et al., 1995). Leptin has a three-dimensional structure with four α -helices that are typical for the IL-6-family of cytokines like IL-6, IL-12 and IL-15 (F. Zhang et al., 1997). The leptin receptor (Ob-R) has at least six isoforms (Tartaglia et al., 1995) and leptin acts through the full-length functional isoform of Ob-R (Ob-Rb) (Tartaglia et al., 1995), which is expressed by many cell types, e.g. lymphocytes, monocytes and endothelial cells (Lord et al., 1998). It has been shown that both leptin and leptin receptor (Ob-Rb) are expressed in the human lung in bronchial and alveolar epithelial cells, alveolar type II pneumocytes, macrophages and bronchial smooth muscle cells (Bruno et al., 2005; Nair et al., 2008; Vernooij et al., 2009).

There are several intra-cellular pathways involved in mediating the effects of leptin on immune cells e.g. Janus kinase 2 (JAK2)/-signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinases (MAPKs) p38 and extracellular-signal-regulated kinase (ERK), and phosphatidylinositol 3 kinase (PI3K) (Banks et al., 2000).

Circulating leptin levels correlate positively with the mass of the adipose tissue (Maffei et al., 1995). In addition, the sex hormones have been reported to exert effects on its production so that testosterone reduces the concentration of leptin (Friedman & Halaas, 1998; Luukkaa et al., 1998) whereas oestrogens increase its production (Friedman & Halaas, 1998). Thus higher serum leptin levels have been detected in females than in males, even when the values were adjusted for age and body mass index (BMI) (Friedman & Halaas, 1998).

The primary role of leptin is regarded to be in the control of appetite and energy metabolism through the central nervous system, but it also has importance in the regulation of both innate and adaptive immunity and inflammatory processes (La Cava & Matarese, 2004). In innate immunity, leptin increases the production of many pro-inflammatory cytokines such as TNF- α , IL-6 and IL-12 in monocytes and macrophages (Gainsford et al., 1996; Loffreda et al., 1998). Leptin also modulates the activity and function of neutrophils by increasing chemotaxis (Mancuso et al., 2002) and by inducing oxidative stress through the production of inducible nitric-oxide synthase (iNOS) and reactive oxygen species (ROS) (Caldefie-Chezet et al., 2001). In addition, leptin enhances phagocytosis in macrophages (Mancuso et al., 2002) and increases the activities of natural killer cells (Tian et al., 2002).

The effect of leptin in adaptive immunity is mediated by lymphocytes. Leptin acts on thymic homeostasis by increasing lymphopoiesis thus increasing CD4⁺ T-lymphocyte proliferation as well as by reducing the rate of thymic T cell apoptosis (Howard et al.,

1999). Leptin also promotes the switch towards Th1-type immune responses by increasing INF- γ and TNF- α secretion and suppressing the production of the Th2 cytokine IL-4 (Lord et al., 1998). Increased leptin levels have been reported in synovial fluid of patients with osteoarthritis (Vuolteenaho et al., 2012) and in OA cartilage leptin has been shown to enhance the synthesis of pro-inflammatory mediators such as IL-6, IL-8 (Vuolteenaho et al., 2009) and MMPs such as MMP-1, -3 and -13 (Koskinen, Vuolteenaho et al., 2011). Hence leptin is considered to be a pro-inflammatory adipokine.

2.5.2 Leptin in asthma

Because epidemiological studies have shown that the prevalence of both asthma and obesity have increased concomitantly during recent decades (Ford, 2005), it was interesting to investigate if an obese gene product leptin would be associated with asthma. In fact, several human studies have indicated that a high serum leptin concentration is associated with asthma (Sood, 2010; Ali Assad & Sood, 2012), especially in premenopausal women (Sood et al., 2006), and in children (Guler et al., 2004; Gurkan et al., 2004; Nagel et al., 2009), especially in obese children (Mai et al., 2004). Interestingly, Sood et al reported that adjustment for leptin did not affect the association between asthma and BMI in women suggesting that the relationship between obesity and asthma was not mediated solely via leptin (Sood et al., 2006). In addition, the severity of asthma symptoms has been associated with serum leptin levels (Kattan et al., 2010).

Shore et al have demonstrated that in leptin-deficient mice the exogenous administration of leptin can increase airway hyperreactivity and the allergen specific IgE levels in serum (Shore et al., 2005), pointing to a causal role for leptin in murine asthma. However, in humans with mild atopic asthma, inhalation allergen challenge did not acutely affect the serum leptin concentration (Sood et al., 2009). Leptin itself did not promote smooth muscle proliferation (Nair et al., 2008), but it has been reported to increase the release of vascular endothelial growth factor (VEGF) from airway smooth muscle cells and leptin could therefore in this way influence angiogenesis and airway remodelling (Shin et al., 2008).

Although there are many reports supporting a role for leptin in asthma, some studies have not shown any association between asthma and circulating leptin levels (Kim et al., 2008; Jartti et al., 2009; Sutherland et al., 2009). Thus the current knowledge on the association between leptin and asthma is still controversial and the relationship between leptin and asthma in non-obese adults is not known.

2.5.3 Leptin in COPD

The expression of leptin is increased in bronchial epithelial cells and alveolar macrophages in ex-smokers with or without severe COPD as compared to never smokers (Vernooy et al., 2009), and the level of leptin expression is associated with the severity of COPD (Bruno et al., 2005; Vernooy et al., 2009). As in asthma, high circulating leptin levels have been reported especially in female and overweight patients with COPD (Breyer et al., 2011) suggesting that sex and BMI are significant confounding factors also in the association between leptin and COPD. On the other hand, some groups have not found any differences in serum leptin levels between patients with COPD and healthy controls or any associations between leptin levels and the severity of COPD (Kirdar et al., 2009; Dickens et al., 2011).

The circulating leptin levels in COPD may also be affected by the phenotype of the patient, as lower leptin levels have been reported in COPD patients with either osteoporosis (Vondracek et al., 2009) or emphysema (Schols et al., 1999). However, these results may be affected by the lower fat mass and BMI in the subjects with osteoporosis or emphysema as lower circulating leptin levels have been reported in COPD patients with either low (Takabatake et al., 1999) or normal (Eker et al., 2010) BMI. Higher circulating leptin levels are also related to systemic inflammatory activity (Breyer et al., 2012) and COPD exacerbations (Creutzberg et al., 2000; Krommidas et al., 2010). Thus the precise role of leptin in the pathogenesis of COPD, particularly in different phenotypes remains unresolved.

2.5.4 Leptin in interstitial lung diseases

The role of leptin in interstitial lung diseases is not known. So far, there are no previous studies investigating the role of leptin in asbestos-induced lung diseases. There is one study published about adipokines in silica exposure (Sauni et al., 2012). They found no association between leptin and silica exposure and plasma leptin levels did not differ between silica-exposed workers and non-exposed controls, although increased plasma levels of other adipokines were reported (Sauni et al., 2012).

2.6 Nesfatin-1

2.6.1 Structure and functions of nesfatin-1

Nesfatin-1 is a 55 kDa protein, which is synthesized from its precursor protein nucleobindin 2 (NUCB2) (later termed as NUCB2-encoded satiety- and fat-influencing protein), whose gene is located on chromosome 11 (Miura et al., 1992). Nesfatin-1 was discovered in 2006 by a Japanese group, who showed that nesfatin-1 was expressed in the appetite-control nuclei in rat brain and that intracerebroventricular injection of nesfatin-1 could

reduce appetite and body weight (Oh-I et al., 2006). Later, nesfatin-1 expression was also identified in human adipose tissue, assigning it as a novel adipocytokine (Ramanjaneya et al., 2010). Stimulation of subcutaneous adipose tissue with TNF- α , IL-6, insulin and dexamethasone has been shown to increase the secretion of nesfatin-1 (Ramanjaneya et al., 2010). Nesfatin-1 levels in human plasma have not been demonstrated to differ between males and females (Barnikol-Watanabe et al., 1994; Li et al., 2010) and nesfatin-1 does not seem to be associated with BMI (Li et al., 2010; Hofmann et al., 2013; Cetinkaya et al., 2013).

2.6.2 Nesfatin-1 in different diseases

The role of nesfatin-1 in human disorders, especially in respiratory diseases, remains largely unknown and there are only a few studies published on this topic. Lower fasting plasma nesfatin-1 levels have been measured in patients with type II diabetes as compared to healthy controls and to type I diabetes patients, (Li et al., 2010). On the other hand, nesfatin-1 plasma levels are lower in patients with lung cancer and weight loss (Cetinkaya et al., 2013). Nesfatin-1 may also have a role in the modulation of emotions, since it has been associated with higher scores of anxiety in female obese subjects (Hofmann et al., 2013). In patients with cystic fibrosis, the circulating nesfatin-1 levels were highest in those subjects with severe disease and low fat mass, and the levels were not associated with serum levels of TNF- α or IL-6 (Cohen et al., 2013). Nesfatin-1 has been claimed to have anti-inflammatory and anti-apoptotic effects in rat brain after traumatic brain injury (Tang et al., 2012) while it exerted pro-inflammatory effects in human chondrocytes (Scotece, Conde, Abella et al., 2014). Nesfatin-1 also seems to have a cardio-protective effect, as it has been shown to protect the heart against ischemia (Angelone et al., 2013) and decreased plasma levels of nesfatin-1 have been measured in acute myocardial infarction (Dai et al., 2013). However, at present, there are no studies which would have attempted to clarify the role of nesfatin-1 in asthma, COPD or parenchymal lung diseases.

2.7 Resistin

2.7.1 Structure and functions of resistin

Resistin, also known as FIZZ3 (“found in inflammatory zone”), belongs to the family of resistin-like molecules (RELMs) and it received its name from the observation that it induced insulin resistance in mice (Steppan et al., 2001). The family of resistin-like molecules is encoded by 4 genes in the mouse (mresistin, mRELM α , - β , - γ) and by two genes in humans (hresistin and hRELM β) (Gerstmayer et al., 2003). The human resistin gene is located on chromosome 19 (Steppan & Lazar, 2004).

In human adipose tissue, macrophages, not adipocytes, are the most important source of resistin (L. Patel et al., 2003) suggesting that in humans, resistin is more implicated in inflammation rather than in metabolism. This is supported by a finding that there is no clear relationship between the levels of resistin, obesity and insulin resistance in humans (Lee et al., 2003). Resistin gene expression is induced in human mononuclear cells by pro-inflammatory cytokines IL-1, IL-6 and TNF- α (Kaser et al., 2003) and resistin itself promotes the expression of TNF- α and IL-6 and thus also enhances its own activity via a positive feedback (Bokarewa et al., 2005). Resistin circulates in two forms, the more prevalent high-molecular-weight hexamer and the less prevalent, but more bioactive low-molecular-weight complex (S. D. Patel et al., 2004). A toll-like receptor 4 (TLR4) serves as the receptor for the pro-inflammatory effects of resistin in human cells (Tarkowski et al., 2010) and they are transmitted intracellularly by the NF- κ B signalling pathway (Silswal et al., 2005).

The recent data from rodents and human studies indicate that resistin is involved in cardiac pathology by participating in the development of vascular endothelial cell dysfunction, angiogenesis and smooth muscle cell proliferation (Schwartz & Lazar, 2011; Jamaluddin et al., 2012). Interestingly, plasma resistin levels have also been reported to correlate with oxidative stress and myocardial injury in patients undergoing cardiac surgery (Laurikka et al., 2014).

2.7.2 Resistin in inflammatory lung diseases

Resistin has been shown to be expressed in murine lungs in both bronchial epithelial cells and in type II pneumocytes (Holcomb et al., 2000). However, the role of resistin in inflammatory lung diseases is not clear, as increased (Al Mutairi et al., 2011; Kumor-Kisielewska et al., 2013), decreased (Kim et al., 2008) and unchanged (Arshi et al., 2010) circulating resistin levels have been reported in patients with asthma as compared to healthy controls. A negative correlation between resistin and bronchial hyperreactivity has been demonstrated (Kim et al., 2008) evidence for a protective role against asthma, but on the other hand, resistin has also been associated with the severity of asthma in steroid treated patients (Larochelle et al., 2007).

The results on the role of resistin in COPD are also conflicting, as both increased and unchanged levels of circulating resistin have been described in COPD (Breyer et al., 2011; Al Mutairi et al., 2011; Kumor-Kisielewska et al., 2013). However, in the studies observing the increased resistin concentrations in COPD, there was also an association between resistin levels and the severity of airway obstruction (Al Mutairi et al., 2011; Kumor-Kisielewska et al., 2013).

Increased plasma resistin concentrations have been reported in subjects who had been exposed to silica (Sauni et al., 2012), but nothing is known on whether resistin plays any

role in interstitial lung diseases. It seems that resistin may have associations with different inflammatory lung diseases, but it still remains unclear whether it has disease promoting or protective functions.

2.8 Visfatin

2.8.1 Structure and functions of visfatin

Visfatin, also called NAMPT (nicotinamide phosphoribosyltransferase) and previously identified as pre-B cell colony-enhancing factor (PBEF) is a 52 kDa protein encoded by the PBEF gene located on chromosome 7 (Samal et al., 1994). It was originally discovered in lymphocytes, bone marrow, liver and muscles (Samal et al., 1994), but later identified in many other organs including the lungs (Adeghate, 2008). Leucocytes, especially granulocytes and monocytes, are the major sources of visfatin (Friebe et al., 2011), but also macrophages express this adipokine, even in higher amounts than adipocytes (Curat et al., 2006). Visfatin is expressed in visceral adipose tissue and high circulating levels are seen in obesity, especially in children (Friebe et al., 2011; Jaleel et al., 2013). However, also lower (Derosa et al., 2013) and unchanged (Olszanecka-Glinianowicz et al., 2012) visfatin plasma levels have been reported in obese compared to normal weight persons. The plasma concentration of visfatin did not correlate with insulin resistance (Oki et al., 2007) but it has been claimed to be a pleiotropic protein, functioning as a growth factor, a cytokine and an enzyme and it may play a role in regulation of glucose and lipid metabolism (L. Q. Zhang et al., 2011).

Visfatin is also involved in the regulation of inflammation and since it can influence innate immunity. Visfatin expression is induced in mammals by LPS and pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6 (Ognjanovic & Bryant-Greenwood, 2002) and in monocytes visfatin itself has been reported to induce the production of these same pro-inflammatory cytokines by stimulating the p38 MAPK and MEK-1 pathways (Moschen et al., 2007). Visfatin expression positively correlated with serum levels of IL-6 and CRP (Oki et al., 2007). Taken together, these data support visfatin's role as a pro-inflammatory adipokine.

2.8.2 Visfatin in lung diseases

Visfatin has been shown to inhibit neutrophil apoptosis in patients with sepsis (Jia et al., 2004) and individuals infected with pandemic H1N1 influenza, where the inhibition of visfatin expression attenuated inflammation (Gao et al., 2011). Visfatin has also been proposed as a biomarker in acute lung injury (ALI) where both serum and bronchoalveolar lavage fluid levels of visfatin were reported to increase (Ye et al 2005). There are only two

previous publications on visfatin and COPD. Liu et al described higher plasma visfatin levels in underweight men with COPD in comparison with normal-weight controls (X. Liu et al., 2009). In addition, they detected positive correlations between the levels of visfatin and CRP and TNF- α , but no correlation was found between visfatin concentrations and BMI values (X. Liu et al., 2009). In another study, significantly lower visfatin levels were observed in normal-weight patients with COPD than in controls with similar BMI values (Eker et al., 2010).

AIMS OF THE STUDY

Several cell types and cytokines are involved in the complex inflammation processes present in both obstructive pulmonary diseases asthma and COPD as well as in the interstitial lung disease induced by exposure to asbestos fibres. Adipokines are hormone-like mediators identified first as products of adipose tissue involved in the regulation of energy metabolism and appetite. Later they have been found to be produced by several other cells and tissues, also in the lungs and to regulate inflammatory responses. Therefore it was hypothesised that adipokines could be involved in the inflammatory activity in lung diseases. The present study was designed to evaluate the association of adipokines to asthma, COPD and asbestos-induced interstitial lung disease and to investigate if adipokines might have value as biomarkers of disease severity and/or treatment response in these lung diseases.

The detailed aims were:

1. to study if adipokines are associated with the activity of airway inflammation, with lung function impairment or with symptoms in asthma and COPD (I, III–IV).
2. to examine if adipokines could predict responsiveness to glucocorticoids in asthma or COPD (I, III–IV).
3. to clarify whether adipokines are associated with the systemic inflammation in asthma or COPD (I, III–IV).
4. to investigate if adipokines are related to systemic inflammation and the severity of pulmonary fibrosis in asbestos-exposed subjects (II)

SUBJECTS AND METHODS

1 Subjects and Study Protocols

1.1 Adipokines in asthma (I)

Thirty-five steroid-naive, non-smoking female patients with asthma (mean age 34 yrs, range 20–57 yrs) with BMI ≤ 30 kg/m² (range 18–30 kg/m²) were recruited by the Department of Respiratory Medicine at Tampere University Hospital. The diagnosis of asthma was based on symptoms and reversible or variable airway obstruction (β_2 -agonist induced increase in FVC or FEV₁ $\geq 12\%$ and 200 ml, or diurnal variability in PEF $\geq 20\%$, or exercise induced decrease in FEV₁ $\geq 15\%$). Thirty-two age- and sex-matched non-smoking healthy controls with similar BMI values, no asthmatic symptoms and normal lung function served as controls. Both groups were free from any other chronic diseases.

Lung function, asthma symptom score, plasma levels of adipokines, serum levels of IgE, eosinophil cationic protein (ECP), eosinophil protein X (EPX), myeloperoxidase (MPO), interleukin 6 (IL-6), blood eosinophil count (EOS), and exhaled nitric oxide (NO) were measured in the patients and the controls. The asthmatics also filled in an asthma symptom questionnaire (Lehtimäki, Kankaanranta, Saarelainen, Turjanmaa et al., 2001). The same measurements were repeated in eleven asthmatics after 8 weeks of treatment with inhaled fluticasone propionate (500 μ g b.i.d. during weeks 1–4, and 250 μ g b.i.d. during weeks 5–8; Flixotide Diskus, GSK, Ware, UK).

1.2 Adipokines in asbestos-exposed workers (II)

A total of 118 men with moderate or heavy occupational exposure to asbestos [estimation of at least 20 fibre years considered sufficient to cause asbestosis (Asbestos, asbestosis, and cancer: The Helsinki criteria for diagnosis and attribution, 1997)] were recruited by the Department of Occupational Medicine at Tampere University Hospital. In order to exclude other pulmonary diseases that might affect the inflammatory markers, the following exclusion criteria were applied: FEV₁/FVC < 0.70 , bronchiectasis or any signs of emphysema on high resolution computed tomography (HRCT) of the lungs, diagnosed asthma, or the use of asthma medication. All the subjects were never smokers or had quit smoking for at least 5 years prior to the study.

Spirometry was performed and a venous blood sample was drawn in all subjects. HRCT of the lungs was taken and pulmonary diffusing capacity for carbon monoxide ($D_{L,CO}$) was measured in the asbestos-exposed subjects, and they were divided into three groups based on interstitial findings on HRCT: (1) normal interstitial (= parenchymal) finding, (2) borderline interstitial findings with minor sporadic fibrotic changes only, or (3) mild to extreme pulmonary fibrosis i.e. asbestosis.

Of the 118 asbestos-exposed men, 33 had to be excluded based on the above mentioned exclusion criteria (16 due to $FEV_1/FVC < 0.70$, 11 due to emphysema or bronchiectasiae on HRCT and 6 due to diagnosed asthma or use of asthma medication) and thus 85 were included in the study. Of these 85 subjects, 35 subjects had normal interstitial findings on HRCT (fibrosis class 0), 31 subjects had borderline interstitial changes (fibrosis classes 0.5–1.5), and 19 subjects exhibited signs of pulmonary fibrosis (fibrosis classes ≥ 2.0) and were regarded as exhibiting asbestosis. Twenty-eight non-smoking healthy men with normal spirometry values, no respiratory symptoms and no known exposure to asbestos or other harmful agents served as the control group.

1.3 Adipokines in COPD (III–IV)

This study involved forty-three steroid-naïve male patients with COPD among subjects who had been referred from primary care for diagnostic assessment to the Department of Respiratory Medicine at Tampere University Hospital. The diagnosis of COPD was based on GOLD strategy paper (GOLD 2013) and the inclusion criteria were smoking history of at least 20 pack-years, symptoms of COPD (cough, sputum production and dyspnoea), post bronchodilator $FEV_1/FVC < 0.7$, reversibility of FVC and FEV_1 induced by β_2 -agonist $< 12\%$ or 200 ml and pulmonary emphysema visible on HRCT of the lungs. None of the subjects had a diagnosis or a clinical history of asthma or diabetes. Ten (23%) of the patients had hypertension and five (12%) had hypercholesterolemia while the number of patients with other diseases was too small to allow any statistical analysis. Forty-one age-matched non-smoking healthy males with normal lung function served as controls.

Spirometry, body plethysmography, fractional exhaled nitric oxide concentration (F_eNO) and pulmonary diffusing capacity per unit of alveolar volume standardized for haemoglobin ($Hb-D_{L,CO}/V_A$) were measured, HRCT of the lungs was performed and the symptoms being scored with the St George's Respiratory Questionnaire (SGRQ) in patients with COPD. A venous blood sample was drawn from all subjects. The same measurements excluding HRCT were repeated in twenty-seven patients with COPD after 4 weeks of treatment with inhaled fluticasone propionate (500 μ g b.i.d. Flixotide Diskus; GlaxoSmithKline, Ware, UK).

The basic characteristics of the subjects (both the patients and the controls) in different studies in the present thesis is summarized in Table 2.

Table 2. Subject characteristics in studies I (asthma), II (asbestos-exposed) and III–IV (COPD).

	Study I Asthma	Study II Asbestos-exposed			Study III–IV COPD
		No fibrosis	Borderline	Fibrotic i.e. asbestosis	
N (F/M)	35 (35/0)	35 (0/35)	31 (0/31)	19 (0/19)	43 (0/43)
Age (yrs)	33.9 ± 2.1	62 ± 1	67 ± 1	68 ± 1	59.5 ± 1.2
BMI (kg/m ²)	23.1 ± 0.5	30 ± 1	29 ± 1	27 ± 1	25.8 ± 0.6
FEV ₁ (% pred)	90 ± 1.9	92 ± 2	85 ± 3;	82 ± 3	53 ± 2
Smoking status	non-smokers	non-smokers or quit smoking for ≥ 5 years prior			≥ 20 pack-years
	Study I Controls	Study II Unexposed controls		Study III–IV Controls	
N (F/M)	32 (32/0)	28 (0/28)		41 (0/41)	
Age (yrs)	33.8 ± 2.1	62 ± 1		62.5 ± 1.0	
BMI (kg/m ²)	22.8 ± 0.5	27 ± 1		26.7 ± 0.6	
FEV ₁ (% pred)	96 ± 3.2	*		*	
Smoking status	non-smokers	non-smokers or quit smoking for ≥ 5 years prior			non-smokers

N, total number of subjects; F, female; M, male; BMI, body mass index; FEV₁, forced expiratory volume in 1 second; * normal in every subject
Values are presented as mean ± SEM.

1.4 Ethics

All the studies were approved by the Regional Ethics Committee of the Expert Responsibility area of Tampere University Hospital, Tampere, Finland and complied with the Declaration of Helsinki. All subjects provided their written informed consent.

2 Methods

2.1 Adipokines

Venous blood was collected for the assessment of plasma levels of adipokines.

In asthmatics and in subjects with asbestos-exposure adipokines adiponectin, adipisin, leptin and resistin concentrations were measured by enzyme-immuno-assay (EIA) using commercial reagents (DuoSet ELISA, R&D Systems Europe Ltd, Abindgon, U.K). The detection limits and inter-assay coefficients of variation, respectively, were 15.6 ng/l and 2.0% (I) and 2.4% (II) for adiponectin, 4.0 ng/l and 3.8% (I) and 1.8% (II) for adipisin,

15.6 ng/l and 3.9% (I) and 1.9% (II) for leptin and 15.6 ng/l and 4.0% (I) and 7.3% (II) for resistin.

In the patients with COPD, the levels of adipokines adiponectin (III), adipsin (unpublished data), leptin (III), nesfatin-1 (IV), resistin (unpublished data) and visfatin (IV) were determined by EIA by using the following reagents: R&D Systems Europe Ltd, Abingdon, U.K (adiponectin, adipsin, leptin, nesfatin-1 and resistin) and Phoenix Pharmaceuticals Inc., Karlsruhe, Germany (visfatin). The detection limits and inter-assay coefficients of variation, were 31.3 pg/ml and 6.7% for adiponectin, 31.3 pg/ml and 4.4% for adipsin, 15.6 pg/ml and 4.5% for leptin, 7.8 pg/ml and 11.6% for nesfatin-1, 15.6 pg/ml and 7.3% for resistin and 0.1 ng/ml and 8.3% for visfatin.

All adipokine measurements were conducted in the laboratory of the Immunopharmacology Research Group, University of Tampere School of Medicine and Tampere University Hospital, Tampere, Finland between 2008–2014.

2.2 Other inflammatory markers in blood

In the asthma study, venous blood was collected for the assessment of serum levels of immunoglobulin E (IgE), eosinophil cationic protein (ECP), eosinophil protein X (EPX), myeloperoxidase (MPO), interleukin 6 (IL-6), and blood eosinophil count (EOS). Radioimmunoassay (ECP RIA, EPX RIA and MPO RIA, Pharmacia AB, Uppsala, Sweden) was used to measure ECP, EPX and MPO levels. Immunoluminometry was used to estimate IgE, and IL-6 was assayed by EIA (PeliPair ELISA, Sanquin, Amsterdam, The Netherlands). The detection limits and inter-assay coefficients of variation, respectively, were 2.0 µg/l and 4.2% for ECP, 3.0 µg/l and 5.4% for EPX, 8.0 µg/l and 6.2% for MPO and 0.6 ng/l and 6.1% for IL-6.

In workers with asbestos-exposure, venous blood was collected for the assessment of serum levels of IL-6 and interleukin 8 (IL-8), and blood erythrocyte sedimentation rate (ESR). IL-6 and IL-8 were measured by EIA (PeliPair ELISA, Sanquin, Amsterdam, The Netherlands). The detection limits and inter-assay coefficients of variation, respectively, were 0.6 ng/l and 2.9% for IL-6 and 1.56 ng/l and 0.9% for IL-8.

In patients with COPD, plasma concentrations of IL-6, IL-8, matrix metalloproteinase 9 (MMP-9) and TNF- α were determined by EIA with the following reagents: MMP-9 and tumour necrosis factor alpha (TNF- α): R&D Systems Europe Ltd, Abingdon, U.K, IL-6: Sanquin, Amsterdam, The Netherlands and IL-8: BD Biosciences San Diego, CA, USA. The detection limits and inter-assay coefficients of variation, were 0.3 pg/ml and 7.6% for IL-6, 1.56 pg/ml and 7.0% for IL-8, 7.8 pg/ml and 6.0% for MMP-9 and 0.5 pg/ml and 6.5% for TNF- α .

2.3 Exhaled nitric oxide (NO) measurement

Exhaled NO was measured with a Sievers NOA 280[®] NO-analyzer (Sievers Instruments, Boulder, CO, USA). The analyzer was calibrated daily with a known NO concentration [103 parts per million (ppm), AGA, Sweden] and before every subject with filtered NO-free air. Bronchial NO flux ($J_{aw}NO$) and alveolar NO concentration ($C_A NO$) were calculated for asthmatic subjects based on measurements at flow rates of 100, 175 and 370 ml/s using the method described by Tsoukias and George (Tsoukias & George, 1998; Lehtimäki, Kankaanranta, Saarelainen, Hahtola et al., 2001) and the fractional exhaled NO concentration at an exhalation flow rate of 50 mL/s ($F_{\epsilon}NO_{0,05}$) was used in the analysis of patients with COPD.

2.4 Lung function

Spirometry (Vmax 20 C spirometer, Sensor-Medics, Yorba Linda, CA, USA) and body plethysmography (Autobox 6200, Sensor-Medics, Yorba Linda, CA, USA) were measured before and after 400 µg of inhaled salbutamol. Pulmonary diffusing capacity for carbon monoxide ($D_{L,CO}$) was assessed with Vmax 20 C, Sensor-Medics.

2.5 High-resolution computed tomography (HRCT) of the lungs

HRCT was scanned with a Siemens Somatom Plus 4 (Siemens Medical, Erlangen, Germany). In the study on asbestos-exposure, 1 mm slices were taken at 3 cm intervals using imaging parameters of 130–140 kV and 100–111 mA, and in the COPD studies, a section thickness of 1mm was used with a 10-mm inter-slice spacing at 140 kV and 206 mA. The subjects were lying in the supine (COPD) or prone (asbestos-exposed subjects) position and performing full inspiration. The HRCT images were scored according to a consensus by two experienced thoracic radiologists blinded to the medical information of the patients.

2.5.1 HRCT grading of asbestos-induced changes

Pulmonary fibrosis, emphysema, parietal pleural plaques, and pulmonary nodules were scored separately as described previously (Oksa et al., 1994; Huuskonen et al., 2001). The semi-quantitative scoring of the HRCT findings indicating interstitial lung fibrosis (septal thickening, subpleural lines, parenchymal bands or honeycombing) in both lungs was made according to a scale of classes from 0 to 5. Fibrosis class 0 represents normal parenchymal finding, class 1 (0.5–1.5) represents borderline parenchymal finding with minor sporadic changes, and classes 2 to 5 represent mild to severe diffuse pulmonary fibrosis (Huuskonen

et al., 2001; Leivo-Korpela et al., 2012). If the readers could not match the findings exactly with any given fibrosis class, five subclasses (0.5, 1.5, 2.5, 3.5, 4.5) were used. Fibrosis class 2 has been considered as a threshold for the diagnosis of asbestosis (Huuskonen et al., 2001).

2.5.2 HRCT grading of emphysema and bronchial changes in COPD

The extent of emphysema was estimated visually to the nearest 5% on each image section excluding the two most cranial and caudal images, as described by Desai and colleagues (Desai et al., 2007). The mean value of emphysema percent on all image sections was taken as the final emphysema score for each subject. In the evaluation of the airway wall thickness, the external diameter (D) and the luminal diameter (L) of the right upper lobe bronchus were measured in each subject. Only images showing the bronchus as a straight cross-section without an angle were selected. Airway wall thickness (T) was calculated as $(D-L)/2$. The thickness-to-diameter ratio (TDR) and the percentage wall area (PWA) were calculated as $TDR=T/D$, and $PWA = [(D/2)^2\pi - (L/2)^2\pi]/[(D/2)^2\pi] \times 100\%$ (Orlandi et al., 2005). TDR and PWA were used in statistical analysis.

2.6 Symptoms

2.6.1 Asthma symptoms

Asthma symptoms were recorded by using a written symptom questionnaire. Cough, chest tightness, wheezing and nocturnal asthma symptoms were each scored from 0 to 3 yielding a total score ranging from 0 to 12 points as previously described (Lehtimäki et al., 2001).

2.6.2 Symptoms in the patients with COPD

The subjects filled in the Finnish translation of the SGRQ containing questions which has scoring on three aspects of the disease (symptom frequency and severity, activities that cause or are limited by breathlessness, and the impact of the disease on social functioning including psychological disturbances resulting from the disease) to obtain a total score. The scale has a range from 0 to 100, with higher scores representing more severe disease.

2.7 Cell culture

Human THP-1 monocyte / macrophage cell line (American Type Culture Collection, Manassas, VA, USA) was used. The cells were cultured at 37 °C in a humidified 5% carbon dioxide atmosphere in RPMI 1640 medium adjusted to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/l glucose, and 1.5 g/l bicarbonate, and

supplemented with 10% heat-inactivated fetal bovine serum (all obtained from Lonza Verviers SPRL, Belgium), penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (250 ng/ml) (all obtained from Invitrogen, Paisley, UK), and 0.05 mM 2-mercaptoethanol. The cells were differentiated into macrophages by adding the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA, 100 nM) for 72 h at the time of seeding of the cells on 24-well plates. Cells were serum starved for 16 h before the experiments were started. Resistin (recombinant human resistin; PeproTech, Inc., Rocky Hill, NJ, USA) and fluticasone (Sigma Chemical Co, St. Louis, MO, USA) were added into the fresh culture medium, and the cells were incubated for 24 h. Culture medium was collected and stored at -20°C until assayed. The concentrations of human IL-6 (PeliPair ELISA, Sanquin, Amsterdam, The Netherlands) and human TNF- α (R&D Systems, Minneapolis, MN, USA) were determined by ELISA. The detection limits and intra-assay coefficients of variation, were for 7.8 ng/l and 4.8% for TNF- α and 0.6 ng/l and 6.0% for IL-6, respectively.

2.8 Statistics

SPSS versions 15.0.1 and 19 (SPSS Inc., Chicago, Illinois, USA) and InStat 3.05 (GraphPad Software, Inc., San Diego, CA, USA) softwares were used in the statistical analysis and p-values < 0.05 were considered as significant. The normality of the distributions was analysed with Kolmogorov-Smirnov's test. Some of the skewed data could be normalised by log-transformation. The results are presented as mean \pm SEM for normally distributed data and as median [interquartile range] for non-normally distributed data.

To study between-group differences, t-test, and one-way analysis of variance (with either Games-Howell or Dunnett's post-test) were used in the normally distributed data, while Mann-Whitney test, and Kruskal-Wallis test (with Dunn's post-test) were used for non-normal data. Paired t-test and Wilcoxon's test were used for repeated measurements in analysing the effects of glucocorticoid treatment.

Pearson's r and Spearman's rho were used to study correlations for normally and non-normally distributed data, respectively. Since BMI affects the circulating levels of some adipokines, the possible confounding effect of BMI was controlled for on the associations between adipokines and the other measured parameters either by dividing the measured adipokine levels by BMI (I) or by using partial correlation with BMI as a control variable (II, III). In study I, a stepwise multiple linear regression analysis was also used to further determine if the correlations between lung function indices and the levels of serum adipokines could be attributable to BMI.

SUMMARY OF THE RESULTS

1 Adiponectin in inflammatory lung diseases

1.1 Adiponectin in asthma (I) and COPD (III)

In patients with COPD, plasma adiponectin levels correlated positively with airway resistance (Raw) ($r = 0.362$, $p = 0.019$) and functional residual capacity (FRC) ($r = 0.355$, $p = 0.046$) indicating that in these patients high plasma adiponectin concentrations were associated with peripheral obstruction and hyperinflation. However, the adiponectin level did not correlate with FEV₁ or FEV₁/FVC, plasma inflammatory markers, the degree of emphysema or airway wall thickness on HRCT or with St George's Respiratory questionnaire (SGRQ) scores. In patients with asthma, BMI-adjusted adiponectin level displayed no correlations with spirometric parameters of lung function, symptoms, exhaled NO or serum markers of inflammation. There were no significant differences in BMI-adjusted plasma adiponectin levels between asthmatics ($n = 35$) and healthy controls. Furthermore, plasma adiponectin levels did not differ between the patients with COPD ($n = 43$) and their controls nor between ex-smokers ($n = 10$) and current smokers ($n = 33$) in the COPD group.

Treatment with inhaled fluticasone did not induce any statistically significant changes in plasma BMI-adjusted adiponectin levels in asthma or adiponectin levels in COPD. However, it was interesting that in patients with COPD, the baseline adiponectin concentration correlated negatively with the fluticasone induced changes in health status as assessed by the SGRQ total score ($r = -0.410$, $p = 0.042$), symptoms measured by SGRQ symptom score ($r = -0.413$, $p = 0.040$) and in FRC% pred ($r = -0.428$, $p = 0.003$), i.e. higher baseline plasma adiponectin levels were associated with better relief of symptoms and dynamic hyperinflation in response to fluticasone treatment in COPD (III).

1.2 Adiponectin in asbestos-induced interstitial lung disease (II)

Plasma adiponectin levels were not related to the degree of interstitial fibrosis in subjects with asbestos-exposure and no correlations were found between adiponectin and lung function, pleural plaques or the inflammatory markers measured in blood. There were no significant differences in plasma adiponectin concentrations between the subjects exposed to asbestos and the unexposed, healthy controls.

Thus, high plasma levels of adiponectin were associated with peripheral airway obstruction and dynamic hyperinflation as well as with a favourable response to fluticasone treatment in patients with COPD, but the adiponectin level was not related to asthma or its severity. No relationship was detected between plasma adiponectin concentrations and asbestos-induced interstitial lung disease.

2 Adipsin in inflammatory lung diseases

2.1 Adipsin in asthma (I) and COPD (unpublished data)

The plasma adipsin level correlated positively with fractional exhaled nitric oxide ($F_{\epsilon}NO_{0.05}$) ($r = 0.437$, $p = 0.004$) in patients with COPD, but BMI-adjusted adipsin values exhibited no correlations with indices of lung function, symptoms, exhaled NO or serum markers of inflammation in patients with asthma. There were no significant differences in BMI-adjusted plasma adipsin levels between patients with asthma ($n = 35$) and their controls. Furthermore, the plasma adipsin levels did not differ between the patients with COPD ($n = 43$) and healthy controls. Treatment with inhaled fluticasone decreased the BMI-adjusted plasma adipsin levels in a statistically significant manner (I) in patients with asthma. A declining trend was also seen in plasma adipsin levels in patients with COPD during inhaled fluticasone treatment ($883 \pm 27 \rightarrow 842 \pm 24$ ng/ml, $p = 0.092$).

2.2 Adipsin in asbestos-induced interstitial lung disease (II)

In subjects with a history of moderate to heavy exposure to asbestos ($n = 85$), the adipsin concentration in plasma correlated positively with the degree of interstitial fibrosis on HRCT ($\rho = 0.412$, $p < 0.001$). There was also a statistically significant ($p < 0.0001$) increasing linear trend in serum adipsin levels when the asbestos-exposed subjects were divided into three groups of increasing interstitial involvement; subjects with normal interstitial findings ($n = 35$), borderline interstitial changes ($n = 31$) or fibrosis i.e. asbestosis ($n = 19$) on HRCT (Figure 2). Accordingly, adipsin levels were significantly higher in

asbestos-exposed subjects with pulmonary fibrosis as compared to subjects exposed to asbestos with no interstitial fibrosis or to unexposed control subjects (Figure 2).

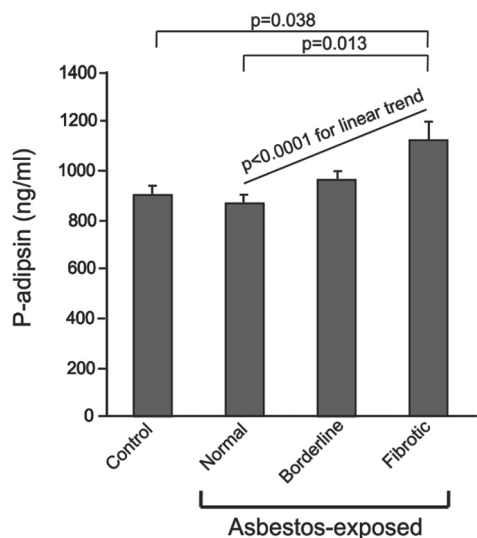


Figure 2. Plasma adipsin levels in asbestos-exposed subjects with normal, borderline or fibrotic (i.e. asbestosis) interstitial findings on HRCT of the lungs, and in unexposed controls (Reprinted with permission from: Leivo-Korpela et al. 2012, Respiratory Medicine 106: 1435–1440 © Elsevier Ltd.)

The association between adipsin and the severity of asbestos-induced lung reaction was further supported by the findings that plasma adipsin levels correlated negatively with pulmonary diffusing capacity ($D_{L,CO}$) (Figure 3A) and positively with the extent of pleural plaques in the asbestos-exposed subjects ($r = 0.245$, $p = 0.043$), i.e. the higher the adipsin level, the poorer the diffusing capacity and the more pleural plaques that were present.

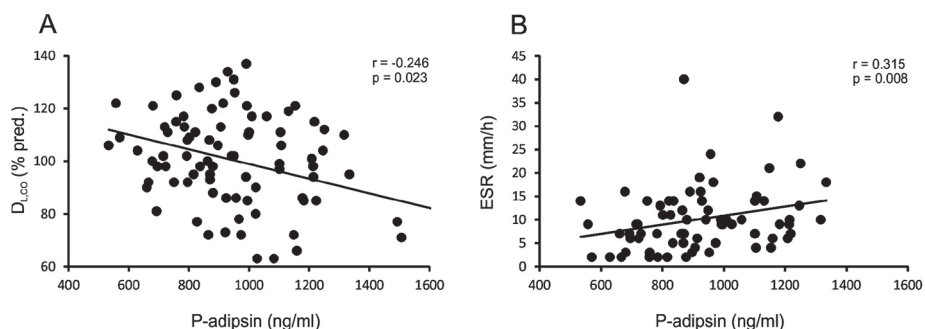


Figure 3. Plasma adipsin levels correlated negative with pulmonary diffusing capacity for carbon monoxide ($D_{L,CO}$) (A) and positively with erythrocyte sedimentation rate (ESR) (B) in subjects exposed to asbestos ($n = 85$). (Reprinted with permission from: Leivo-Korpela et al. 2012, Respiratory Medicine 106: 1435–1440. © Elsevier Ltd., modified)

The plasma adipsin concentrations also correlated with ESR (Figure 3B) and with plasma levels of IL-6 ($r = 0.256$, $p = 0.018$) revealing an association between adipsin and systemic inflammation in those subjects with moderate or heavy occupational exposure to asbestos.

In summary, adipsin was found to be associated with the degree of pulmonary fibrosis and systemic inflammation in subjects exposed to asbestos but plasma adipsin levels were not associated with lung function, symptoms or indices of inflammation in asthma. In patients with COPD, the plasma adipsin levels correlated positively with fractional exhaled NO. Further, treatment with inhaled glucocorticoids reduced plasma adipsin levels.

3 Leptin in inflammatory lung diseases

3.1 Leptin in asthma (I) and COPD (III)

In patients with asthma, BMI-adjusted leptin levels correlated positively with the asthma symptom score ($\rho = 0.371$, $p = 0.031$) and negatively with lung volumes VC% predicted ($\rho = -0.445$, $p = 0.007$), FVC% predicted ($\rho = -0.406$, $p = 0.016$; Figure 4A) and with FEV₁% predicted ($\rho = -0.345$, $p = 0.045$; Figure 4B), i.e. the higher the leptin level, the poorer the lung function and the more experienced symptoms by the patient (I). It was confirmed with a stepwise multiple linear regression analysis that leptin was a predictor of VC% predicted, FVC% predicted and FEV₁% predicted independently of BMI.

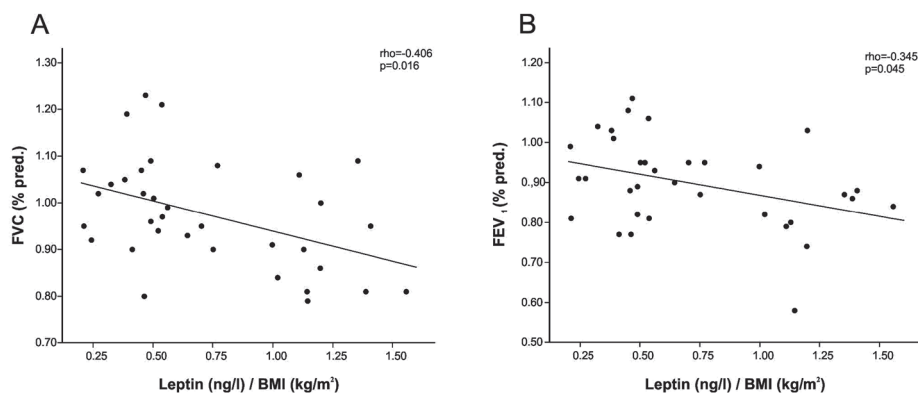


Figure 4. Correlation between BMI-adjusted plasma leptin levels and lung function parameters FVC (A) and FEV₁ (B) in patients with steroid-naïve asthma (n = 35). (Reprinted with permission from Leivo-Korpela et al. 2011, Journal of Inflammation 8:12. © BioMed Central Ltd.)

In patients with COPD, the leptin levels did not correlate with any indices of lung function measured with spirometry or body plethysmography, with the inflammatory markers measured in plasma, with the degree of emphysema or airway wall thickness on HRCT or with the health status and symptoms assessed by SGRQ scores. BMI-adjusted plasma leptin levels did not differ in patients with asthma when compared to healthy controls. The plasma leptin levels did not differ between the patients with COPD and their healthy controls or between ex-smokers and current smokers in the COPD group.

Treatment with inhaled fluticasone increased plasma leptin levels in a statistically significant manner ($6.0 \pm 1.0 \rightarrow 7.1 \pm 1.2$ ng/ml, $p = 0.018$) in patients with COPD but had no major effect on BMI-adjusted leptin concentrations in asthmatics. Before the treatment with inhaled fluticasone, BMI-adjusted leptin tended to correlate positively with the change in serum levels of ECP ($\rho = 0.545$, $p = 0.083$) and EPX ($\rho = 0.445$, $p = 0.170$) in patients with asthma.

3.2 Leptin in asbestos-induced interstitial lung disease (II)

The plasma leptin levels did not differ between asbestos-exposed and unexposed subjects, and leptin was not related to the degree of lung fibrosis or any of the other measured markers of disease severity or inflammation in subjects exposed to asbestos.

In summary, higher plasma leptin levels were associated with poor lung function and more severe symptoms in asthma, but leptin was not related to disease severity in COPD. There did not appear to be any relationship between the leptin concentration in plasma and asbestos-induced interstitial lung disease.

4 Resistin in inflammatory lung diseases

4.1 Resistin in asthma (I) and COPD (unpublished data)

The treatment with inhaled fluticasone had no effect on BMI-adjusted plasma resistin levels in patients with asthma (I) or on plasma resistin levels in patients with COPD ($16.3 \pm 0.8 \rightarrow 15.6 \pm 0.7$ ng/ml, $p = 0.277$). Interestingly baseline BMI-adjusted resistin levels correlated negatively with changes in serum levels of ECP ($\rho = -0.745$, $p = 0.013$), EPX ($\rho = -0.733$, $p = 0.016$; Figure 5A) and MPO ($\rho = -0.721$, $p = 0.019$; Figure 5B) in asthmatics during fluticasone treatment, i.e. the higher the pre-treatment resistin levels, the better the response to inhaled fluticasone in patients with asthma.

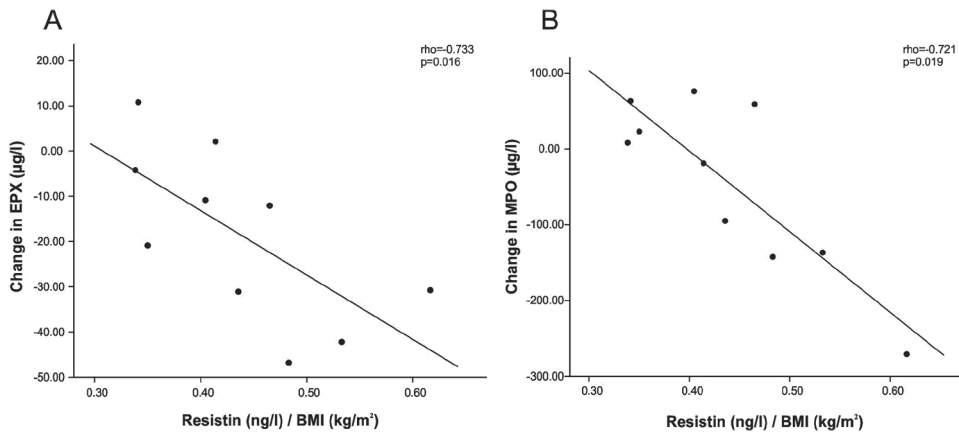


Figure 5. Correlations between baseline BMI-adjusted plasma resistin levels and fluticasone-induced changes in serum markers of inflammation EPX (A) and MPO (B). (Reprinted with permission from Leivo-Korpela et al. 2011, Journal of Inflammation 8:12. © BioMed Central Ltd., modified)

Since resistin levels were associated with favourable anti-inflammatory activity of fluticasone in asthma (I), it was decided to investigate the effects of this adipokine on human THP-1 macrophages. Interestingly, resistin (0.1–2 µg/ml) increased the production of pro-inflammatory cytokines IL-6 (Figure 6A) and TNF-α (Figure 6B) in THP-1 cells in a concentration-dependent manner. Moreover, fluticasone (10 and 100 nM) significantly reduced resistin-induced IL-6 and TNF-α production (Figure 6).

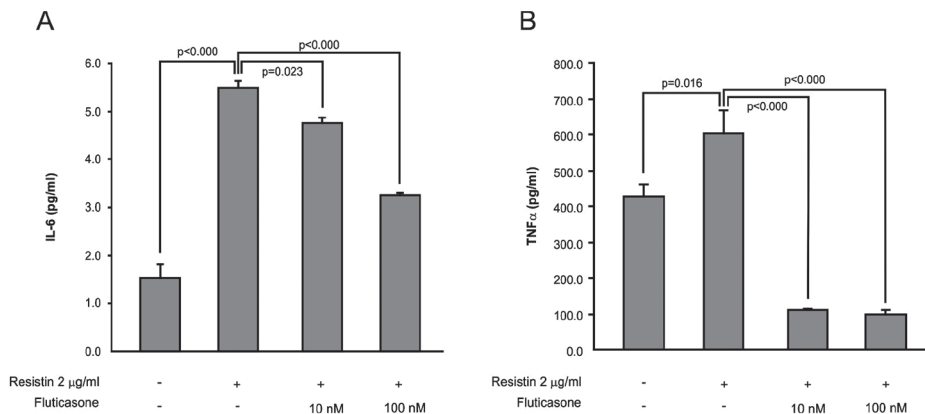


Figure 6. Resistin enhanced cytokine production in human macrophages, and this effect could be reversed by fluticasone. Human THP-1 macrophages were cultured for 24 h with resistin (2 µg/ml) in the absence or in the presence of fluticasone (10–100 nM). Thereafter interleukin 6 (IL-6) (A) and tumour necrosis factor alpha (TNF-α) (B) concentrations were measured in the culture media by ELISA. (Reprinted with permission from Leivo-Korpela et al. 2011, Journal of Inflammation 8:12. © BioMed Central Ltd.)

In patients with COPD, plasma resistin levels were higher than in controls (19.2 ± 0.7 vs 16.7 ± 0.8 ng/ml, $p = 0.022$), but in asthma, the BMI-adjusted plasma resistin levels did not differ between the patients and controls (I). In patients with COPD, plasma resistin levels did not correlate with the parameters of lung function as measured either by spirometry or body plethysmography, with the inflammatory markers measured in plasma (IL-6, MPO, MMP-9), with exhaled NO, with the degree of emphysema or airway wall thickness on HRCT or with health status and symptoms as assessed by SGRQ scores at baseline. Furthermore, the plasma resistin level did not predict the anti-inflammatory effect of glucocorticoids in COPD.

4.2 Resistin in asbestos-induced interstitial lung disease (II)

A positive correlation was found between plasma levels of resistin and IL-6 ($r = 0.291$, $p = 0.007$), but the level of resistin did not correlate with the degree of interstitial fibrosis, pleural plaques or lung function. There were no significant differences detected in plasma resistin concentrations between asbestos-exposed subjects and unexposed, healthy controls. Furthermore, the plasma resistin levels did not differ between the subgroups of exposed subjects with different findings on HRCT of the lungs.

High plasma resistin levels predicted favourable anti-inflammatory effect of inhaled glucocorticoids in patients with asthma suggesting that resistin may be a marker of the steroid-sensitive phenotype in asthma. In patients with COPD, plasma resistin levels were elevated but the levels were not related to the disease severity or steroid responsiveness. In asbestos-induced interstitial lung disease, the resistin concentration in plasma correlated with plasma IL-6 levels.

5 Nesfatin-1 and visfatin in COPD (IV)

In COPD, the plasma concentrations of both nesfatin-1 and visfatin correlated positively with those of IL-6, and furthermore, nesfatin-1 correlated with IL-8 and TNF- α and also visfatin tended to correlate positively with TNF- α . In addition, a negative correlation was detected between visfatin and pulmonary diffusing capacity ($Hb-D_{L/CO}/V_A$) in patients with COPD. The correlations between nesfatin-1 and visfatin and other parameters in patients with COPD are listed in Table 3.

Table 3. Correlations of nesfatin-1 and visfatin plasma levels with the other parameters assessed in the patients with COPD (n=43).

	Nesfatin-1	Visfatin
FEV ₁ (% pred)	rho= 0.097 p = 0.535	r = 0.127 p = 0.422
F _i NO _{0.05} (ppb)	rho = -0.035 p = 0.823	r =0.040 p = 0.802
Hb-D _{L,CO} /V _A (% pred)	rho = -0.103 p = 0.512	r = -0.369 p = 0.016
Emphysema percentage (%)	rho = 0.076 p = 0.626	r =0.204 p = 0.194
IL-6 (pg/ml)	rho = 0.401 p = 0.008	r = 0.341 p = 0.027
IL-8 (pg/ml)	rho = 0.321 p = 0.036	r = 0.121 p = 0.443
TNF-α (pg/ml)	rho = 0.329 p = 0.033	r = 0.305 p = 0.052

FEV₁, forced expiratory volume in 1 second; F_iNO_{0.05}, fractional exhaled NO concentration at exhalation flow rate of 50 mL/s; Hb-D_{L,CO}/V_A pulmonary diffusing capacity of carbon monoxide per unit of alveolar volume standardized for haemoglobin concentration; IL, interleukin; TNF, tumour necrosis factor.

Visfatin levels were reduced in patients with COPD (7.5 ± 0.2 vs 8.9 ± 0.4 ng/ml, $p = 0.002$), but plasma nesfatin-1 levels did not differ from controls ($75.0 [26.2-103.1]$ vs $43.1 [17.9-86.6]$ pg/ml, $p = 0.117$). In addition, nesfatin-1 and visfatin plasma levels were similar in ex-smokers ($n = 10$) and current smokers ($n = 33$).

There were no changes in the plasma levels of nesfatin-1 or visfatin during 4 weeks of treatment with inhaled fluticasone (IV). The baseline plasma levels of these adipokines did not correlate with the degree of fluticasone induced changes in lung function, symptom scores or circulating inflammatory factors in COPD.

Taken together, the plasma levels of nesfatin-1 and visfatin correlated with the systemic inflammation and, in addition, the visfatin level was associated with the parenchymal impairment in patients with emphysematous COPD.

6 Summary of the associations between adipokines and asthma, COPD and asbestos-exposure (I–IV)

According to the present results, leptin and resistin levels in plasma were associated with asthma, whereas those of adiponectin, adipsin, nesfatin-1 and visfatin were associated with COPD. Levels of resistin and especially those of adipsin were associated with asbestos-exposure as can be seen in more detail in Table 4.

Table 4. Summary of the associations (YES/NO) between adipokines and inflammatory lung diseases asthma (I), COPD (III–IV) and asbestos-exposed subjects (II).

<p>ADIPONECTIN</p> <ul style="list-style-type: none"> ● COPD: YES <ul style="list-style-type: none"> – Positive correlations with peripheral obstruction (Raw) and hyperinflation (FRC).* – High pre-treatment adiponectin predicted favourable relief of symptoms and hyperinflation during treatment with inhaled glucocorticoids. ● Asthma: NO ● Asbestos-exposed subjects: NO
<p>ADIPSIN</p> <ul style="list-style-type: none"> ● Asbestos-exposed subjects: YES <ul style="list-style-type: none"> – Positive correlations with the degree of interstitial fibrosis and extend of pleural plaques. – Positive correlations with circulating inflammatory markers ERS and IL-6. – Negative correlation with $D_{L,CO}$. ● COPD: YES <ul style="list-style-type: none"> – Positive correlation with fractional nitric oxide ($F_eNO_{0.05}$).* ● Asthma: NO
<p>LEPTIN</p> <ul style="list-style-type: none"> ● Asthma: YES <ul style="list-style-type: none"> – BMI-adjusted leptin correlated positively with asthma symptoms. – BMI-adjusted leptin correlated negatively with lung function (VC, FVC and FEV₁% pred). ● COPD: NO ● Asbestos-exposed subjects: NO
<p>RESISTIN</p> <ul style="list-style-type: none"> ● Asthma: YES <ul style="list-style-type: none"> – High pre-treatment BMI-adjusted resistin predicted greater decrease in serum levels of inflammatory markers ECP, EPX and MPO during treatment with inhaled glucocorticoids. ● Asbestos-exposed subjects: YES <ul style="list-style-type: none"> – Positive correlation with plasma IL-6 concentration. ● COPD: NO
<p>NESFATIN-1</p> <ul style="list-style-type: none"> ● COPD: YES <ul style="list-style-type: none"> – Positive correlations with plasma IL-6, IL-8 and TNF-α concentration. ● Not analysed in patients with asthma or asbestos-exposed subjects.
<p>VISFATIN</p> <ul style="list-style-type: none"> ● COPD: YES <ul style="list-style-type: none"> – Positive correlations with plasma IL-6 and TNF-α concentration. – Negative correlation with $D_{L,CO}/V_A$. ● Not analysed in patients with asthma or asbestos-exposed subjects.

* partial correlation controlling for body mass index (BMI)

DISCUSSION

1 Adipokines in lung diseases

Adipokines are a group of hormone-like mediators secreted by adipose tissue and initially linked to energy metabolism (Fantuzzi, 2005). The recent literature suggests that adipokines are also involved in the regulation of inflammation and in the immune response (Tilg & Moschen, 2006; Ouchi et al., 2011) and their role in inflammatory lung diseases, asthma and COPD has also been extensively discussed (Ali Assad & Sood, 2012). Lately also associations with other lung diseases such as bronchopulmonary dysplasia (Korhonen et al., 2014), lung cancer (Mou et al., 2014; Ntikoudi et al., 2014), acute respiratory failure (Walkey et al., 2010) and chronic respiratory failure due to sleep apnea (Blüher, 2013) or due to overlap syndrome and obesity hypoventilation (Verbraecken & McNicholas, 2013) have been examined.

Studies I, III and IV in this thesis expand information on the role of adipokines in asthma and COPD, also in non-obese patients, and particularly offer novel data on the association between plasma adipokine concentrations and glucocorticoid responsiveness. Study II provides unique evidence on the association of adipokines, especially of adipisin, with asbestos-induced interstitial lung disease.

2 Adipokines in asbestos-induced interstitial lung disease

2.1 Plasma levels of adipokines in asbestos-exposed workers

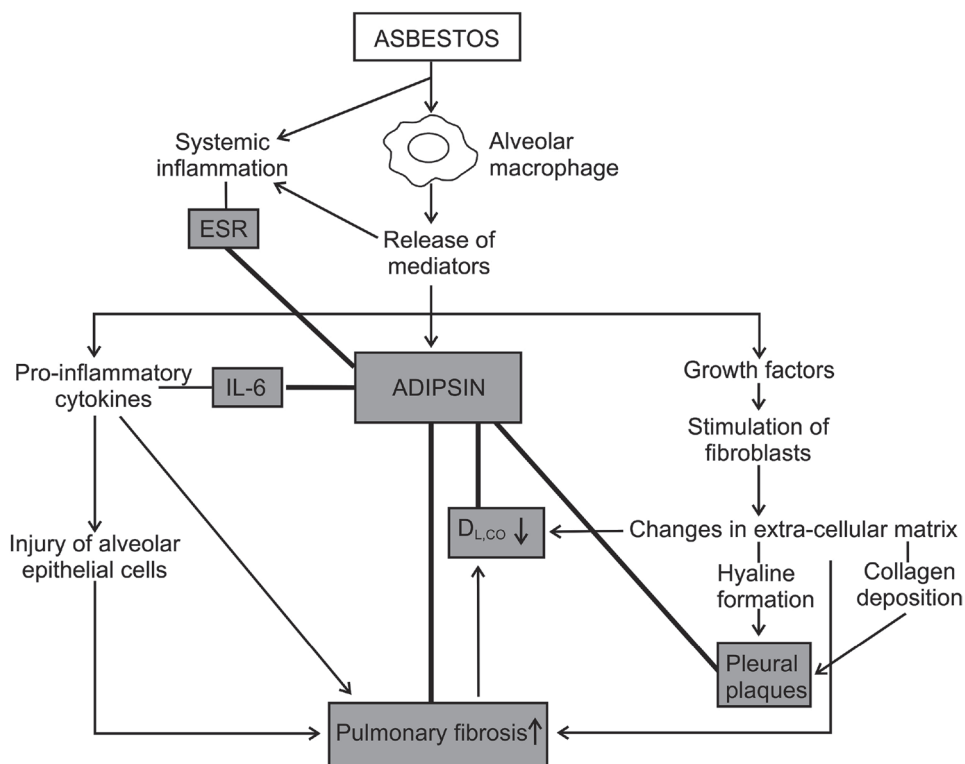
Plasma levels of adipisin were increased in asbestos-exposed subjects with interstitial fibrosis as compared to those with no interstitial changes or to un-exposed controls. There is no previous data on plasma adipokine levels in patients with asbestos-induced tissue reactions in the lungs; i.e. this is a novel and unique finding, which can be explained by the pro-inflammatory properties of adipisin as described below. There were no differences in adiponectin, leptin or resistin plasma levels between these groups.

2.2 Adipsin in asbestos-induced interstitial lung disease

The pathogenesis of asbestosis is poorly understood. Previously the levels of a few inflammatory markers in exhaled air (Lehtonen et al., 2007; Lehtimäki et al., 2010) or induced sputum (Prince et al., 2008; Setta et al., 2008) have been shown to correlate with the asbestos-induced lung injury, but this is the first study to reveal an association between adipsin and asbestos-induced lung disease. In the present study, adipsin was associated with the degree of pulmonary fibrosis, the extent of pleural plaques, the markers of systemic inflammation and the degree of impaired pulmonary diffusing capacity in asbestos-exposed workers. These results indicate that adipsin may have a role in the pathogenesis of asbestos-induced lung injury, which is of particular interest because the pathogenesis of asbestos-induced inflammatory response leading to lung fibrosis, i.e. asbestosis is not understood in detail and there are no disease-modifying treatments for this debilitating disease.

Asbestosis is characterized by slowly progressing interstitial fibrosis in the lung walls and increased numbers of activated alveolar macrophages (Robledo & Mossman, 1999). It has been postulated that the central factor leading to the development of interstitial fibrosis is the impairment of alveolar macrophage-mediated clearance of asbestos fibres (Oberdorster, 2002). Asbestos causes alveolar macrophages to attempt to phagocytize the fibres, and due to this process, these cells start to secrete a wide variety of inflammatory cytokines and growth factors and there is also the generation of reactive oxygen and nitrogen species (ROS, RNS) (Robledo & Mossman, 1999; G. Liu et al., 2013). In addition, ROS produced by alveolar macrophages and alveolar epithelial cells (AEC) stimulate AEC apoptosis, which also leads to the release of pro-inflammatory cytokines and growth-factors such as TNF- α , TGF- β , IL-1 β and gremlin (G. Liu et al., 2013). It is known that alveolar macrophages not only secrete IL-6 (Lemaire & Ouellet, 1996) that stimulates fibroblast proliferation (Robledo & Mossman, 1999), but macrophages are also an important source of adipsin (White et al., 1992). Adipsin is the rate-limiting enzyme in the alternative complement cascade (White et al., 1992) and further, adipsin expression is regulated by TNF- α (Min & Spiegelman, 1986), pointing to a pro-inflammatory role in the regulation of inflammatory responses.

In asbestos-exposure it is possible that adipsin is one of the key factors secreted by activated alveolar macrophages and due to its pro-inflammatory nature, it might participate in promoting the pro-fibrotic and inflammatory process encountered in interstitial fibrosis and asbestosis. This hypothesis is supported by the many connections found between circulating adipsin levels and the degree of interstitial fibrosis, systemic inflammation and impaired gas-exchange. As only a minority of asbestos-exposed subjects ever develop asbestos-induced interstitial pulmonary fibrosis, there is a need for a reliable marker of the ongoing disease process so that those exposed subjects who are at risk of developing the disease could be identified. The associations found and the hypothesised roles of adipsin in asbestos-induced fibrosis are presented in Figure 7.



$D_{L,CO}$ pulmonary diffusing capacity of carbon monoxide; ESR, erythrocyte sedimentation rate; IL, interleukin

Figure 7. The hypothesised role of adiponectin in asbestos-induced pulmonary fibrosis. The factors investigated in this study are shown in grey boxes and the associations based on the current results are presented by bolded lines without arrows. The general pathophysiological changes of asbestos-induced lung injury have been modified from Liu et al 2013.

3 Adipokines in asthma

Murine studies suggest that at least two adipokines, leptin and adiponectin play a role in inflammatory pulmonary conditions like asthma (Shore et al., 2005; Shore et al., 2006). Both of these adipokines participate in the regulation of energy metabolism and plasma leptin levels are known to increase and plasma adiponectin levels to decrease in obesity (Arita et al., 1999; Fantuzzi & Faggioni, 2000). Therefore the association between adipokines and asthma was initially associated with increased levels of pro-inflammatory leptin in obesity-linked asthma (Sood, 2010), in fact the literature about adipokines in non-obese asthma is still rather limited. This present study provided novel evidence for an association between adipokine levels and asthma in non-obese patients.

3.1 Plasma levels of adipokines in asthma

In the non-obese female asthmatics no differences were found in plasma levels of any of the adipokines measured when compared to age-matched healthy women. However, some of the earlier studies have hinted that higher circulating leptin and lower adiponectin levels could be associated with asthma independently of obesity (Sood, 2010; Ali Assad & Sood, 2012). There are reports that leptin levels are increased more often in children (Guler et al., 2004; Gurkan et al., 2004; Nagel et al., 2009) than in adults (Sood et al., 2006). This discrepancy may be related to differences in confounding factors such as the type of asthmatic inflammation, age at onset of asthma and previous use of glucocorticoids, as described later. Thus, it seems that there is no universal relationship between any of the adipokines and asthma, but sex, BMI and the phenotype of asthma are important factors determining these associations.

3.2 Leptin and asthma severity

The present study found that high circulating leptin levels were associated with poorer lung function and more symptoms independent of BMI in non-obese female patients with asthma, suggesting that the link between leptin and asthma is not restricted to obesity. These results are supported by the previous findings of Guler et al. (Guler et al., 2004) and Sood et al. (Sood et al., 2006). Interestingly, an inverse correlation between leptin levels and lung function has been reported also in non-obese healthy subjects (Sin & Man, 2003) evidence that leptin is associated with lung function regardless of asthma and BMI. The results on the association between leptin and asthma symptoms in the present study are in line with those of Kattan et al who have also reported a positive relationship between circulating leptin levels and asthma symptoms in female asthmatics (Kattan et al., 2010).

The association between leptin and asthma severity may be explained, at least partly, by the fact that leptin induces the production of pro-inflammatory mediators such as TNF- α , IL-6 and IL-12 (Loffreda et al., 1998), which may further augment asthmatic inflammation. Moreover, it has been shown that human eosinophils express leptin receptors and that leptin delays apoptosis of eosinophils (Conus et al., 2005). This can further enhance eosinophilic inflammation, which was evidenced in the patients of the present study as increased serum levels of EPX and IgE, and an elevated blood eosinophil count and greater bronchial NO flux.

The prevalences of asthma and obesity are increasing concomitantly and obesity is a recognized risk factor for asthma (Ford, 2005). The effects of obesity on lung mechanics, systemic inflammation, co-morbidities and energy regulating hormones like adipokines are thought to explain this relationship (Shore, 2008), but the mechanistic basis for this connection has not been recognised. However, there are recent clinical findings suggesting that leptin and other adipokines, the production of which is known to increase in obesity,

influence obesity associated asthma through direct effects on the airways, such as inducing airway remodelling, rather than by enhancing airway inflammation (Sideleva et al., 2012). Interestingly, it has also been reported that bariatric surgery improves asthma symptoms and alleviates airway hyperreactivity in obesity associated with late-onset asthma with low serum IgE (Dixon et al., 2011). Based on the previous and the current results, it can be assumed that the higher leptin levels present in obesity together with obesity-related worsening of lung function could be one of the factors determining the relationship between obesity and asthma. However, Sood et al reported that adjustment for leptin level did not significantly affect the association between asthma and BMI in women, suggesting that the relationship between obesity and asthma is not mediated solely via leptin (Sood et al., 2006).

3.3 Resistin and the treatment response to inhaled glucocorticoids in asthma

The present study found that high pre-treatment resistin levels were associated with a more pronounced decrease in serum markers of inflammation during fluticasone treatment suggesting that resistin may be a biomarker of the steroid-sensitive phenotype of asthma. This relationship may be explained by the finding that resistin is an endogenous agonist of Toll-like receptor 4 (TLR4) (Tarkowski et al., 2010). TLR4 activation is known to enhance the expression of various genes involved in asthmatic inflammation through NF- κ B pathway and further, glucocorticoids exert their anti-inflammatory effects largely by inhibiting the NF- κ B dependent transcription (Barnes, 2005). It was also demonstrated in vitro that resistin was able to enhance the expression of pro-inflammatory cytokines IL-6 and TNF- α in human macrophages and this effect was inhibited by fluticasone. It has also been shown that the expression of resistin itself can be enhanced by some inflammatory factors e.g. IL-1, IL-6, TNF- α and by LPS in a NF- κ B dependent manner (Kaser et al., 2003; Silswal et al., 2005). Therefore high resistin levels may reflect an asthmatic phenotype characterized by increased NF- κ B activity and hence a favourable response to glucocorticoids.

3.4 Adipsin and adiponectin in asthma

There are no previous studies assessing adipsin levels in asthma. The present study observed no relationship between asthma and adipsin. Therefore it is likely that adipsin is not a major factor in asthmatic inflammation but it seems to be associated with interstitial lung diseases after exposure to asbestos (II) or silica (Sauni et al., 2012).

In murine asthma models, adiponectin has been linked to asthmatic inflammation (Shore et al., 2006), but there did not be any relationship between adiponectin and asthma in non-obese female patients in the current study. However, adiponectin has been linked

to increased symptoms in male patients with asthma (Sood et al., 2011). Therefore, the role of adiponectin in human asthma remains somewhat unclear and there may also be gender-related features.

4 Adipokines in COPD

There is increasing evidence that the most widely studied adipokines, adiponectin and leptin, are involved in the inflammatory process of COPD (Ali Assad & Sood, 2012), but overall, the data on adipokines in COPD is still inconclusive. Very little, even nothing, is known about the roles and functions of other adipokines such as nesfatin-1 and visfatin in COPD.

4.1 Plasma levels of adipokines in COPD

The data on the levels of adipokines in COPD is also inconclusive. The current study found that in emphysematous male COPD patients with on average normal BMI, the plasma levels of visfatin were decreased when compared to healthy controls, but the levels of other adipokines were normal. This finding on visfatin is in line with a previous study showing decreased visfatin levels in normal weight patients with COPD (Eker et al., 2010), but interestingly, elevated visfatin levels have been reported in COPD patients with low BMI (X. Liu et al., 2009). Leptin levels in COPD have been claimed to be increased in female COPD patients (Breyer et al., 2011) but decreased in male counterparts with emphysema (Schols et al., 1999). In addition, reduced circulating leptin levels have been described also in COPD patients with both normal (Eker et al., 2010) and low BMI (Takabatake et al., 1999). However, many of the studies have reported unchanged leptin levels in COPD (Kirdar et al., 2009; Dickens et al., 2011), which is in line with present results. In addition, there are reports that adiponectin levels may be either unchanged (Dickens et al., 2011; Breyer et al., 2011) or increased (Tomoda et al., 2007; Kirdar et al., 2009; Krommidas et al., 2010; Chan et al., 2010; Breyer et al., 2012; Carolan et al., 2013) in COPD. However, the higher circulating adiponectin levels have often been linked to female gender (Breyer et al., 2011; Breyer et al., 2012), smoking (Dickens et al., 2011), COPD exacerbation (Kirdar et al., 2009; Krommidas et al., 2010) or lower BMI (Carolan et al., 2013) (Table 4). In the current study with stable, normal-weight male COPD patients and non-smoking controls no statistically significant difference was observed between adiponectin levels, although plasma adiponectin tended to be higher in patients with COPD. As with asthma, these discrepancies in adipokine levels between different studies are likely related to differences in patient characteristics such as gender, BMI, disease phenotype, smoking status and the level of systemic inflammation.

4.2 Adipokines and systemic inflammation in COPD

Systemic inflammation is an important feature in COPD (Fabbri & Rabe, 2007; Sinden & Stockley, 2010), and adipose tissue related inflammation has been proposed to represent a link connecting pulmonary tissue injury with the systemic inflammation in COPD (Wouters et al., 2009) although the mechanisms have remained poorly understood. Franssen et al have proposed that systemic and adipose tissue hypoxia, which are often present in COPD, may be important factors inducing the expression and secretion of pro-inflammatory cytokines and thus augmenting adipose tissue dysfunction and adipokine secretion in COPD (Franssen et al., 2008). Altered adipokine secretion may also be one connecting link between COPD and its comorbidities, for example the presence of insulin resistance, has been reported in normal weight patients with COPD (Bolton et al., 2007). The current study observed adipokines nesfatin-1 and visfatin to be novel inflammatory factors in COPD as their levels correlated positively with serum levels of IL-6, IL-8 and TNF- α . This connection may be attributable to the fact that IL-6 increases the expression of both visfatin (Ognjanovic & Bryant-Greenwood, 2002) and nesfatin-1 (Ramanjaneya et al., 2010), while the expression of nesfatin-1 is induced also by TNF- α (Ramanjaneya et al., 2010).

4.3 Adipokines and lung function in COPD

The current study found that high plasma adiponectin levels were associated with peripheral airway obstruction in patients with emphysematous COPD. There are many causes for the airway obstruction in COPD such as mucosal oedema, contraction of the airway smooth muscle, small airway fibrosis and loss of parenchymal support to small airways due to emphysema (Barnes et al., 2003). In the present study, it was observed that plasma adiponectin levels correlated with Raw and FRC but not with FEV₁ and FEV₁/FVC. This is interesting, as FEV₁ mostly reflects obstruction in the larger airways and is not very sensitive to changes occurring in the small airways. Furthermore, in cases of severe small airway obstruction and airway closure during exhalation causing dynamic restriction, FVC decreases in addition to FEV₁ and thus the ratio FEV₁/FVC may not reflect the severity of small airway obstruction. Raw provides a more sensitive estimate of small airway obstruction than FEV₁ or FEV₁/FVC, and FRC is considered as a good marker of dynamic hyperinflation in COPD. (Pellegrino et al., 2005) Thus, the present results suggest that adiponectin is more closely related to peripheral than central airway obstruction in COPD. This is in line with the earlier results of Tomoda and colleagues who reported that the plasma adiponectin level correlated with hyperinflation but not with FEV₁ in their groups of COPD patients (Tomoda et al., 2007) (Table 5).

The relationship between adiponectin levels in plasma and small airway obstruction indicates that adiponectin may be a marker or even a mediator of parenchymal inflammation

and the tissue destruction present in emphysematous COPD, which then leads to small airway obstruction and hyperinflation. These results are supported by the data in a cohort from the COPD Gene study, in which Carolan et al detected an association between plasma adiponectin and CT-assessed emphysema in patients with COPD (Carolan et al., 2013) (Table 5). Interestingly, the degenerative cartilage changes seen in (osteo)arthritis seem to display similarities with tissue matrix degrading events leading to pulmonary emphysema in COPD. Adiponectin has been reported to contribute to cartilage matrix destruction in arthritis (Gomez et al., 2011; Koskinen et al., 2011) by inducing the production of certain pro-inflammatory cytokines and degrading metalloproteinase enzymes which are also involved in COPD and emphysema (Barnes et al., 2003). In addition, adiponectin may have a direct effect on airway function, as human airway smooth muscle cells express adiponectin receptors (Shin et al., 2008) and adiponectin is known to increase contractility in smooth muscle cells (Ding et al., 2012). Thus, it is possible that the increased levels of circulating adiponectin on one hand increase the contractility of airway smooth muscle but on the other hand enhance inflammation and tissue destruction as is evident in COPD, and those effects together contribute to the peripheral obstruction associated with adiponectin levels in COPD. The findings on adiponectin in human COPD are summarized in Table 5.

In the present study it was also demonstrated that visfatin could be linked with pulmonary function in COPD since the high plasma visfatin levels were associated with parenchymal impairment, i.e. lower pulmonary diffusing capacity in patients with COPD. The connection between visfatin and parenchymal impairment in emphysematous COPD might be explained by the previously mentioned pro-inflammatory properties of visfatin contributing to the tissue inflammation and fibrosis found in COPD (Hogg, 2004). However, visfatin levels were not associated with the degree of macroscopic emphysema visible on HRCT, suggesting that visfatin is related to the microscopic processes affecting pulmonary diffusing capacity. The latter proposal is supported by a recent finding that human endothelial cells, which are also present in alveolar capillaries, can both synthesize and release visfatin, particularly in response to inflammation (Romacho et al., 2013). The present hypothesis that visfatin can cause inflammation and oedema in lung tissue rather than smooth muscle contraction is supported by the findings of Machura et al, who were unable to detect any correlation between visfatin and a variety of spirometric parameters (Machura et al., 2012).

Table 5. Summary of human studies evaluating adiponectin in COPD.

Primary findings	total N (F/M)	Age (yrs)	BMI (kg/m ²)	FEV ₁ (% pred)	Smoking status COPD/controls	Stable=S (N)/ Exacer=E (N)	Authors (Year)
P-adiponectin <ul style="list-style-type: none"> • no difference in COPD vs controls • positive correlations with Raw and FRC i.e. hyperinflation • high pre-treatment adiponectin level predicted favourable response to inhaled glucocorticoids 	43 (0/43)	59.5 ± 1.2	25.8 ± 0.6	53 ± 2	current or ex-smokers (≥20 pack years)/ non-smokers	S (43)	Leivo-Korpela et al. (2014)
P-adiponectin <ul style="list-style-type: none"> • higher level in certain COPD phenotype • higher adiponectin level associated with emphysema, lower BMI, bronchial reversibility, female gender and older age 	432 (192/240)	66 ± 1	27.2 ± 0.3	50.4 ± 1.0	current or ex-smokers (≥10 pack years) both COPD and controls	S (432)	Carolan et al. (2013)
S-adiponectin <ul style="list-style-type: none"> • higher level in COPD vs controls • lower in male vs female both in COPD and controls • negative correlation with CRP 	186 (92/94)	58 [52–63]	28 [23–32]	51 [39–62]	current or ex-smokers (≥10 pack years)/ current, ex- or non- smokers	S (186)	Breyer et al. (2012)
P-adiponectin <ul style="list-style-type: none"> • no difference in COPD vs controls • lower level in male vs female both in COPD and controls • no correlations with CRP, IL-6 or TNF-α levels 	91 (45/46)	62.0 (55.0–70.0)	24.3 (20.6–29.4)	40.0 (27.0–53.2)	current or ex-smokers/ non-smokers	S (91)	Breyer et al. (2011)
P-adiponectin <ul style="list-style-type: none"> • no difference in COPD vs non-smoker controls • higher levels in COPD vs ex-smoker controls 	201 (55/146)	64.5 6.0)	26.9 (5.5)	43.8 (17.2)	current or ex-smokers/ ex- or non-smokers	S (92)/ E (109)	Dickens et al. (2011)
P-adiponectin <ul style="list-style-type: none"> • higher level in COPD vs both control groups • negative correlation with FEV₁, IL-6 and CRP levels 	71 (0/71)	70 ± 9	20.7 ± 3.7	37 ± 13	current or ex-smokers/ current, ex- or non- smokers	S (71)	Chan et al. (2010)
P-adiponectin <ul style="list-style-type: none"> • higher level at resolution vs admission of exacerbation (E) or stable disease (S) • negative associations with CRP, IL-6 and TNF-α on admission of E 	63 (9/54)	67.4 ± 9.1	27.7 ± 5.3	42.8 ± 13.4	current or ex-smokers/ no controls	E (63)	Krommidas et al. (2010)
S-adiponectin <ul style="list-style-type: none"> • higher level in COPD • higher in exacerbation vs stable disease • no correlation with FEV₁ 	36 (0/36)	65.6 ± 7.8	25.2 ± 4.0	71.51 ± 21.7 (S) 54.28 ± 24.2 (E)	current smokers both COPD and controls	S (15)/ E (21)	Kirdar et al. (2009)
P-adiponectin <ul style="list-style-type: none"> • higher level in COPD • positive correlation with RV i.e. hyperinflation • no correlation with FEV₁ 	31 (0/31)	71 ± 1	20.1 ± 0.6 BMI ≥ 25: 23.8 ± 0.5 BMI < 25: 17.7 ± 0.4	45.6 ± 5.7 (BMI ≥ 25) 44.0 ± 3.8 (BMI < 25)	smoking history not determined	S (31)	Tomoda et al. (2007)

N, number of subjects; F, female; M, male; BMI, body mass index; FEV₁, forced expiratory volume in 1 second; S, stable disease; E, COPD exacerbation; P-, plasma; S-, serum; RV, residual volume; Raw, airway resistance; FRC, functional residual capacity. Values are presented as mean ± SEM or mean (SD).

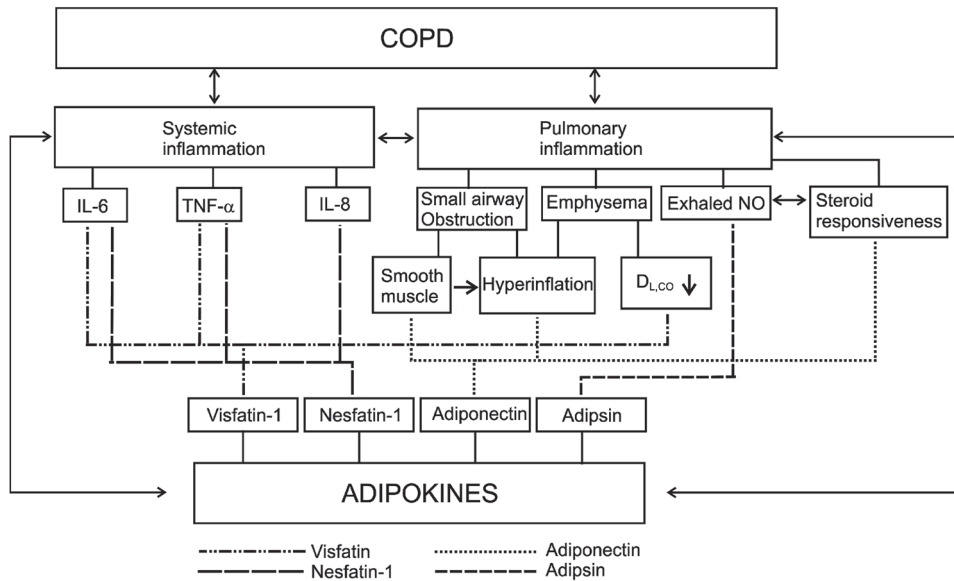
4.4 Adiponectin and treatment response to inhaled glucocorticoids in COPD

In the present study, higher adiponectin levels were associated with a favourable effect of inhaled fluticasone treatment on both symptoms and dynamic hyperinflation in patients with COPD. This finding is of particular clinical interest because the action of inhaled glucocorticoids in COPD is not fully understood and at present there are no reliable clinical tests or biomarkers which can differentiate between steroid responders and non-responders. The present findings suggest that there is a relationship between the type of lung inflammation and the circulating adiponectin levels such that adiponectin is related to the steroid-sensitive inflammation in COPD. This was the first study to evaluate the association between adipokines and steroid responsiveness in COPD.

In addition to the anti-inflammatory action of glucocorticoids, specific inhibition of the adiponectin induced smooth muscle contraction might be another mechanism through which this adipokine can function to alleviate small airway obstruction and the symptoms in COPD. This is supported by the fact that glucocorticoids are known to inhibit the expression of adiponectin receptors (AdipoR1 and AdipoR2) in rat (de Oliveira et al., 2011) and human (Jang et al., 2008) muscle cells. Therefore, it may be that the contractile effect of adiponectin on airway smooth muscle is alleviated when adiponectin receptor density is reduced during glucocorticoid treatment. In addition, glucocorticoids have been shown to decrease adiponectin expression (de Oliveira et al., 2011) and this might also be a potential mechanism linking adiponectin and the responsiveness to glucocorticoid treatment in COPD. However, most of the studies (Lewandowski et al., 2006; J. V. Patel et al., 2006; Man et al., 2009) have reported that glucocorticoids, delivered either orally or by inhalation, do not alter plasma levels of adiponectin. This is in line with the present finding that fluticasone treatment had no effect on the circulating adiponectin concentrations per se, although pre-treatment adiponectin levels were associated with the steroid-responsiveness.

4.5 Summary of the association between adipokines and COPD in the present study

The findings in current study suggest that adipokines may have a role in the pathogenesis of emphysematous COPD acting as pro-inflammatory mediators. The main findings of this thesis on the associations of adipokines and COPD are presented in Figure 8.



IL, interleukin; TNF- α , tumour necrosis factor alpha; $D_{L,CO}$ pulmonary diffusing capacity of carbon monoxide; NO, nitric oxide.

Figure 8. Associations between adipokines and different aspects of COPD based on the current results.

5 The effect of inhaled glucocorticoids on plasma adipokine levels

There is very little information available on the influence of treatment with inhaled glucocorticoids on the circulating levels of adipokines. In the asthma study conducted here, inhaled glucocorticoids decreased only the plasma levels of adipsin but had no effect on the other adipokines, while in the COPD study, inhaled fluticasone thereby increased the plasma levels of leptin. Interestingly, a declining trend was also seen in plasma adipsin levels in patients with COPD and in patients with asthma plasma leptin levels also increased but the change was statistically insignificant. These effects may be explained by direct effects of glucocorticoids on the expression of adipsin and leptin genes, since glucocorticoids have been reported to directly down-regulate the expression of the adipsin gene (Spiegelman et al., 1989) and up-regulate the expression of the leptin gene (Sukumaran et al., 2011). Most clinical studies (Lewandowski et al., 2006; J. V. Patel et al., 2006; Man et al., 2009) have reported that glucocorticoids, delivered either orally or by inhalation, do not alter plasma levels of adiponectin, which is consistent to the present findings in both asthma and COPD. On the other hand glucocorticoids have been shown to decrease the adiponectin expression (de Oliveira et al., 2011). Thus it seems that the effect of inhaled glucocorticoids on adiponectin levels can vary in different diseases and the results are not consistent. Furthermore, the dose and the duration of glucocorticoid treatment can have an influence on the changes in serum adipokine levels as seen in patients with rheumatoid arthritis where

short-term oral glucocorticoid treatment has increased levels of leptin and adiponectin and did not change the levels of resistin and visfatin, but long-term glucocorticoid treatment only reduced the serum levels of resistin (Klaasen et al., 2012).

6 Weaknesses of the study and confounding factors in measuring adipokines

Due to the cross-sectional nature of this study, it was not possible to verify whether the measured changes in adipokine levels were the cause or consequence of the disease. Longitudinal studies would be needed to confirm these changes and the associations. The numbers of patients studied were limited and the study cohorts were restricted only to one gender, which on the other hand did help to overcome the confounding effect of gender.

There are several patient-related contributory factors to be taken into account in adipokine studies e.g. age, gender, BMI and fat distribution in the body, menopause, atopy, comorbidities, drugs and smoking, but at present there is insufficient data on the detailed effects, mechanisms and significance of these factors.

6.1 Obesity

All of the patients in present study were non-obese, which made it possible to investigate the association of adipokines with lung diseases independent of the obvious effects of obesity; however, there was still some variation in BMI within the patient groups although the patients and control groups did not differ from each other in their average BMI. It has been shown that in general, obesity increases the circulating levels of adipisin, leptin and resistin, while levels of adiponectin and visfatin are decreased as compared to those in non-obese subjects (Derosa et al., 2013).

Unfortunately, in many of the published studies, the results have not been adjusted for the confounding effect of obesity, despite correlations seen between BMI and certain adipokines. In the current study BMI adjustment was used in the statistical analyses as described in the Methods. The adiponectin level correlated negatively with BMI in patients with COPD, in subjects with asbestos-exposure and in controls, but not in patients with asthma. The plasma adipisin concentration correlated positively with BMI in patients with asthma and with COPD, but no correlation was seen between it and BMI values in the asbestos-exposed subjects or in any of the control groups. Leptin levels correlated positively with BMI in all study populations in the current study and also in healthy controls. In contrast the levels of resistin did not correlate with BMI in any of the groups and furthermore, those of nesfatin-1 and visfatin did not correlate with BMI in the patients with COPD or controls.

6.2 Gender differences

Gender-related differences in adipokine levels have been reported in many studies and it has been noted that adipokine levels are altered in both asthma and COPD, particularly in women (Sood et al., 2008; Breyer et al., 2011; Breyer et al., 2012). This may be explained by different proportions of adipose tissue in the body and possibly also partly by an interesting finding indicating that androgens can inhibit leptin secretion whereas it is stimulated by estradiol (Machinal-Quelin et al., 2002). Therefore, also the studies in children must be interpreted separately from those done in adults. To overcome the confounding effect of gender on some adipokine levels, only either men or women were included into the studies and the controls were matched for sex. However, this obviously prevented conducting comparisons between the two genders.

6.3 Impact of disease heterogeneity

Asthma and COPD are heterogeneous diseases with different inflammatory profiles (Barnes, 2008) and clinical outcomes (Carolan & Sutherland, 2013) and it has been postulated that the levels and roles of adipokines quite likely vary between these different inflammatory phenotypes (Sood & Shore, 2013). The present asthma and COPD study populations were well defined and steroid-naïve. The asthmatics in current study could be categorized to represent Th2-associated, allergic asthma and at least partly phenotyped as early-onset allergic asthma (Wenzel, 2012). All the patients in the COPD studies had an emphysematous stable COPD and they could be phenotyped as infrequent exacerbators with emphysema (Miravittles et al., 2013). Unfortunately in many of the published adipokine studies, the exact characterization of the phenotypes of study subjects has been lacking and often alterations in circulating adiponectin and leptin levels have been associated with exacerbations, especially in COPD (Creutzberg et al., 2000; Krommidas et al., 2010).

6.4 Impact of smoking

Smoking could affect circulating adipokine levels by either changing the pulmonary adipokine production or by altering the adipokine production in adipose tissue by inducing systemic inflammation. The effect of smoking on the expression and levels of adipokines is still unclear and the data is very limited. In the present COPD population, there did not appear to be differences in any of the adipokines measured between ex-smokers and current smokers with COPD. However, in one previous study, exposure to tobacco smoke has been claimed to reduce the expression of adiponectin in humans (Miller et al., 2009).

6.5 Impact of patient selection

The differences in patient selection could also have affected the partly conflicting results. All of the asthma and COPD patients in the current study were referred from primary care to specialist health care, so the first patient selection had been conducted already in primary care and thus the study population might have consisted of patients with a more severe disease or difficulties in diagnostics. It is known that the severity of the diseases can vary extensively in different medical settings, but the strength of this work was that all asthma and COPD patients were newly diagnosed, steroid-naïve and well characterized, which was also the case in the study with asbestos-exposed workers.

6.6 Different isoforms of adiponectin

In the present COPD study, plasma adiponectin levels were not higher in COPD than controls, which can be explained by the fact that only male patients with COPD were investigated. Previously Peake et al have reported that there is a sexual dimorphism in the concentrations of the circulating adiponectin i.e. women had higher absolute concentrations of circulation total adiponectin and its isoforms (Peake et al., 2005). In addition, the proportions of isoforms differed between males and females, such that males had lower proportions of the high molecular isoform which has been proposed to be the most biologically active isoform of this molecule (Hara et al., 2006), also in COPD (Daniele et al., 2012). In this study the total amount of circulating adiponectin was assayed, however the measurement of the high molecular isoform separately would possibly have strengthened the positive results found here.

7 Future prospects and possible clinical applications

7.1 Adipokines in predicting diseases severity and prognosis in inflammatory lung diseases

In the present study, levels of both leptin and adiponectin were associated with disease severity, i.e. a higher leptin concentration correlated with poorer lung function and more symptoms in asthma whereas a higher adiponectin was associated with more severe dynamic hyperinflation in COPD. Interestingly, elevated systemic adiponectin levels have been associated with lower survival in subjects suffering acute respiratory failure (Walkey et al., 2010) and with an increased risk of mortality in patients with COPD (Waschki et al., 2011). Thus, the present findings together with the published data suggest that certain adipokines can indeed predict disease severity in some patient groups and in certain diseases, but more confirmatory studies will be needed. In addition, it is not known if

alterations in the adipokine plasma levels are associated with their role in the pathogenesis of the disease or if the adipokines are mainly part of the body's protective response to combat inflammation; more investigations are necessary to clarify adipokines' detailed role in the pathophysiological changes occurring in inflammatory lung diseases.

There is a clinical need for biomarkers that would reveal the current activity of asbestos-induced immune response in order to help to assess the individual risk for development or further progression of pulmonary fibrosis among asbestos-exposed subjects. According to the present results, adipsin may have some value as a biomarker of the asbestos-induced inflammatory activity but additional prospective studies will be needed to determine if measurements of plasma adipsin levels can be used to predict disease progression and to select the individuals who would benefit from follow-up. If adipsin can be confirmed to be a biomarker revealing a more active inflammatory tissue reaction, it may be beneficial to combine the measurement of plasma adipsin and pulmonary function tests in order to focus HRCT examinations only on high risk patients and thus to reduce radiation exposure of other asbestos-exposed workers.

However, it is not clear whether adipsin is specifically associated with asbestos-related macrophage activation or if this finding may be extended to include other forms of fibrosing lung diseases. The latter hypothesis is supported by a previous study showing increased plasma adipsin levels in subjects with a heavy occupational exposure to silica (Sauni et al., 2012). Further studies will be required confirm this hypothesis in other interstitial lung diseases and to elucidate the role of adipsin in the course of the fibrosing process.

7.2 The usefulness of adipokines in predicting steroid responsiveness and in other types of clinical characterization

Based on the results of the current study, one could postulate that adipokines may have a role in predicting steroid responsiveness in asthma and COPD and thus help to identify responsive patients. The mechanisms of action of inhaled glucocorticoids in COPD and in steroid resistance in asthma are not fully understood. The current study has hinted that adiponectin in COPD and resistin in asthma may have a role in predicting steroid responsiveness.

According to these results, the measurement of plasma resistin levels may be a potential biomarker for steroid-sensitivity in asthma and could well be a way to reassess treatment strategies in asthma patients who do not respond to traditional anti-inflammatory therapy with inhaled glucocorticoids. Since the present asthma study population was small, the investigation should be repeated with a larger patient group with both genders and in both obese and non-obese patients with asthma. It would be of special interest to test if these results would be true also in obese asthmatics, since obesity-related asthma phenotype is often associated with a diminished response to glucocorticosteroids (Wenzel, 2012). If

so, it would be useful if elevated resistin levels could help to select the steroid responders among obese patients; and thus the other patients (i.e. steroid non-responders which had been identified via their low resistin levels) could be treated with other drug combinations instead of increasing doses of glucocorticoids and thus side-effects of glucocorticoids such as impaired glucose tolerance could be avoided in these already obese patients.

The steroid responsiveness in COPD is also of considerable interest, because of the potential usefulness of these drugs in preventing exacerbations and on the other hand, in avoiding their adverse effects such as increased pneumonia risk. At present there are no specific diagnostic tools or biomarkers in clinical practice to distinguish steroid responsiveness in COPD, but many trials are ongoing to find the most suitable markers. The results of the current study suggest that adiponectin may serve as a biomarker of the steroid-sensitive phenotype in COPD and specific inhibition of the adiponectin-induced smooth muscle contraction is proposed to be one mechanism by which inhaled glucocorticoids may act to alleviate small airway obstruction in COPD.

Additional studies will be required to evaluate whether this finding is reproducible in other COPD populations, i.e. in females and non-emphysematous COPD in order to clarify whether the plasma adiponectin level may also serve as a biomarker of glucocorticoid treatment responses in other COPD phenotypes.

Recently it was reported that serum adiponectin levels were associated with the clinical phenotype of emphysema (Carolan et al., 2013) and further, higher adiponectin (Wouters et al., 2007; Krommidas et al., 2010) and leptin levels (Creutzberg et al., 2000; Krommidas et al., 2010) have been associated with COPD exacerbations. Taking these findings in conjunction with those in this thesis may indicate that adipokines would be useful in subgrouping the patients with COPD. Interestingly, Mou et al recently reported that an increase in the serum leptin level may be a useful marker for the improved prognosis in patients with lung adenocarcinoma undergoing cisplatin/pemetrexed chemotherapy (Mou et al., 2014), but further studies will be necessary before one can be sure that adipokines represent an additional tool for phenotypic characterization.

7.3 Adipokines as new targets for pharmacological intervention

New agents and targets for pharmacological interventions are needed especially for incurable emphysema and pulmonary fibrosis. In addition for asthma and COPD, phenotype specific personalized new treatments are being sought. At present, it is not known whether modulation of adipokine levels or their effects would be helpful in the prevention and/or treatment of these disorders. It could be, however, hypothesised that the down-regulation of adipokine expression, the use of anti-adipokine antibodies to prevent adipokines from binding to their receptors or the direct blocking of the adipokine receptors could be useful in influencing adipokine related unfavourable effects in inflammatory lung diseases.

Adiponectin deficiency in adiponectin knockout mice has been associated with an emphysema-like phenotype as well as with systemic inflammation and extra-pulmonary manifestations such as cachexia and osteoporosis suggesting that reduced adiponectin levels could be one causal mechanism linking COPD with comorbidities (Nakanishi et al., 2011). On the other hand, it has also been demonstrated that adiponectin deficiency in adiponectin knockout mice can protect against tobacco-induced inflammation and the progression of emphysema, evidence that adiponectin may exert a pro-inflammatory influence in the lungs (Miller et al., 2010). In the present study, adiponectin concentrations in COPD patients correlated with the degree of peripheral obstruction and steroid responsiveness. This finding raises the question if a reduction of adiponectin levels would have beneficial effects in COPD. That proposal is supported by the fact that airway smooth muscle cells express adiponectin receptors (Shin et al., 2008) and by the experimental findings that the contractility of smooth muscle cells is increased by adiponectin (Ding et al., 2012). However, further experimental and clinical studies will be needed to evaluate the possible beneficial effects of reduced adiponectin activity in patients with COPD.

Furthermore, nesfatin-1 and visfatin levels were associated with systemic inflammation, and those of visfatin also with a reduced pulmonary diffusing capacity in patients with COPD. Therefore, it is interesting to speculate whether the blocking of nesfatin-1 and / or visfatin could exert an anti-inflammatory effect on the systemic inflammation present in COPD and also whether inhibition of visfatin's effects would slow down the parenchymal changes in COPD. The latter concept is supported by the findings that in acute lung injury caused by influenza A (H1N1) virus, an inhibition of visfatin could attenuate lung inflammation by inhibiting the expression of pro-inflammatory cytokines IL-6, IL-8 and TNF- α (Gao et al., 2011), the same cytokines which were linked to visfatin and nesfatin-1 levels in the present COPD study. In addition, the blockade of visfatin expression has been shown to reduce the local inflammatory responses in the pulmonary endothelium (Gao et al., 2011), although no clear effect on circulating pro-inflammatory cytokines was detected.

According to the present results in the asthma study where leptin was associated with disease severity, it could be hypothesised that preventing the effects of leptin could be useful in management of this disorder. A monoclonal antibody against leptin has been developed (Mahmoudian et al., 2012) and in mice infected with influenza A virus subtype H1N1, the anti-leptin antibody exerted an anti-inflammatory effect and improved survival (A. J. Zhang et al., 2013). Those findings support the rationale to investigate anti-leptin treatment in the management of asthma. Weight loss is another means known to reduce circulating leptin levels (Maffei et al., 1995) and weight loss after bariatric surgery has been shown to improve asthma symptoms and to alleviate airway hyperreactivity (Dixon et al., 2011). Thus, dieting is also one effective way to reduce circulating leptin levels and thereby possibly to relieve the severity of asthma.

The promising results on adipsin as a potential factor in the pathogenesis of asbestos-induced pulmonary fibrosis in current study will hopefully encourage future studies to identify the role of adipsin in the disease mechanisms as well as providing a possible therapeutic target. Further studies should be conducted to determine if the modulation or blocking of adipsin, known also as complement factor D which is a part of alternative complement cascade (White et al., 1992), could be useful in preventing the progression of pulmonary fibrosis. The first complement inhibitor, eculizumab, which binds to the complement protein C5, has been shown to be effective for the treatment of paroxysmal nocturnal hemoglobinuria (Risitano, 2013) and it has been proposed to be effective in attenuating allergen-induced responses (Smith et al., 2012).

At the same time one needs to take into account the fact that modulation of circulating adipokine levels could also have harmful effects by blocking the beneficial metabolic effects of certain adipokines, e.g. the roles of adiponectin and leptin in energy homeostasis where these adipokines regulate both glucose and lipid metabolism with adiponectin also governing the sensitivity to insulin (Kadowaki & Yamauchi, 2005; Tilg & Moschen, 2006).

SUMMARY AND CONCLUSIONS

Adipokines are known to participate in energy metabolism and also to regulate inflammatory responses. Asthma and COPD are heterogeneous inflammatory diseases of the lower airways and express as phenotypes with different inflammatory profiles, clinical outcomes and treatment responses. Exposure to asbestos induces a chronic inflammation in the lungs predisposing pulmonary fibrosis which can proceed to asbestosis. The aim of the present study was to investigate if adipokines are associated with inflammatory activity or disease severity in asthma, COPD and/or asbestos-induced interstitial lung disease. An additional aim was to study if adipokines could be useful in predicting the response to glucocorticoid treatment in asthma or COPD. All of the findings are based on clinical studies (and in a single case on experiments in human macrophage cell culture). Adipokine measurements were performed on human plasma samples.

The major findings and conclusions were:

- A.** Plasma adiponectin levels were associated with peripheral airway obstruction and dynamic hyperinflation in patients with COPD. In addition, visfatin may mediate interstitial changes associated with decreased pulmonary diffusing capacity in emphysematous COPD.

B. High plasma leptin levels were associated with poorer lung function and increased symptoms in patients with asthma indicating that leptin is related to the severity of asthma also in non-obese patients.
- A.** Higher pre-treatment adiponectin levels predicted more favourable relief of symptoms and hyperinflation during inhaled glucocorticoid treatment in patients with COPD suggesting that adiponectin may be a predictive biomarker of treatment responses. These findings also support the experimental data that adiponectin can act as a pro-inflammatory mediator capable of inducing tissue matrix degradation and evoking smooth muscle contraction in emphysematous COPD.

B. Higher pre-treatment plasma resistin levels were associated with more favourable anti-inflammatory effects of inhaled glucocorticoids in steroid-naïve non-obese patients with asthma suggesting that resistin may be a marker of steroid-sensitive phenotype of asthmatic inflammation.
- Plasma nesfatin-1 and visfatin levels were introduced as novel factors associated with systemic inflammation in COPD. This suggest that nesfatin-1 and visfatin may have a pro-inflammatory role in the pathogenesis of emphysematous COPD.

4. Plasma levels of adipsin were associated with the degree of interstitial pulmonary fibrosis, the impairment of pulmonary diffusing capacity, the extent of pleural plaques and the systemic markers of inflammation in asbestos-exposed subjects. This indicates that adipsin may play a role in the pathogenesis of asbestos-induced lung injury and it could serve as a marker of ongoing disease activity and risk of progression of the fibrotic process in the lungs.

The results of the present study series revealed that the association between adipokines and asthma is not restricted to obesity. Evidence was presented that adipokines are associated with disease severity in asthma and COPD and that certain adipokines could be used as predictors of steroid-responsiveness. Another original finding was that adipsin is associated with the pulmonary fibrosis caused by asbestos-exposure. Novel data was also provided on the role of two adipokines, nesfatin-1 and visfatin, in stable emphysematous COPD.

These findings open new avenues for further studies to elucidate if adipokines could be used as biomarkers for phenotyping asthma and/or COPD or as tools to predict for disease severity and/or progression. It will also be of interest to determine whether the modulation of adipokines would be helpful in the prevention of disease progression or even initiation or if adipokines could represent a new target for anti-inflammatory treatment strategies in inflammatory lung diseases.

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RESEARCH

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Adipokine resistin predicts anti-inflammatory effect of glucocorticoids in asthma

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Abstract

Background: Adipokines are protein mediators secreted by adipose tissue. Recently, adipokines have also been involved in the regulation of inflammation and allergic responses, and suggested to affect the risk of asthma especially in obese female patients. We assessed if adipokines predict responsiveness to glucocorticoids and if plasma adipokine levels are associated with lung function or inflammatory activity also in non-obese (body mass index (BMI) ≤ 30 kg/m²) women with newly-diagnosed steroid-naïve asthma.

Methods: Lung function, exhaled NO, plasma levels of adipokines leptin, resistin, adiponectin and adipsin, and inflammatory markers were measured in 35 steroid-naïve female asthmatics and in healthy controls. The measurements were repeated in a subgroup of asthmatics after 8 weeks of treatment with inhaled fluticasone. Adipokine concentrations in plasma were adjusted for BMI.

Results: High baseline resistin concentrations were associated with a more pronounced decrease in serum levels of eosinophil cationic protein (ECP) ($r = -0.745$, $p = 0.013$), eosinophil protein X (EPX) ($r = -0.733$, $p = 0.016$) and myeloperoxidase (MPO) ($r = -0.721$, $p = 0.019$) during fluticasone treatment. In asthmatics, leptin correlated positively with asthma symptom score and negatively with lung function. However, no significant differences in plasma adipokine levels between non-obese asthmatics and healthy controls were found. The effects of resistin were also investigated in human macrophages in cell culture. Interestingly, resistin increased the production of proinflammatory factors IL-6 and TNF- α and that was inhibited by fluticasone.

Conclusions: High resistin levels predicted favourable anti-inflammatory effect of inhaled glucocorticoids suggesting that resistin may be a marker of steroid-sensitive phenotype in asthma. High leptin levels were associated with a more severe disease suggesting that the link between leptin and asthma is not restricted to obesity.

Background

Asthma is a chronic inflammatory airway disease characterised by cough, chest tightness and wheezing, and it is associated with reversible or variable airway obstruction. However, the diagnosis and follow-up of the disease are currently based on symptoms and lung function measurements rather than on assessing the underlying inflammatory process [1]. Several asthmatic phenotypes with different inflammatory mechanisms have been described suggesting that asthma is not a single disease entity but a syndrome with different

underlying causes and mechanisms [2]. The efficacy of treatment with inhaled glucocorticoids seems to vary between asthmatic phenotypes, and phenotype-specific predictors of treatment response are needed.

Adipokines like leptin, adiponectin, resistin and adipsin are protein mediators secreted by adipocytes and macrophages within the adipose tissue [3]. Leptin and resistin are usually pro-inflammatory, while adiponectin has mainly anti-inflammatory properties [3]. Leptin levels increase in obesity [4] and leptin has therefore been suggested to belong to the factors explaining the relation between obesity and asthma. Some studies suggest that leptin affects asthma also independently of body mass index (BMI) [5,6]. Adiponectin has been demonstrated to have anti-inflammatory properties [3,7]

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and it is associated with lower risk for asthma in women regardless of BMI [8]. There are only a few publications on resistin in human asthma with conflicting results [9-11]. Larochelle et al [9] found higher resistin levels in asthmatics and the levels were increased with disease severity, while Kim et al [10] suggested that resistin may have a protective effect against asthma. The role of adipisin in asthmatic inflammation has not been studied previously. There is limited data on adipokines in non-obese asthmatics and only a little information how treatment with inhaled glucocorticoids influence the circulating levels of adipokines.

As discussed above, there are some evidence suggesting connections between adipokines and asthma. However, further studies are needed to understand the role of adipokines in the pathogenesis of, and more importantly, in predicting treatment responses in different phenotypes of asthma. Nuclear factor κ B (NF- κ B) is a transcription factor inducing the expression of many pro-inflammatory genes. Inhaled glucocorticoids exert their anti-inflammatory effects through a wide variety of mechanisms, of which inhibition of NF- κ B is one of the most important [12]. Interestingly, also adipokine resistin has been linked to NF- κ B at two levels; its expression is enhanced by inflammatory factors IL-1, IL-6, TNF- α and LPS [13] which all are known activators of NF- κ B. In addition, pro-inflammatory effects of resistin are partly mediated through activation of the NF- κ B pathway [14]. Therefore resistin may have a role as a factor or a predictor in steroid-responsive asthma.

The aim of the present study was to assess if plasma levels of resistin or other adipokines would predict the responsiveness to inhaled corticosteroids, and if adipokines are associated with lung function, symptoms or inflammatory activity in newly diagnosed asthma in non-obese (BMI \leq 30 kg/m²) female subjects. We found that high baseline resistin levels predicted favourable response to inhaled fluticasone, while high leptin levels were associated with poor lung function and more symptoms.

Methods

Subjects

Thirty-five steroid-naive, non-smoking female asthmatics (mean age 34 yrs, range 20-57 yrs) with BMI \leq 30 kg/m² (range 18-30 kg/m²) were recruited. The diagnosis of asthma was based on symptoms and reversible or variable airway obstruction (β_2 -agonist induced increase in FVC or FEV₁ \geq 12% and 200 ml, or diurnal variability in PEF \geq 20%, or exercise induced decrease in FEV₁ \geq 15%). Thirty-two age- and sex-matched non-smoking healthy controls with similar BMI, no asthmatic symptoms and normal lung function served as

controls. Both groups were free from any other chronic diseases.

Study protocol

Lung function, asthma symptom score, plasma levels of adipokines, serum levels of other inflammatory markers, and exhaled nitric oxide (NO) were measured in asthmatics and in controls. The asthmatics also filled in an asthma symptom questionnaire. The same measurements were repeated in 11 asthmatics after 8 weeks of treatment with inhaled fluticasone propionate (Flixotide Diskus, GSK, Ware, UK, 500 μ g b.i.d. during weeks 1-4, and 250 μ g b.i.d. during weeks 5-8). The study was approved by the ethics committee of Tampere University Hospital and all subjects gave their written informed consent.

Adipokines and inflammatory markers

Venous blood was collected for the assessment of plasma levels of adipokines (resistin, leptin, adiponectin, adipisin), serum levels of immunoglobulin E (IgE), eosinophil cationic protein (ECP), eosinophil protein X (EPX), myeloperoxidase (MPO), interleukin 6 (IL-6), and blood eosinophil count (EOS). Adipokines were determined by enzyme-immuno-assay (EIA) by using commercial reagents (DuoSet ELISA, R&D Systems Europe Ltd, Abingdon, U.K). As plasma adipokine levels are dependent on the amount of adipose tissue, adipokine levels were adjusted for BMI by dividing the measured concentration by BMI. Radioimmunoassay (ECP RIA, EPX RIA and MPO RIA, Pharmacia AB, Uppsala, Sweden) was used to measure ECP, EPX and MPO levels. Immunoluminometry was used to measure IgE, and IL-6 was measured by EIA (PeliPair ELISA, Sanquin, Amsterdam, Netherlands). The detection limits and inter-assay coefficients of variation, respectively, were 15.6 ng/l and 4.0% for resistin, 15.6 ng/l and 3.9% for leptin, 15.6 ng/l and 2.0% for adiponectin, 4.0 ng/l and 3.8% for adipisin, 2.0 μ g/l and 4.2% for ECP, 3.0 μ g/l and 5.4% for EPX, 8.0 μ g/l and 6.2% for MPO and 0.6 ng/l and 6.1% for IL-6.

Exhaled NO and lung function

Exhaled NO was measured with a Sievers NOA 280[®] NO-analyzer (Sievers Instruments, Boulder, CO, USA) at exhalation flow rates of 100, 175 and 370 ml/s with a mouth pressure of 9 cmH₂O. The analyzer was calibrated daily with a known NO concentration (103 parts per million (ppm), AGA, Sweden) and before every subject with filtered NO-free air. Bronchial NO flux and alveolar NO concentration were calculated for each subject using the method described by Tsoukias and George [15,16]. Airway function was measured with Vmax 20 C

spirometer (Sensor-Medics, Yorda Linda, CA, USA) before and after 400 µg of inhaled salbutamol.

Asthma symptoms questionnaire

Asthma symptoms were recorded by using written symptom questionnaire. Cough, chest tightness, wheezing and nocturnal asthma symptoms were each scored from 0 to 3 yielding a total score from 0 to 12 points [17].

Cell culture

Human THP-1 monocyte/macrophage cell line (American Type Culture Collection, Manassas, VA, USA) was used. The cells were cultured at 37°C in humidified 5% carbon dioxide atmosphere in RPMI 1640 medium adjusted to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/l glucose, and 1.5 g/l bicarbonate, and supplemented with 10% heat-inactivated fetal bovine serum (all obtained from Lonza Verviers SPRL, Belgium), penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (250 ng/ml) (all obtained from Invitrogen, Paisley, UK), and 0.05 mM 2-mercaptoethanol. The cells were differentiated to macrophages by adding the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA, 100 nM) for 72 h at the time of seeding of the cells on 24-well plates. Cells were serum starved for 16 h before the experiments were started. Resistin (recombinant human resistin; Pepro-Tech, Inc., Rocky Hill, NJ, USA) and fluticasone (Sigma Chemical Co, St. Louis, MO, USA) were added in fresh culture medium, and the cells were incubated for 24 h. Culture medium was collected and stored at -20°C until assayed. The concentrations of human IL-6 (PeliPair ELISA, Sanquin, Amsterdam, Netherlands) and human TNF-α (R&D Systems, Minneapolis, MN, USA) were determined by ELISA. The detection limits and intra-assay coefficients of variation, were 7.8 ng/l and 4.8% for TNF-α and 0.6 ng/l and 6.0% for IL-6, respectively.

Statistics

Normality of the distributions of plasma adipokines and other parameters were analysed with Kolmogorov-Smirnov's test. Differences in adipokine levels between asthmatics and controls were analysed with t-test or Mann-Whitney test, where appropriate. Spearman's rho was used to analyse correlations between adipokine levels and lung function indices, other inflammatory markers or symptom scores. Changes in plasma levels of adipokines and other markers of inflammation during fluticasone treatment were analysed with a paired t-test or Wilcoxon's test, where appropriate. A stepwise multiple linear regression analysis was used to determine if the correlations between lung function indices and the levels of plasma adipokines were explained by BMI. Results

from the cell culture experiments were analyzed by using one-way ANOVA followed by Dunnett multiple comparisons test. Results are presented as mean ± SEM for normally distributed data and as median [interquartile range] for non-normally distributed data. A p-value < 0.05 was considered as significant. SPSS 15.0.1 software (SPSS Inc., Chicago, Illinois, USA) was used in the statistical analysis.

Results

Subject characteristics are given in Table 1. There were no differences in age or BMI between asthmatics and controls. Asthmatics had higher serum levels of EPX and IgE, and higher blood eosinophil count and bronchial NO flux than controls.

Leptin and resistin levels were normally distributed, while distribution of adiponectin and adipisin were non-normal. As plasma adipokine levels are dependent on the amount of adipose tissue, adipokine levels were adjusted for BMI by dividing the measured concentration by BMI. There were no significant differences in BMI-adjusted plasma adipokine levels between asthmatics and healthy controls (Table 2).

Predicting treatment responses

Interestingly, pre-treatment resistin levels seemed to predict the anti-inflammatory effect of inhaled fluticasone. Baseline BMI adjusted resistin correlated

Table 1 Subject characteristics.

	Asthmatics	Controls	p-value
N	35	32	
Age (yrs)	33.9 ± 2.1	33.8 ± 2.1	0.980
BMI (kg/m ²)	23.1 ± 0.5	22.8 ± 0.5	0.627
FEV ₁ (% pred)	90 ± 1.9	96 ± 3.2	0.125
ECP (µg/l)	11.2 [6.9 - 19.9]	9.2 [6.1 - 14.4]	0.105
EPX (µg/l)	29.6 [20.8 - 61.1]	18.3 [16.3 - 27.4]	0.003
MPO (µg/l)	218.6 [138.0 - 325.0]	246.8 [155.7 - 317.4]	0.716
EOS (10 ⁹ /l)	0.23 [0.16 - 0.44]	0.15 [0.10 - 0.20]	<0.001
IgE (IU/l)	87.0 [25.0 - 204.0]	24.5 [11.0 - 41.0]	0.002
IL-6 (ng/l)	3.8 [2.6 - 5.3]	3.0 [2.3 - 5.0]	0.327
J _{Br,NO} (nl/s)	2.6 ± 0.3	0.7 ± 0.1	<0.001
C _{Alv} (ppb)	1.2 ± 0.3	1.1 ± 0.1	0.671

BMI, body mass index

FEV₁, forced expiratory volume in 1 second

ECP, eosinophil cationic protein

EPX, eosinophil protein X

MPO, myeloperoxidase

EOS, blood eosinophil count

IgE, immunoglobulin E

IL-6, interleukin 6

J_{Br,NO}, Bronchial NO flux

C_{Alv}, Alveolar NO concentration

Values are presented as mean ± SEM for normally distributed data and as median [interquartile range] for non-normally distributed data.

Table 2 Plasma levels of adipokines in asthmatics and controls.

	Asthmatics	Controls	p-value
N	35	32	
Resistin (ng/l)/BMI (m ² /kg)	0.5 [0.4 - 0.8]	0.5 [0.5 - 0.7]	0.603
Leptin (ng/l)/BMI (m ² /kg)	0.5 [0.5 - 1.1]	0.6 [0.4 - 0.8]	0.366
Adiponectin (ng/l)/BMI (m ² /kg)	165 ± 9.5	176 ± 13	0.490
Adipsin (ng/l)/BMI (m ² /kg)	32 ± 1.3	33 ± 1.3	0.813

Adipokine values were adjusted for BMI (body mass index)
 Values are presented as mean ± SEM for normally distributed data and as median [interquartile range] for non-normally distributed data.

negatively with change in serum levels of ECP (rho = -0.745, p = 0.013), EPX (rho = -0.733, p = 0.016, Figure 1), and MPO (rho = -0.721, p = 0.019, Figure 2) during fluticasone treatment, i.e. the higher the pre-treatment resistin the better the response to inhaled fluticasone. The other adipokines did not correlate significantly with fluticasone-induced changes in the inflammatory markers.

Treatment with inhaled fluticasone decreased plasma adipsin levels but had no effects on other adipokines. Fluticasone treatment decreased also serum levels of ECP and EPX, reduced bronchial NO flux and asthma symptoms, and improved lung function (Table 3).

Correlations between adipokines and other parameters

In asthmatics, BMI adjusted leptin correlated positively with asthma symptom score (rho = 0.371, p = 0.031) and negatively with lung volumes VC% predicted (rho = -0.445, p = 0.007), FVC% predicted (rho = -0.406, p =

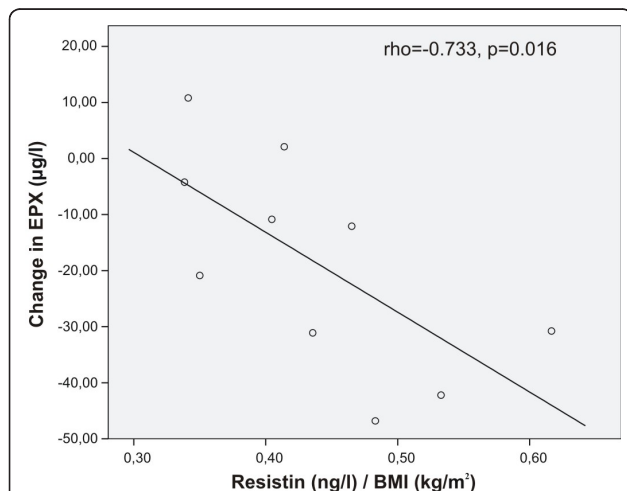


Figure 1 Correlation between baseline resistin and fluticasone-induced change in EPX. Baseline BMI-adjusted resistin correlated negatively with the change in serum levels of eosinophil protein X (EPX) during inhaled fluticasone treatment (Spearman's rank correlation), i.e. the higher the baseline resistin the larger the decrease in EPX levels in response to inhaled fluticasone.

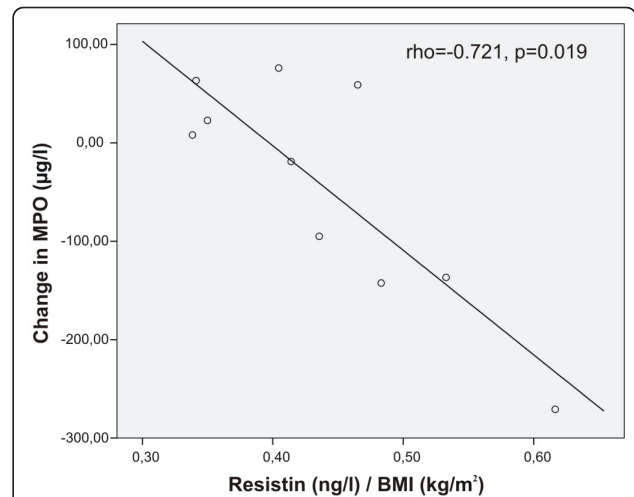


Figure 2 Correlation between baseline resistin and fluticasone-induced change in MPO. Baseline BMI-adjusted resistin correlated negatively with the change in serum levels of myeloperoxidase (MPO) during inhaled fluticasone treatment (Spearman's rank correlation), i.e. the higher the baseline resistin the larger the decrease in MPO levels in response to inhaled fluticasone.

0.016, Figure 3) and with FEV₁% predicted (rho = -0.345, p = 0.045, Figure 4), i.e. the higher the leptin level, the poorer the lung function and the more symptoms. In contrast, BMI adjusted resistin correlated positively with lung volumes VC % predicted (rho = 0.383,

Table 3 Plasma adipokines and other parameters before and after 8-week treatment with fluticasone in 11 asthmatics.

	Before treatment	After treatment	p-value
Resistin (ng/l)/BMI (m ² /kg)	0.4 [0.3 - 0.5]	0.4 [0.4-0.5]	0.722
Leptin (ng/l)/BMI (m ² /kg)	0.5 [0.4 - 1.1]	0.7 [0.2-1.0]	0.722
Adiponectin (ng/l)/BMI (m ² /kg)	154.4 ± 20.1	146.4 ± 21.0	0.271
Adipsin (ng/l)/BMI (m ² /kg)	27.5 ± 1.5	24.9 ± 1.8	0.026
ECP (µg/l)	16.0 [8.5 - 46.8]	12.4 [6.2 - 21.4]	0.026
EPX (µg/l)	47.2 [28.8 - 68.4]	22.3 [16.6 - 45.1]	0.013
MPO (µg/l)	218.6 [163.5 - 409.1]	199.7 [144.7 - 266.8]	0.534
FEV ₁ (% pred)	85 ± 4.0	95 ± 5.5	0.032
J _{Br,NO} (nl/s)	3.6 ± 0.4	0.6 ± 0.1	<0.001
C _{Alv} (ppb)	1.5 ± 0.6	1.3 ± 0.1	0.705
Symptom score	6.0 [4.0 - 10.0]	0 [0.0 - 0.0]	0.005

ECP, eosinophil cationic protein

EPX, eosinophil protein X

MPO, myeloperoxidase

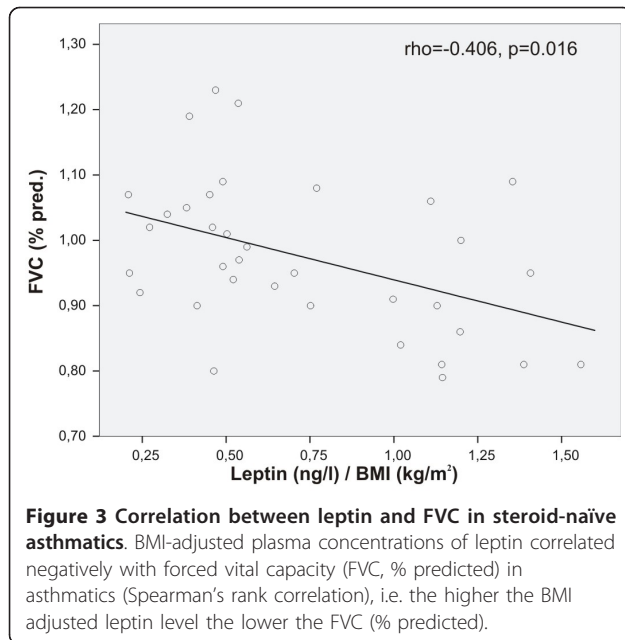
FEV₁, forced expiratory volume in 1 second

J_{Br,NO}, Bronchial NO flux

C_{Alv}, Alveolar NO concentration

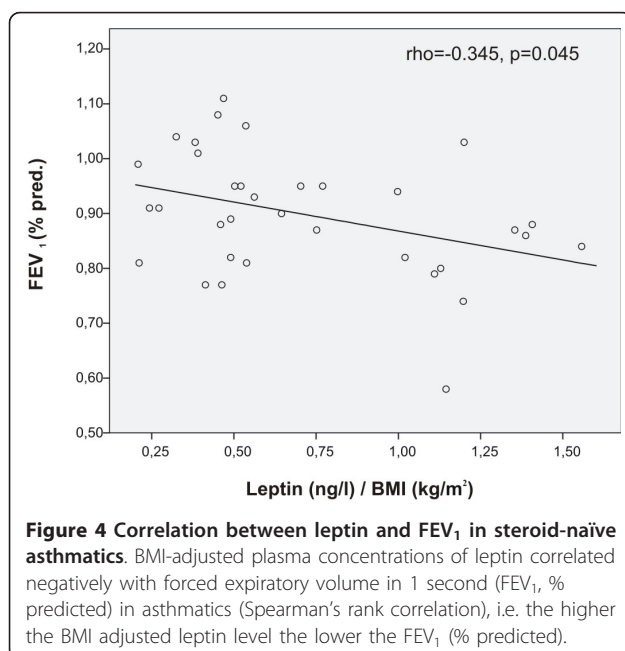
Adipokine values were adjusted for BMI (body mass index).

Values are presented as mean ± SEM for normally distributed data and as median [interquartile range] for non-normally distributed data.



$p = 0.023$) and FVC % predicted ($\rho = 0.439$, $p = 0.008$) in asthmatics. Adiponectin and adipsin had no correlations with indices of lung function, symptoms or serum markers of inflammation.

As both lung function and plasma adipokines are related to BMI, we tested if the above mentioned correlations between adipokines and lung function are explained by BMI. We conducted a stepwise multiple linear regression analysis with lung function as the dependent variable, and BMI and adipokine levels as



independent variables. Correlation of BMI adjusted resistin with VC % predicted and FVC % predicted were explained by changes in BMI. However, BMI adjusted leptin was an independent predictor of VC % predicted, FVC % predicted and FEV₁ % predicted.

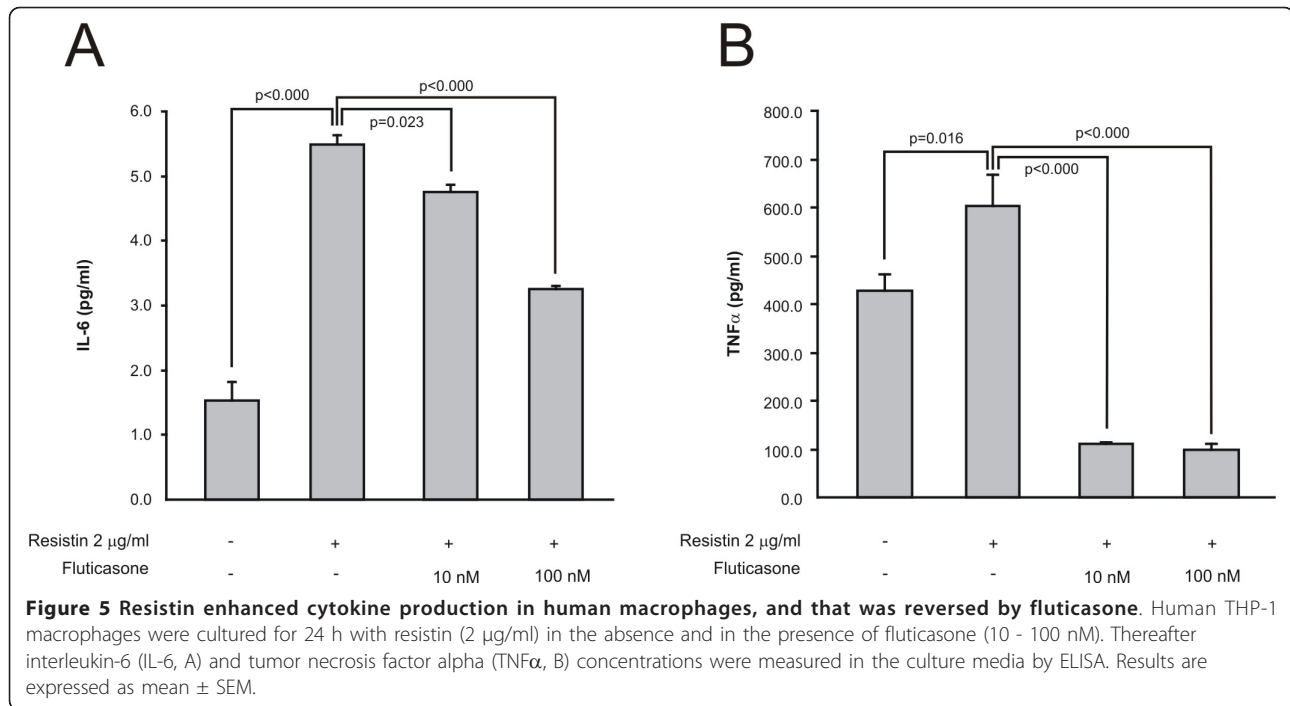
The effects of resistin on IL-6 and TNF α production in human macrophages

Because resistin levels were associated with favourable anti-inflammatory activity of fluticasone, we studied the effects of this adipokine on human THP-1 macrophages. Interestingly, resistin (0.1 - 2 $\mu\text{g/ml}$) increased production of proinflammatory cytokines IL-6 and TNF- α in THP-1 cells in a concentration-dependent manner. Moreover, fluticasone (10 and 100 nM) significantly reduced resistin-induced IL-6 and TNF- α production in (Figure 5).

Discussion

In the present study, we investigated the role of adipokines in asthma in non-obese steroid-naïve female patients. The main finding was that high pre-treatment resistin levels were associated with a more pronounced decrease in serum levels of inflammatory markers during fluticasone treatment indicating a better steroid-response. In addition, high plasma leptin levels were associated with poorer lung function and increased symptoms suggesting that leptin is related to the severity of asthma also in non-obese patients.

Resistin is associated with different inflammatory states [3], but there are only a few previous publications on resistin in patients with asthma. LaRoche et al showed that steroid-treated patients with moderate to severe asthma had higher levels of resistin than controls, and resistin levels were increased with increasing disease severity [9]. On the contrary, Kim and colleagues found that resistin levels were lower in atopic asthmatic children than in healthy controls, and resistin was associated with lower markers of atopy or bronchial responsiveness [10]. However, Arshi et al did not find any differences in resistin levels between pediatric patients with asthma and healthy children [11]. In the present study including non-obese women with newly diagnosed steroid-naïve asthma, we found that baseline resistin concentrations correlated with anti-inflammatory effects of inhaled fluticasone suggesting that resistin may be a feature and biomarker of steroid-sensitive phenotype of asthma. This relation may be explained by the finding that resistin is an endogenous agonist of Toll-like receptor 4 (TLR4) which leads to activation of various genes involved in asthmatic inflammation through NF- κB pathway [18]. Accordingly, we found here that resistin was able to enhance the production of proinflammatory cytokines IL-6 and TNF- α in human macrophages and interestingly, this effect was inhibited with fluticasone. Also, the expression of resistin itself has



been reported to be enhanced by inflammatory factors like IL-1, IL-6, TNF- α and LPS by an NF- κ B dependent manner [13,14]. Therefore high resistin levels may reflect an asthmatic phenotype characterized by increased NF- κ B activity and hence favourable response to glucocorticoids, the anti-inflammatory action of which is primarily based on their suppressive effect on NF- κ B [12].

We found that in non-obese female asthmatics the levels of adipokines were not different from healthy controls. Previously, conflicting results on the levels of adipokines in patients with asthma have been published. Leptin has been reported to be increased [5,6,19,20] or normal [10,21,22] in asthma, resistin either increased [9] or decreased [10], and adiponectin either decreased [8,23] or normal [10,21,22]. There are no previous publications on adipisin in asthma. The conflicting results are likely explained by differences in patient selection. Asthma is often considered as a single disease entity, but it is actually a syndrome with many different pathological pathways ultimately leading to quite similar clinical presentation: variable airway obstruction with chest tightness, wheezing and cough [2]. The role of adipokines quite likely varies between these different inflammatory processes. In addition, there are patient-related contributing factors like age, sex, fat distribution in the body, menopause, atopy, comorbidities and drugs, but there is insufficient data on the detailed effects, mechanisms and significance of these factors so far.

Interestingly, BMI-adjusted leptin levels were associated with poorer lung function and more symptoms in the

present study in non-obese steroid-naïve asthmatics. This is in line with a previous study showing an inverse correlation between leptin levels and lung function in non-obese healthy subjects [24] suggesting that leptin is associated with lung function regardless of BMI. Leptin has been reported to induce the production of pro-inflammatory mediators TNF- α , IL-6 and IL-12 [25]. This may further augment asthmatic inflammation and might explain the association of leptin to asthma severity.

We also found that inhaled glucocorticoids decreased plasma levels of adipisin but had no effect on other adipokines. This may be explained by the previous finding that glucocorticoids down-regulate the expression of adipisin gene [26]. In line with the negative effect of fluticasone on leptin in the present study, Radetti's and Heuck's groups have reported previously that leptin secretion was not affected by inhaled corticosteroids [27,28]. However, there are no previous studies on the effect of inhaled glucocorticoids on the levels of other adipokines than leptin.

Conclusions

In non-obese women with newly-diagnosed steroid-naïve asthma, high resistin levels predicted favourable anti-inflammatory effect of inhaled glucocorticoids suggesting that resistin may be a feature and biomarker of steroid-sensitive phenotype of asthma. High leptin levels were associated with a more severe asthma suggesting that the link between adipokine leptin and asthma is not restricted to obesity.

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Authors' contributions

SL-K and LL performed the statistical analysis and drafted the manuscript. KV carried out the cell culture experiments. LL and EM developed the protocol and equipment and supervised the exhaled NO measurements. RN and EM were responsible for the analyses of adipokines and inflammatory markers. HK and SS handled the patient recruitment and clinical treatment. All authors participated in the design of the study, and they all read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Adipokine adipsin is associated with the degree of lung fibrosis in asbestos-exposed workers

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KEYWORDS

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Summary

Objectives: Asbestos-exposure causes an inflammatory response driven by alveolar macrophages that can lead to pulmonary fibrosis. In addition to classical inflammatory cytokines, macrophages produce adipokines which regulate the inflammatory response. We studied if adipokines are related to the degree of parenchymal fibrosis, impaired lung function and inflammation in asbestos-exposed subjects.

Methods: Eighty-five males with moderate to heavy occupational exposure to asbestos and unexposed controls were studied. We measured plasma levels of adipokines adiponectin, adipsin, leptin and resistin, IL-6, IL-8, erythrocyte sedimentation rate (ERS), spirometry and $D_{L,CO}$. Degree of interstitial lung fibrosis (septal thickening, subpleural lines, parenchymal bands or honeycombing) was scored in classes 0–5 according to a validated scoring system. The subjects were divided into three groups: normal parenchymal finding (fibrosis class 0), borderline changes (classes 0.5–1.5) and fibrosis (i.e. asbestosis; classes 2–5).

Results: Adipsin correlated positively with parenchymal fibrosis ($\rho = 0.412$, $p < 0.001$) and there was a linear increasing trend of mean plasma adipsin levels among the three groups of asbestos-exposed subjects (from normal parenchymal finding to borderline changes and to fibrosis) ($p < 0.0001$). Accordingly, plasma adipsin levels correlated positively with the extent of pleural plaques ($r = 0.245$, $p = 0.043$), and negatively with $D_{L,CO}$ ($r = -0.246$, $p = 0.023$). Also, a positive correlation was found between adipsin and inflammatory markers ESR

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($r = 0.315$, $p = 0.008$) and IL-6 ($r = 0.256$, $p = 0.018$).

Conclusions: Adipsin was associated with the degree of parenchymal fibrosis, impairment of pulmonary diffusing capacity and with inflammatory activity in asbestos-exposed subjects suggesting that adipsin may have a role in the pathogenesis or as a biomarker in asbestos-induced lung disease.

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Introduction

Asbestosis is a diffuse interstitial lung fibrosis caused by an inflammatory response to inhaled asbestos fibres.¹ Alveolar macrophages release various cytokines in response to asbestos-exposure and they play a significant role in the pathogenesis of interstitial fibrosis.² The development of asbestosis requires moderate or heavy exposure to asbestos and takes 15–20 years in most cases. The detailed pathogenesis of asbestosis is poorly known and further understanding of the disease mechanisms is required to lay the background for the development of disease-modifying treatments for asbestosis.

The diagnosis of asbestosis is based on findings of diffuse interstitial fibrosis when a history of sufficient exposure to asbestos exists and other causes of pulmonary fibrosis have been ruled out.³ Diagnostic criteria for diffuse pulmonary fibrosis in asbestos-exposed subjects are based on findings on high-resolution computed tomography (HRCT) of the lungs. However, only a part of asbestos-exposed subjects develop diffuse pulmonary fibrosis, while many of the subjects show either normal HRCT or only borderline parenchymal changes that do not fulfil the diagnostic criteria for asbestosis.^{4,5} According to the recent findings, the borderline parenchymal changes are also associated with increased pulmonary inflammation and they may be an early stage of the process finally leading to frank fibrosis and asbestosis.⁶ Further studies are needed to identify biomarkers of asbestos-induced immunoresponse leading to pulmonary fibrosis.

Adipokines like adiponectin, adipsin, leptin and resistin are protein mediators first identified as products of adipose tissue regulating energy metabolism and appetite. Recently, adipokines have also been found to be produced by macrophages and various other cells and to regulate and mediate inflammatory responses.^{7,8} Leptin and resistin are usually pro-inflammatory, while adiponectin has mainly anti-inflammatory properties.^{7,8} Adipsin is a rate-limiting enzyme in the alternative complement cascade⁹ supporting its function as a pro-inflammatory factor. There is increasing evidence showing that adipokines play a role also in asthma and COPD,¹⁰ but very little is known on adipokines in context of other lung diseases. Recently, we reported that plasma levels of adipsin, and to a lesser extent also those of resistin, were clearly increased in workers with a history of heavy occupational silica exposure suggesting a possible role for adipokines also in parenchymal lung diseases.¹¹ However, there are no previous studies on the role of adipokines in asbestos-induced lung diseases.

As asbestos-exposure causes an inflammatory response in the lungs driven by alveolar macrophages and macrophages are known to secrete adipokines, we hypothesised

that adipokines may be factors involved in asbestos-induced lung inflammation and fibrosis. Therefore we investigated if plasma levels of adipokines adiponectin, adipsin, leptin or resistin are associated with pulmonary fibrosis, impaired lung function or markers of inflammation in asbestos-exposed subjects. The results show that adipsin may be an important factor or biomarker in the development of asbestosis because plasma adipsin levels were associated with parenchymal fibrosis and pleural plaques, with reduced diffusion capacity and with increased levels of inflammatory markers ESR and IL-6.

Methods

Subjects and study design

We recruited 118 men at the Clinic of Occupational Medicine at Tampere University Hospital with moderate or heavy occupational exposure to asbestos. To exclude other pulmonary diseases that might affect the inflammatory markers, the exclusion criteria were $FEV_1/FVC < 0.70$, bronchiectasis or any signs of emphysema on HRCT, diagnosed asthma, or asthma medication. Twenty-eight healthy men with normal spirometry, no respiratory symptoms and no known exposure to asbestos or other harmful agents served as the control group. All the subjects were never smokers or had quit smoking for at least 5 years prior. The protocol was approved by the Ethics Committee of Tampere University Hospital and all the subjects gave their written informed consent.

Spirometry was measured and a venous blood sample was drawn in all subjects. All the subjects in the exposed groups had a history of moderate or heavy occupational exposure to asbestos (estimation of at least 20 fibre years) considered sufficient to cause asbestosis. HRCT of the lungs was taken and pulmonary diffusing capacity for carbon monoxide ($D_{L,CO}$) was measured in asbestos-exposed subjects, and they were divided into three groups based on parenchymal findings on HRCT: (1) normal parenchymal finding, (2) borderline parenchymal finding with minor sporadic changes only, or (3) mild to extreme pulmonary fibrosis (i.e. asbestosis), see Table 1.

Of the 118 asbestos-exposed men, 33 were excluded based on the above mentioned exclusion criteria (16 due to $FEV_1/FVC < 0.70$, 11 due to emphysema or bronchiectasiae on HRCT and 6 due to diagnosed asthma or use of asthma medication) and 85 were included in the study. Of the 85 subjects included in the final analysis, 35 subjects had normal parenchymal findings on HRCT (fibrosis class 0), 31 subjects had borderline parenchymal changes (fibrosis classes 0.5–1.5), and 19 subjects had pulmonary fibrosis (fibrosis classes ≥ 2.0) regarded as asbestosis.

Table 1 Criteria for parenchymal fibrosis and the classification system of fibrosis severity on high-resolution computed tomography of the lungs. Modified from Huuskonen et al.⁴

Criteria for parenchymal fibrosis	Septal thickening Subpleural lines Parenchymal bands Honeycombing
Fibrosis classes	
Class 0, normal parenchymal finding	Normal finding by all the criteria
Class 1, borderline parenchymal changes	1–2 criteria sporadically in the lung periphery; no honeycombing
Class 2, mild fibrosis	At least 2 criteria on both sides and on several slices in the lung periphery; no honeycombing
Class 3, moderate fibrosis	Several criteria, changes extend deeper into the lung than in class 2; honeycombing as a general rule
Class 4, severe fibrosis	Several criteria or associated findings extending deep into the lung; honeycombing; lung architectural change
Class 5, extreme fibrosis	Extremely severe and various fibrotic changes; little normally aerated lung left

HRCT grading

HRCT was scanned (Siemens Somatom Plus 4; Siemens Medical, Erlangen, Germany) with 1 mm slices taken at 3 cm intervals using imaging parameters of 130–140 kV and 100–111 mA. The HRCT images were scored using consensus reading by two experienced thoracic radiologists (RJ and TV) blinded to the medical information of the patients. Pulmonary fibrosis, emphysema, parietal pleural plaques, and pulmonary nodules were scored separately as described previously.^{4,12} The semi-quantitative scoring of the HRCT findings indicating interstitial lung fibrosis (septal thickening, subpleural lines, parenchymal bands or honeycombing) in both lungs was made according to a scale of classes from 0 to 5. Fibrosis class 0 represents normal parenchymal finding, class 1 (0.5–1.5) represents borderline parenchymal finding with minor sporadic changes, and classes 2–5 represent mild to severe diffuse pulmonary fibrosis (Table 1).⁴ If the readers could not match the findings exactly with any given fibrosis class, five subclasses (0.5, 1.5, 2.5, 3.5, 4.5) were used. The fibrosis class 2 has been considered as a threshold for the diagnosis of asbestosis.⁴

Adipokines and inflammatory markers

Venous blood was collected for the assessment of plasma levels of adipokines (adiponectin, adipsin, leptin, resistin) and serum levels of interleukin 6 (IL-6) interleukin 8 (IL-8),

and blood erythrocyte sedimentation rate (ESR). Adipokines were determined by enzyme-immuno-assay (EIA) by using commercial reagents (DuoSet ELISA, R&D Systems Europe Ltd, Abingdon, U.K). IL-6 and IL-8 were measured by enzyme-linked immunosorbent-assay (PeliPair ELISA, Sanquin, Amsterdam, The Netherlands). The detection limits and inter-assay coefficients of variation, respectively, were 15.6 ng/l and 2.4% for adiponectin, 4.0 ng/l and 1.8% for adipsin, 15.6 ng/l and 1.9% for leptin, 15.6 ng/l and 7.3% for resistin, 0.6 ng/l and 2.9% for IL-6, and 1.56 ng/l and 0.9% for IL-8.

Statistics

The distribution of leptin levels was skewed while other adipokines were normally distributed (Kolmogorov–Smirnov's test). Differences in adipokine levels between the subject groups were analysed using one-way ANOVA with Games–Howell post-test (adiponectin, adipsin, resistin) or non-parametric Kruskal–Wallis ANOVA with Dunn's post-test (leptin). Skewed data are presented as median [interquartile range] and normal data as mean \pm SEM. Pearson's *r* was used to analyse the correlations between adipsin or resistin and lung function or other inflammatory markers. As adiponectin and leptin correlated with BMI, partial correlations controlling for BMI were used to analyse the correlations between adiponectin or leptin and lung function or other inflammatory markers. Spearman's rho was used to study correlations between fibrosis class in the HRCT and adipokines. A *p*-value <0.05 was considered as significant. SPSS 15.0.1 (SPSS Inc., Chicago, Illinois, USA) and InStat 3.05 (GraphPad Software, Inc., San Diego, CA, USA) softwares were used in the statistical analysis.

Results

Subject characteristics, indices of pulmonary function and levels of inflammatory markers are given in Table 2. Among the asbestos-exposed subjects, FVC % predicted ($p = 0.051$), FEV₁ % predicted ($p = 0.022$) and *D*_{L,CO} % predicted ($p < 0.001$) were lower while the extent of pleural plaques ($p = 0.066$) was higher in subjects with parenchymal fibrosis than in subjects with normal parenchymal finding on HRCT.

Adipsin, but no other adipokines, correlated positively with the degree of parenchymal fibrosis: Spearman's rho for the correlation between plasma adipsin and fibrosis class determined on HRCT (classes 0–5) was 0.412 ($p < 0.001$). There was also a statistically significant ($p < 0.0001$) increasing linear trend in serum adipsin levels when the asbestos-exposed subjects were divided into three groups, i.e. subjects with normal finding, borderline finding or fibrosis in HRCT (Fig. 1). Accordingly, adipsin levels were higher in asbestos-exposed subjects with pulmonary fibrosis as compared to exposed subjects with no parenchymal fibrosis ($p = 0.013$) or to unexposed control subjects ($p = 0.038$).

In support to the association of adipsin with the severity of asbestos-induced lung reaction, plasma adipsin levels were found to correlate positively with the extent of

Table 2 Subject characteristics.

	No fibrosis	Borderline fibrosis	Fibrotic (asbestosis)	Unexposed, healthy controls
N	35	31	19	28
Age (yrs)	62 ± 1	67 ± 1	68 ± 1	62 ± 1
BMI (kg/m ²)	30 ± 1	29 ± 1	27 ± 1	27 ± 1
FEV ₁ (% pred)	92 ± 2	85 ± 3	82 ± 3	*
FVC (% pred)	92 ± 3	85 ± 3	84 ± 3	*
Hb-D _{L,CO} (% pred)	109 ± 2	98 ± 3	86 ± 4	N.A.
ESR (mm/h)	10 ± 1	10 ± 1	14 ± 5	N.A.
IL-6 (ng/l)	3.5 ± 0.3	4.5 ± 0.6	3.7 ± 0.5	2.6 ± 0.4
IL-8 (ng/l)	10.6 ± 0.7	11.6 ± 1.4	8.2 ± 1.3	10.2 ± 0.6
Adipsin (ng/ml)	871 ± 32	965 ± 34	1129 ± 70	906 ± 34
Adiponectin (ng/ml)	2212 ± 149	2727 ± 202	2969 ± 256	2360 ± 169
Leptin (ng/ml)	8.2 [5.6–13.6]	6.9 [5.3–10.5]	7.5 [6.7–9.7]	5.6 [1.7–8.9]
Resistin (ng/ml)	17.2 ± 1	19.1 ± 1	14.9 ± 1	19.1 ± 1
Pleural plaques (cm ²)	286 ± 24	362 ± 22	490 ± 65	N.A.

Values are presented as mean ± SEM for normal data and as median [interquartile range] for non-normal data.

*, Normal in every subject; and N.A., not analysed.

BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; Hb-D_{L,CO}, pulmonary diffusing capacity for carbon monoxide standardized for hemoglobin; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6; and IL-8, interleukin 8.

pleural plaques in the asbestos-exposed subjects ($r = 0.245$, $p = 0.043$, Table 3) and negatively with pulmonary diffusing capacity ($D_{L,CO}$) ($r = -0.246$, $p = 0.023$, Fig. 2), i.e. the higher the adipsin level the poorer the diffusing capacity and the more pleural plaques. Interestingly, plasma adipsin concentrations correlated also with ESR ($r = 0.315$, $p = 0.008$, Fig. 2) and with plasma IL-6 ($r = 0.256$, $p = 0.018$, Table 3) showing an association with the asbestos-induced inflammation.

There were no significant differences in adiponectin, leptin or resistin levels between the groups. A positive correlation was found between resistin and IL-6 levels but adiponectin or leptin did not correlate significantly with lung function, pleural plaques or the inflammatory markers measured in blood.

Discussion

The main finding in the present study was that plasma levels of adipsin were associated with the degree of parenchymal fibrosis, with impairment of pulmonary diffusing capacity, with the extent of pleural plaques and with the systemic markers of inflammation in asbestos-exposed subjects. This suggests that adipsin may have a role in the pathogenesis of asbestos-induced lung injury.

The pathogenesis of asbestos-induced diseases is associated with persistent inflammatory response to asbestos with cellular and immunological abnormalities.¹ Asbestosis is characterized by slowly progressing interstitial fibrosis in alveolar wall and increased numbers of activated alveolar macrophages.¹³ Alveolar macrophages secrete IL-6¹⁴ that stimulate fibroblast proliferation.¹³ Macrophages also secrete adipokines⁷ that are known to be associated with other inflammatory lung diseases like asthma and COPD.¹⁰ This is the first study to show an association between adipokine adipsin and inflammatory activity and degree of fibrosis in asbestos-exposed subjects. Adipsin may thus be a factor in the pathogenesis of asbestosis and the present results encourage future studies to identify the potential role of adipsin in the disease mechanisms as well as a possible treatment target. That is of particular interest because the pathogenesis of asbestos-induced inflammatory response leading to lung fibrosis is not understood in detail and because of the lack of disease-modifying treatments for asbestosis.

Based on the present results, adipsin may also have a value as a biomarker of the inflammatory activity and/or

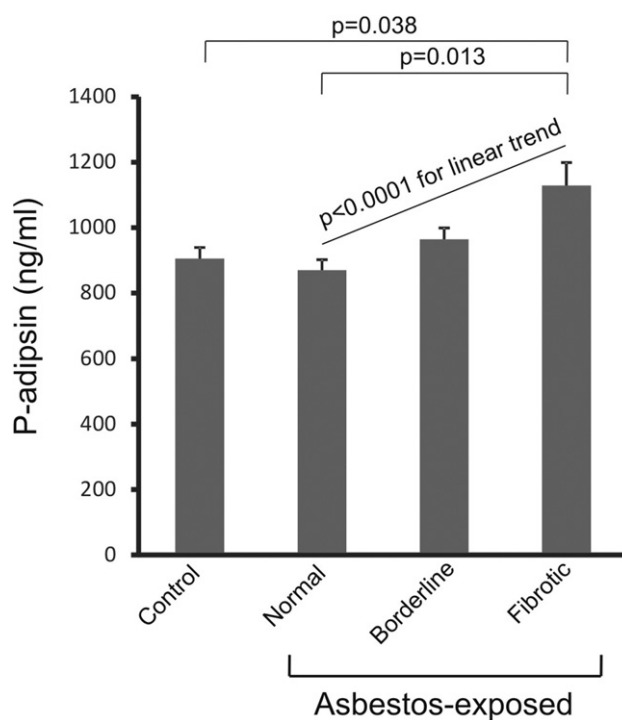


Figure 1 Plasma adipsin levels in asbestos-exposed subjects with normal, borderline or fibrotic (i.e. asbestosis) parenchymal findings on HRCT, and in unexposed controls.

Table 3 Correlations between plasma adipokines and other parameters in asbestos-exposed subjects ($n = 85$).

	Adiponectin ^a	Adipsin	Leptin ^a	Resistin
FVC (% pred)	$r = -0.082$ $p = 0.521$	$r = -0.163$ $p = 0.142$	$r = -0.246$ $p = 0.050$	$r = 0.115$ $p = 0.300$
Hb-D _{L,CO} (% pred)	$r = -0.029$ $p = 0.818$	$r = -0.246$ $p = 0.023$	$r = -0.195$ $p = 0.122$	$r = -0.058$ $p = 0.599$
Pleural plaques (cm ²)	$r = -0.028$ $p = 0.825$	$r = 0.245$ $p = 0.043$	$r = 0.019$ $p = 0.884$	$r = 0.088$ $p = 0.473$
ESR (mm/h)	$r = 0.018$ $p = 0.886$	$r = 0.315$ $p = 0.008$	$r = 0.243$ $p = 0.053$	$r = 0.102$ $p = 0.399$
IL-6 (ng/l)	$r = -0.146$ $p = 0.251$	$r = 0.256$ $p = 0.018$	$r = 0.157$ $p = 0.214$	$r = 0.291$ $p = 0.007$
IL-8 (ng/l)	$r = -0.170$ $p = 0.180$	$r = -0.049$ $p = 0.655$	$r = 0.140$ $p = 0.272$	$r = 0.047$ $p = 0.670$

FVC, forced vital capacity; Hb-D_{L,CO}, pulmonary diffusing capacity for carbon monoxide standardized for hemoglobin; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6; and IL-8, interleukin 8.
 p -value < 0.05 was considered as significant.

^a Because adiponectin and leptin correlated with BMI, partial correlations controlling for BMI were calculated.

progression of the fibrosis in subjects exposed to asbestos. There is a clinical need for biomarkers showing the current activity of asbestos-induced immune response to predict the individual risk for development or further progression of pulmonary fibrosis. We and others have previously reported some promising markers in exhaled air or sputum to be associated with asbestos-induced lung injury,^{6,15–17} but there are no previous studies on the role of adipokines in asbestos-induced lung diseases. The present results do, however, encourage a follow-up study to investigate if adipsin might have a predictive value in assessing the rate of the fibrosing process or the risk to develop asbestosis at individual patient level.

Adipsin is also known as complement factor D, which is a rate-limiting enzyme in the alternative pathway of complement activation.⁹ Adipsin, together with other components of the complement cascade, is primarily expressed in adipocytes and monocyte-macrophages in human subjects.⁹ There are only a few previous studies on adipsin in respiratory diseases. Increased plasma adipsin levels have been found in males with seasonal allergic rhinitis,¹⁸ and adipsin has been associated with an experimental model of pulmonary hypertension.¹⁹ Our present

results suggest that adipsin might act as a pro-inflammatory molecule in asbestos-induced lung injury as adipsin levels correlated positively with IL-6 and ESR and were associated with the degree of interstitial lung fibrosis. This would be in line with the fact that alveolar macrophages have a key role in the pathogenesis of asbestosis²⁰ and macrophages are an important source of adipsin.⁹ However, adipsin could also be merely a marker of activation of alveolar macrophages and further studies are needed to elucidate whether or not adipsin has a true pathogenetic role in asbestosis. Based on the present results it is not clear whether adipsin is specifically associated with asbestos related macrophage activation or is it also associated with other forms of fibrosing lung diseases. The latter assumption is supported by our previous finding of increased plasma adipsin levels in subjects with heavy occupational exposure to silica.¹¹ However, further studies are needed to clarify if other forms of interstitial lung diseases or current cigarette smoking activating pulmonary macrophages could also affect the adipsin levels.

Adipocytes are the most important source of adiponectin and leptin⁷ and circulating leptin levels correlate with adipose tissue mass.²¹ Adiponectin appears to act as an anti-

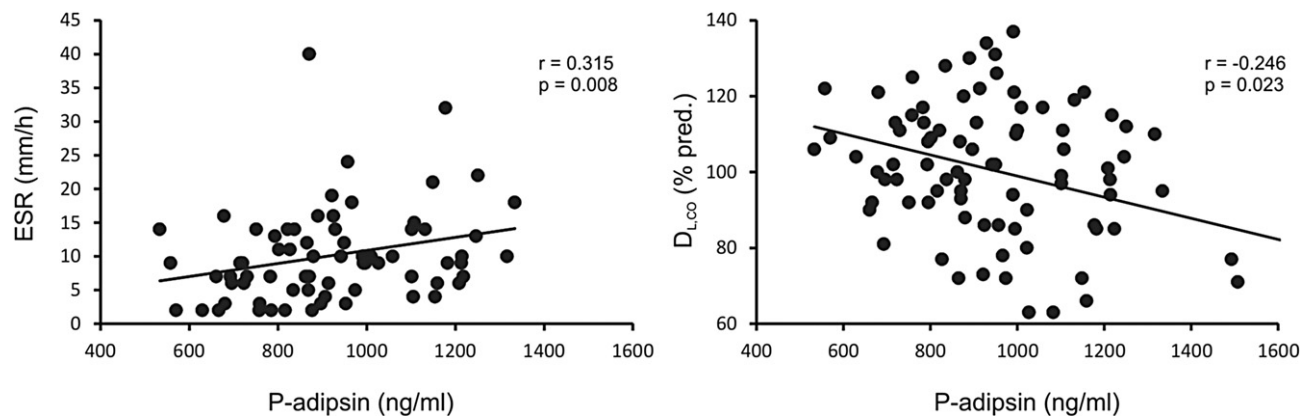


Figure 2 Plasma adipsin levels correlated positively with erythrocyte sedimentation rate (ESR) and negatively with pulmonary diffusing capacity for carbon monoxide ($D_{L,CO}$) in asbestos-exposed subjects ($n = 85$).

inflammatory molecule and it attenuates allergic airway inflammation²² and protects against asthma.²³ Leptin is a pro-inflammatory molecule and high leptin levels are a risk factor for asthma independent of BMI.²⁴ However, the present study did not find any relation between adiponectin or leptin and asbestos-induced lung disease. Resistin is produced by adipocytes and macrophages and it is associated with inflammatory states, but its role is not very well understood.⁸ In asthma, resistin has been reported to be positively correlated with the disease severity²⁵ and we have recently found resistin to predict favourable anti-inflammatory response to inhaled glucocorticoids in asthma.²⁶ However, the current results suggest that resistin does not have a significant role in asbestos-induced lung disease.

In the present study adipokine adipisin was found to be associated with the degree of pulmonary fibrosis and systemic markers of inflammation in asbestos-exposed workers. Thus, adipisin may have a role in the pathogenesis or as a biomarker in asbestos-induced lung diseases. Further studies are needed to elucidate if adipisin could be used as a prognostic tool in the follow-up of asbestos-exposed workers.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Adiponectin is associated with dynamic hyperinflation and a favourable response to inhaled glucocorticoids in patients with COPD



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Summary

Objectives: Adipokines are protein mediators first described as products of adipose tissue regulating energy metabolism and appetite. Recently, adipokines have also been found to modulate inflammation and smooth muscle cell responses. Therefore we investigated the association of two adipokines, adiponectin and leptin, with the degree of emphysema, pulmonary function, symptoms and glucocorticoid responsiveness in patients with COPD.

Methods: Plasma adiponectin and leptin levels, spirometry, body plethysmography and symptoms were measured in 43 male COPD patients with smoking history ≥ 20 pack-years, post bronchodilator FEV₁/FVC < 0.7 and pulmonary emphysema on HRCT. The measurements were repeated in a subgroup of patients after 4 weeks' treatment with inhaled fluticasone.

Results: In patients with COPD, plasma adiponectin levels correlated positively with airway resistance (Raw) ($r = 0.362$, $p = 0.019$) and functional residual capacity (FRC) ($r = 0.355$, $p = 0.046$). Furthermore, the baseline adiponectin concentration correlated negatively with

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the fluticasone induced changes in St George's Respiratory questionnaire (SGRQ) symptom score ($r = -0.413$, $p = 0.040$) and in FRC % pred ($r = -0.428$, $p = 0.003$), i.e. a higher baseline plasma adiponectin level was associated with more pronounced alleviation of symptoms and dynamic hyperinflation. Plasma leptin levels were not related to the measures of lung function, symptoms or glucocorticoid responsiveness.

Conclusions: Plasma adiponectin levels were associated with peripheral airway obstruction and dynamic hyperinflation in patients with COPD. A higher adiponectin level predicted more favourable relief of symptoms and hyperinflation during glucocorticoid treatment. Adiponectin may have a role in the COPD pathogenesis; it may also be a biomarker of disease severity and treatment responses in this disease.

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a persistent airflow limitation with extrapulmonary manifestations [1]. Inflammatory activity is present not only in the lungs [2], but there is also a low-grade systemic inflammation [3] associated with the development of the comorbidities of COPD such as ischaemic heart disease, osteoporosis, diabetes, cachexia and depression [4–6]. It is uncertain whether the systemic inflammation in COPD is a spillover of the inflammation present in the lungs or if pulmonary manifestations are only one form of expression of this systemic disease [5].

It is known that a subset of patients with COPD benefits from inhaled glucocorticoid (GC) therapy, and GCs have been shown to reduce the markers of systemic inflammation in COPD [7]. Unfortunately, current measurements of lung function or pulmonary imaging cannot distinguish the steroid responders from the non-responders, and therefore novel systemic or local markers for steroid-sensitive phenotype of COPD are critically needed.

Adipose tissue releases a variety of factors, also called adipokines, which regulate energy metabolism and appetite and, based on the recent studies, also inflammatory responses [8]. The two best characterized adipokines are adiponectin and leptin. Adiponectin improves insulin sensitivity and, accordingly, plasma adiponectin levels are decreased in obese individuals, especially in those with the metabolic syndrome, type 2 diabetes and atherosclerosis [9]. Adiponectin acts through two specific receptors (AdipoR1 and AdipoR2) and exerts a variety of anti-inflammatory effects [10,11]. On the other hand, adiponectin has also reported to enhance the production of pro-inflammatory cytokines in airway epithelium [12] and to mediate pro-inflammatory and tissue matrix degrading effects in arthritis [13–15].

Discovered in 1994 leptin is another important adipokine [16]. Leptin controls appetite through its regulatory effects in the central nervous system, and it has also a significant role as an effector and regulator in inflammatory responses [17]. Leptin increases the production of many pro-inflammatory mediators and tissue matrix degrading enzymes, and supports the Th1-type immune response [18–20]. Circulating leptin levels directly correlate with the amount of body fat [21] and leptin is linked to several obesity associated inflammatory conditions like cardiovascular [22] and rheumatic diseases [14].

Both adiponectin and leptin are produced in the human lung [12,23,24]. There is recent experimental data indicating that they may also play a role in asthma and COPD by modulating the inflammatory responses [25] and airway smooth muscle cell functions [26]. Adipokines have also been reported to be associated with fibrosing lung diseases [27,28]. Furthermore, adipose-tissue related inflammation has been proposed to represent a link connecting pulmonary damage with the systemic inflammation in COPD [3] but the mechanisms remain poorly understood.

Since adiponectin and leptin both seem to be involved in the regulation of inflammation and smooth muscle function as well as in the tissue matrix destruction, we hypothesized that they may play a role in COPD. Therefore, we investigated if plasma levels of adiponectin and leptin would be associated with the degree of obstruction, hyperinflation and emphysema, with other markers of inflammation, with the degree of symptoms and/or with the response to inhaled glucocorticoids in patients with COPD.

Methods

Subjects and study design

We recruited forty-three steroid-naïve male patients with COPD among subjects referred from primary care for diagnostic assessment to the Department of Respiratory Medicine at Tampere University Hospital. COPD diagnosis was based on GOLD strategy paper [1] and the inclusion criteria were smoking history of at least 20 pack-years, symptoms of COPD (cough, sputum production and dyspnoea), post bronchodilator $FEV_1/FVC < 0.7$, reversibility of FVC or FEV_1 induced by β_2 -agonist $< 12\%$ and 200 ml and pulmonary emphysema visible on high-resolution computed tomography (HRCT) of the lungs. None of the subjects had a diagnosis or a clinical history of asthma. Forty-one age-matched non-smoking healthy males with normal lung function served as controls.

Spirometry and body plethysmography were measured, high-resolution computed tomography (HRCT) of the lungs was performed and symptoms were scored with the St George's Respiratory Questionnaire (SGRQ) in patients with COPD. A venous blood sample was drawn in both groups. The same measurements excluding HRCT were repeated in

twenty-seven patients with COPD after 4 weeks of treatment with inhaled fluticasone propionate (Flixotide Diskus 500 µg b.i.d.; GlaxoSmithKline, Ware, UK).

Adipokines and inflammatory markers

Plasma concentrations of adiponectin, leptin, myeloperoxidase (MPO), interleukin 6 (IL-6) and matrix metalloproteinase 9 (MMP-9) were determined by enzymeimmunoassay by using the following reagents: adiponectin, leptin and MMP-9: R&D Systems Europe Ltd, Abingdon, UK; MPO: Hycult Biotechnology, Uden, Netherlands; and IL-6: Sanquin, Amsterdam, Netherlands. The detection limits and inter-assay coefficients of variation, were 31.3 pg/ml and 6.7% for adiponectin, 15.6 pg/ml and 4.5% for leptin, 0.4 ng/ml and 8.7% for MPO, 0.3 pg/ml and 7.6% for IL-6, and 7.8 pg/ml and 6.0% for MMP-9.

Lung function and HRCT

Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁) were measured with the Vmax 20 C spirometer (Sensor-Medics, Yorba Linda, CA, USA) before and after 400 µg of inhaled salbutamol. Airway resistance (Raw) and functional residual capacity (FRC) were measured with Autobox 6200 body plethysmography (Sensor-Medics, Yorba Linda, CA, USA).

In the HRCT examinations (Siemens Somatom Plus 4, Siemens Medical, Erlangen, Germany), a section thickness of 1 mm was used with a 10-mm inter-slice spacing at 140 kV and 206 mA s. The subjects were lying in the supine position and performing full inspiration. The HRCT images were scored according to a consensus by two experienced thoracic radiologists (RJ and LK) blinded to the medical information of the patients.

The extent of emphysema was estimated visually to the nearest 5% on each image section excluding the two most cranial and caudal images, as described by Desai and colleagues [29]. The mean value of emphysema percent on all image sections was taken as the final emphysema score for each subject. In the evaluation of the airway wall thickness, the external diameter (*D*) and the luminal diameter (*L*) of the right upper lobe bronchus were measured in each subject. Only images showing the bronchus as a cross-section without an angle were selected. Airway wall thickness (*T*) was calculated as $(D - L)/2$. The thickness-to-diameter ratio (TDR) and the percentage wall area (PWA) were calculated as $TDR = T/D$, and $PWA = (D/2)^2\pi - (L/2)^2\pi / (D/2)^2\pi \times 100\%$ [30]. TDR and PWA were used in statistical analysis.

Symptom scoring

The subjects filled in the Finnish translation of the St George's Respiratory Questionnaire (SGRQ) containing questions and scoring on three aspects of the disease (symptom frequency and severity, activities that cause or are limited by breathlessness, and the impact of the disease on social functioning including psychological disturbances resulting from the disease) to obtain a total score. The scale has a range from 0 to 100, with higher scores representing more severe disease.

Statistics

The distributions of both adiponectin and leptin levels were skewed but they became normal after log-transformation, which was used in the statistical analyses. *t*-Test was used to examine differences between healthy controls and patients with COPD. To test if adiponectin or leptin correlate with markers of disease severity, partial correlation controlling for body mass index (BMI) was used as both log-adiponectin and log-leptin correlated with BMI. Changes in plasma levels of adipokines and other measures during fluticasone treatment were analyzed with paired *t*-test. The results are presented as mean ± SEM. A *p*-value < 0.05 was considered as significant. SPSS 19 software (SPSS Inc., Chicago, Illinois, USA) was used in the statistical analysis.

Ethics

The study was approved by the ethics committee of Tampere University Hospital, Tampere, Finland and complies with the declaration of Helsinki. All subjects provided their written informed consent.

Results

The subject characteristics are shown in Table 1. There were no differences with respect to the age or BMI between the patients with COPD and the controls but, as expected, plasma MMP-9 levels were higher in those individuals with emphysematous COPD. The adiponectin level correlated negatively with BMI ($r = -0.588$, $p < 0.001$), while there was a positive correlation between leptin concentration and BMI ($r = 0.700$, $p < 0.001$), as expected. There were no statistically significant differences in plasma adiponectin

Table 1 Characteristics of the subjects.

	COPD	Controls	<i>p</i> -Value
<i>N</i>	43	41	
Age (yrs)	59.5 ± 1.2	62.5 ± 1.0	0.057
BMI (kg/m ²)	25.8 ± 0.6	26.7 ± 0.6	0.332
FEV ₁ (% pred)	53 ± 2	^a	–
Raw (% pred)	213 ± 14	N.A.	–
FRC (% pred)	123 ± 5	N.A.	–
TLC (% pred)	108 ± 2	N.A.	–
MPO (ng/ml)	158.9 ± 11.6	139.6 ± 4.9	0.131
IL-6 (pg/ml)	1.5 ± 0.2	1.5 ± 0.2	0.888
MMP-9 (ng/ml)	40.6 ± 2.7	33.9 ± 2.0	0.048
Adiponectin ^b (ng/ml)	3034 ± 328	2538 ± 191	0.210
Leptin ^b (ng/ml)	7.5 ± 0.9	7.1 ± 1.0	0.703

Values are presented as mean ± SEM.

N.A., not analyzed.

BMI, body mass index; FEV₁, forced expiratory volume in 1 s; Raw, airway resistance; FRC, functional residual capacity; TLC, total lung capacity; MPO, myeloperoxidase; IL-6, interleukin 6; MMP-9, matrix metalloproteinase 9.

^a Normal in every subject.

^b Because distributions of adiponectin and leptin were skewed, log-transformed values were used in the statistical analysis.

and leptin levels between COPD patients and healthy controls, or between ex-smokers ($n = 10$) and current smokers ($n = 33$) in the COPD group.

In the COPD patients, body plethysmography was carried out to evaluate small airway obstruction (measured as airway resistance, Raw) and dynamic hyperinflation (measured as functional residual capacity, FRC). Interestingly, adiponectin levels correlated positively with airway resistance (Raw % predicted, $r = 0.362$, $p = 0.019$, Table 2) and functional residual capacity (FRC % predicted, $r = 0.355$, $p = 0.046$, Table 2), i.e. high plasma adiponectin concentrations were associated with peripheral obstruction and hyperinflation. Neither adiponectin nor leptin levels correlated with FEV₁ or FEV₁/FVC, with the inflammatory markers measured in plasma, with the degree of emphysema or airway wall thickness on HRCT or with SGRQ scores at baseline.

The effect of 4 weeks' fluticasone treatment on the adipokine levels and other parameters are shown in Table 3. St George's Respiratory Questionnaire (SGRQ) total and symptom scores decreased and leptin levels increased during the treatment, but there were no other significant changes. Interestingly, the baseline adiponectin level correlated negatively with fluticasone induced changes in SGRQ total score ($r = -0.410$, $p = 0.042$), SGRQ symptom score ($r = -0.413$, $p = 0.040$) and FRC % predicted ($r = -0.428$, $p = 0.003$), indicating that high baseline adiponectin level was associated with favourable relief of symptoms and hyperinflation in response to fluticasone treatment.

Discussion

The main findings of the present study were that high plasma adiponectin levels were associated with obstruction

Table 2 BMI-adjusted partial correlations between adiponectin and leptin and other parameters in COPD patients ($n = 43$).

	Adiponectin ^a	Leptin ^a
FEV ₁ (% pred)	$r = 0.058$ $p = 0.717$	$r = 0.000$ $p = 0.999$
Raw (% pred)	$r = 0.362$ $p = \mathbf{0.019}$	$r = -0.089$ $p = 0.576$
FRC (% pred)	$r = 0.355$ $p = \mathbf{0.046}$	$r = -0.225$ $p = 0.216$
Emphysema percentage (%)	$r = 0.208$ $p = 0.186$	$r = -0.217$ $p = 0.167$
MPO	$r = 0.117$ $p = 0.483$	$r = 0.160$ $p = 0.338$
IL-6	$r = -0.012$ $p = 0.942$	$r = 0.172$ $p = 0.276$
MMP-9	$r = -0.121$ $p = 0.463$	$r = 0.029$ $p = 0.860$

FEV₁, forced expiratory volume in 1 s; Raw, airway resistance; FRC, functional residual capacity; MPO, myeloperoxidase; IL-6, interleukin 6; MMP-9, matrix metalloproteinase 9.

Statistically significant p -values ($p < 0.05$) are bolded.

^a Because distributions of adiponectin and leptin were skewed, log-transformed values were used in the statistical analysis.

Table 3 Adipokines and other parameters before and after fluticasone treatment in COPD patients.

	Before treatment	After treatment	p -Value
Adiponectin ^a (ng/ml)	3594 ± 486	3460 ± 457	0.598
Leptin ^a (ng/ml)	6.0 ± 1.0	7.1 ± 1.2	0.018
FEV ₁ (% pred)	53 ± 3	56 ± 3	0.288
Raw (% pred)	214 ± 19	210 ± 16	0.656
FRC (% pred)	123 ± 6	121 ± 6	0.467
MPO (ng/ml)	166 ± 16	171 ± 17	0.768
IL-6 (pg/ml)	1.62 ± 0.29	1.67 ± 0.26	0.747
MMP-9 (ng/ml)	39.1 ± 3.5	44.9 ± 4.2	0.268
SGRQ total score	36.4 ± 3.1	30.8 ± 3.1	0.015
SGRQ symptom score	55.2 ± 4.3	38.0 ± 4.5	< 0.001

Values are presented as mean ± SEM, $n = 27$.

FEV₁, forced expiratory volume in 1 s; Raw, airway resistance; FRC, functional residual capacity; MPO, myeloperoxidase; IL-6, interleukin 6; MMP-9, matrix metalloproteinase 9; SGRQ, St George's Respiratory Questionnaire.

Statistically significant p -values ($p < 0.05$) are bolded.

^a Because distributions of adiponectin and leptin were skewed, log-transformed values were used in the statistical analysis.

in peripheral airways and predicted a favourable effect of inhaled fluticasone treatment on both symptoms and dynamic hyperinflation in patients with COPD. This indicates that adiponectin may have a role in the pathogenesis of COPD and in addition, it may serve as a biomarker of disease severity.

Originally, adipose-tissue derived adipokines were found to regulate energy metabolism and be associated with the chronic low-grade inflammation present in obesity-related metabolic disturbances and inflammatory diseases [11,31]. More recently, adiponectin and leptin have also been linked with inflammatory lung diseases like asthma and COPD [25]. The majority of the studies have hinted that high circulating leptin and low adiponectin levels seem to be associated with asthma independently of the presence of obesity [25,32]. Accordingly, we have recently found that increased leptin levels were associated with more severe asthma also in non-obese patients [33]. The data on adiponectin and leptin levels in human COPD are less conclusive [25]. Breyer et al. hypothesized that adipokine metabolism is altered in patients suffering from COPD, particularly in women [34]. Some human studies have demonstrated higher plasma or serum adiponectin concentrations also in male patients with COPD when they are compared to healthy controls [34–37]. Carolan et al. found increased levels of adiponectin especially in subjects with very severe COPD [38]. In the present study, the mean adiponectin level was somewhat higher in COPD patients than in healthy controls although the difference was not statistically significant. There was variation in the adiponectin levels in COPD patients, and interestingly, higher adiponectin levels were associated with more severe airway obstruction and hyperinflation in patients with COPD.

It is thought that there are many causes for the airway obstruction in COPD such as mucosal oedema, contraction of the airway smooth muscle, small airway fibrosis and loss of

parenchymal support to small airways due to emphysema [2]. In the present study, we observed that plasma adiponectin levels correlated with Raw and FRC but not with FEV₁ and FEV₁/FVC. This is interesting, as FEV₁ and FEV₁/FVC are mostly reflecting obstruction in the larger airways and these parameters are not very sensitive to changes occurring in the small airways. Furthermore, in cases of severe small airway obstruction and airway closure during exhalation causing dynamic hyperinflation, FCV reductions in addition to FEV₁ and the ratio FEV₁/FVC may not reflect the severity of small airway obstruction. Raw is a more sensitive measure of small airway obstruction than FEV₁ or FEV₁/FVC, and FRC is believed to be a good marker of dynamic hyperinflation in COPD [39]. Thus, our results suggest that adiponectin may be more closely related to peripheral than central airway obstruction in COPD. This is in line with the earlier results of Tomoda and colleagues who reported that the plasma adiponectin level correlated with hyperinflation but not with FEV₁ in their sample of COPD patients [35].

The relation between adiponectin levels in plasma and small airway obstruction suggests that adiponectin may be a marker or even a mediator of parenchymal inflammation and the tissue destruction present in emphysematous COPD, which then leads to small airway obstruction and hyperinflation. Our results are supported by the data in a cohort of COPDGene study, in which Carolan et al. found an association between plasma adiponectin and CT-assessed emphysema in patients with COPD [38]. Interestingly, the degenerative cartilage changes seen in (osteo)arthritis seem to display similarities with tissue matrix degrading events leading to pulmonary emphysema in COPD. Adiponectin has been reported to contribute to cartilage matrix destruction in arthritis by inducing the production of the same pro-inflammatory cytokines and degrading metalloproteinase enzymes that are also involved in COPD and emphysema [2,14]. In addition, adiponectin may have a direct effect on airway function, as human airway smooth muscle cells express adiponectin receptors [26] and adiponectin is known to increase contractility in smooth muscle cells [40]. Thus, it may be that increased levels of circulating adiponectin enhance the contractility of airway smooth muscle and are involved in inducing the airway obstruction, and also contribute to the inflammation and tissue destruction which is evident in COPD.

We also found that high baseline levels of adiponectin predicted good symptom relief and alleviation of hyperinflation in response to inhaled fluticasone treatment. This finding also indicates that there is a relationship between lung inflammation and the circulating adiponectin levels and further suggests that adiponectin is related to the steroid-sensitive phenotype in COPD. On the other hand, specific inhibition of the adiponectin induced smooth muscle contraction might be one of the mechanisms through which glucocorticoids act to alleviate small airway obstruction and the symptoms in COPD. This is supported by the fact that glucocorticoids can inhibit the expression of adiponectin receptors (AdipoR1 and AdipoR2) as shown in rat [41] and human muscle cells [42]. Therefore it may be that the contractile effect of adiponectin on airway smooth muscle is alleviated if adiponectin receptor density is reduced during glucocorticoid treatment. In addition, glucocorticoids have been shown to decrease adiponectin

expression under some conditions [41] and this might also be a potential mechanism linking adiponectin and the response to steroid treatment. However, most of the studies [43–45] have reported that glucocorticoids, delivered either orally or by inhalation, do not alter plasma levels of adiponectin. This is in line with our present finding that fluticasone treatment had no effect on the circulating adiponectin concentrations.

Based on data from animal studies, adiponectin has also been proposed to exert a protective role against emphysema, as adiponectin deficiency has been reported to lead to increased levels of two pro-inflammatory mediators, TNF- α and MMP-12, and to an emphysema-like phenotype in the mouse lung [46,47]. In addition, exposure to tobacco smoke has been reported to reduce the expression of adiponectin in both mice [48] and humans [12], but adiponectin is highly expressed in the lungs of patients with the emphysematous form of COPD [12]. However, we observed no correlation between the degree of emphysema on HRCT and plasma levels of adiponectin in these subjects with emphysematous COPD. Furthermore, there was no difference in plasma levels of adiponectin between healthy non-smoking controls and patients with emphysematous COPD. This is in line with the results of the large ECLIPSE study [49], although there are also reports of increased serum levels of adiponectin in COPD [34–38].

We detected no difference in plasma levels of leptin between controls and subjects with emphysematous COPD. Previous data on leptin is controversial, as some studies have shown increased [50], others decreased [51,52] and some unchanged [34,36] circulating leptin levels in patients with COPD. These differences are most likely attributable to the heterogeneity of the COPD patient populations and, may also be affected by the gender-dependent differences in adipokine metabolism. As in the present study, Kirdar et al. found no significant differences in plasma leptin levels between male patients with stable COPD and healthy controls [36]. In the study of Breyer et al., leptin levels did not differ between subjects with COPD and healthy controls, but in both groups, the leptin levels were higher in females than in males [34]. In addition, leptin levels correlated with CRP, IL-6 and fibrinogen only in females but not in males with COPD [34]. This is evidence of an important gender-related difference in leptin metabolism and it may be explained by different proportions of adipose tissue of the body composition and possibly also partly by an interesting finding indicating that androgens can inhibit leptin secretion whereas it is stimulated by oestradiol [53].

Leptin may also be associated with some features or comorbidities of COPD. In fact, alterations in leptin levels are often associated with COPD exacerbations [54,55]. Leptin has been shown to stimulate the production of vascular endothelial growth factor (VEGF) in human airway smooth muscle *in vitro* and leptin could therefore affect angiogenesis and promote airway remodelling in COPD [26]. On the other hand, leptin does not evoke contractile responses in human airway smooth muscle cells [56], and this is in line with our findings that the leptin level did not correlate with the markers of airway obstruction. Interestingly, we found that treatment with inhaled fluticasone increased plasma levels of leptin in the COPD patients. Glucocorticoids have been reported to stimulate leptin

expression in adipose tissue [57] and to increase plasma leptin levels in patients with polymyalgia rheumatica [58]. This may be attributable to a direct effect of glucocorticoids on leptin gene expression, as the glucocorticoid responsive element (GRE) has been identified in the leptin gene promoter [59].

In the present study, high plasma levels of adiponectin were associated with peripheral airway obstruction and dynamic hyperinflation in patients with COPD. Furthermore, higher plasma levels of adiponectin also predicted more favourable relief of symptoms and hyperinflation during glucocorticoid treatment, supporting the experimental data that adiponectin is a pro-inflammatory mediator able to induce tissue matrix degradation and to evoke smooth muscle contraction. However, further studies will be needed to confirm the role of adiponectin in the pathogenesis of COPD and whether it is useful as a marker of the steroid-sensitive phenotype in this disease.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Research Article

Adipokines NUCB2/Nesfatin-1 and Visfatin as Novel Inflammatory Factors in Chronic Obstructive Pulmonary Disease

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COPD (chronic obstructive pulmonary disease) is a common lung disease characterized by airflow limitation and systemic inflammation. Recently, adipose tissue mediated inflammation has gathered increasing interest in the pathogenesis of the disease. In this study, we investigated the role of novel adipocytokines nesfatin-1 and visfatin in COPD by measuring if they are associated with the inflammatory activity, lung function, or symptoms. Plasma levels of NUCB2/nesfatin-1 and visfatin were measured together with IL-6, IL-8, TNF- α , and MMP-9, lung function, exhaled nitric oxide, and symptoms in 43 male patients with emphysematous COPD. The measurements were repeated in a subgroup of the patients after four weeks' treatment with inhaled fluticasone. Both visfatin and NUCB2/nesfatin-1 correlated positively with plasma levels of IL-6 ($r = 0.341$, $P = 0.027$ and $\rho = 0.401$, $P = 0.008$, resp.) and TNF- α ($r = 0.305$, $P = 0.052$ and $\rho = 0.329$, $P = 0.033$, resp.) and NUCB2/nesfatin-1 also with IL-8 ($\rho = 0.321$, $P = 0.036$) in patients with COPD. Further, the plasma levels of visfatin correlated negatively with pulmonary diffusing capacity ($r = -0.369$, $P = 0.016$). Neither of the adipokines was affected by fluticasone treatment and they were not related to steroid-responsiveness. The present results introduce adipocytokines NUCB2/nesfatin-1 and visfatin as novel factors associated with systemic inflammation in COPD and suggest that visfatin may mediate impaired pulmonary diffusing capacity.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a disorder characterized by persistent airflow limitation with systemic manifestations [1]. In addition to the inflammatory process in the lungs, there is a low-grade systemic inflammation which is linked to the pathogenesis of the comorbidities present in COPD [2–4]. It is not known whether systemic inflammation is a spillover of the inflammation present in

the lungs or if pulmonary manifestations are one form of expression of this systemic disease [3, 5]. Smoking, lung hyperinflation, tissue hypoxia, and skeletal muscle dysfunction have been suggested as possible factors involved in the pathogenesis of the systemic inflammation in COPD [2]. Recently, adipose tissue mediated inflammation has gathered increasing interest as a significant mechanism in inducing and promoting systemic inflammation in COPD [4].

Adipokines (also known as adipocytokines) are protein mediators secreted by adipose tissue and they are involved not only in the regulation of energy metabolism but also in inflammatory responses in many chronic inflammatory diseases [6, 7]. There is increasing body of evidence supporting a significant role of adipokines adiponectin and leptin in the inflammatory processes in COPD [8], but only a little or nothing is known about the other adipokines like nesfatin-1 and visfatin in inflammatory lung diseases. So far, there are no previous publications on nesfatin-1 and only a few reports on visfatin in COPD.

Nesfatin-1 is a novel adipokine discovered in 2006 and at first linked to appetite and body weight control in rats [9]. Nesfatin-1 is expressed in human adipose tissue and TNF- α , IL-6, insulin, and dexamethasone have been shown to increase its secretion [10]. Nesfatin-1 has also been shown to regulate inflammatory responses and cell apoptosis in rats [11] and to have cardioprotective effects in human studies [12]. There are only two previous publications on nesfatin-1 in lung diseases: one concerning changes in fat mass in lung cancer [13] and the other on cystic fibrosis [14].

Visfatin, also known as nicotinamide phosphoribosyltransferase (NAMPT) and previously identified as pre-B cell colony-enhancing factor (PBEF), was originally discovered in lymphocytes, bone marrow, liver, and muscle [15]. Later, visfatin was identified also in the lungs [16], and, interestingly, the proinflammatory cytokine IL-1 has been reported to enhance visfatin expression in pulmonary epithelial and endothelial cells *in vitro* [17]. Also proinflammatory cytokines IL-6 [18] and TNF- α [19] have been shown to induce the expression of visfatin. Granulocytes and monocytes are major sources of visfatin [20], and it is also produced by macrophages and adipocytes [21]. Visfatin is a proinflammatory cytokine involved in the regulation of inflammation and innate immunity [22, 23]. In the lungs, visfatin is associated with acute lung injury (ALI) [24] and the inhibition of visfatin synthesis has been shown to attenuate inflammation and apoptosis associated with severe virus infection in lung endothelium [25].

In the present study, we measured the plasma levels of visfatin and NUCB2/nesfatin-1 in patients with COPD and in controls and investigated if these adipokines are associated with other markers of inflammation, lung function, the degree of emphysema, and symptoms or with the response to inhaled glucocorticoids in patients with COPD.

2. Materials and Methods

2.1. Subjects and Study Design. Forty-three steroid-naïve male patients with COPD were recruited among subjects referred from primary care for diagnostic assessment to the Department of Respiratory Medicine at Tampere University Hospital, Tampere, Finland. COPD diagnosis was based on GOLD strategy paper [1] and the inclusion criteria were smoking history of at least 20 pack-years, symptoms of COPD (cough, sputum production, and dyspnoea), post-bronchodilator FEV₁/FVC < 0.7, reversibility of FVC or FEV₁ induced by β_2 -agonist < 12% or 200 mL, and pulmonary

emphysema visible on high resolution computed tomography (HRCT) of the lungs. Patients with a diagnosis or clinical history of asthma or diabetes were excluded. Ten (23%) of the patients had hypertension and five (12%) had hypercholesterolemia while the number of patients with other diseases was too small for any statistical analysis. Forty-one age-matched nonsmoking healthy males with normal lung function served as controls.

Spirometry, fractional exhaled nitric oxide concentration (FENO), and pulmonary diffusing capacity per unit of alveolar volume standardized for haemoglobin concentration ($Hb \cdot D_{L,CO}/V_A$) were measured, high resolution computed tomography (HRCT) of the lungs was performed, and symptoms were scored with St. George's Respiratory Questionnaire (SGRQ) in patients with COPD. A venous blood sample was drawn in both groups. The same measurements excluding HRCT were repeated in twenty-seven patients with COPD after 4 weeks of treatment with inhaled fluticasone propionate (Flixotide Diskus 500 μ g b.i.d.; GlaxoSmithKline, Ware, UK).

2.2. Adipokines and Inflammatory Markers. Plasma concentrations of NUCB2/nesfatin-1, visfatin, interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor alpha (TNF- α), and matrix metalloproteinase 9 (MMP-9) were determined by enzyme immunoassay by using the following reagents: NUCB2/nesfatin, TNF- α , and MMP-9: R&D Systems Europe Ltd., Abingdon, UK; visfatin: Phoenix Pharmaceuticals Inc., Karlsruhe, Germany; IL-6: Sanquin, Amsterdam, Netherlands; and IL-8: BD Biosciences, San Diego, CA, USA. According to the manufacturer, the nesfatin-1 antibody detects the protein nucleobinding-2 (NUCB2) in addition to nesfatin-1 which is derived from NUCB2 by posttranslational processing. Therefore, the term NUCB2/nesfatin-1 is used when referring to our own measurements. The detection limits and interassay coefficients of variation were 7.8 pg/mL and 11.6% for NUCB2/nesfatin-1, 0.1 ng/mL and 8.3% for visfatin, 0.3 pg/mL and 7.6% for IL-6, 1.56 pg/mL and 7.0% for IL-8, 0.5 pg/mL and 6.5% for TNF- α , and 7.8 pg/mL and 6.0% for MMP-9.

2.3. Lung Function, Exhaled NO, HRCT, and Symptom Scoring. Spirometry was measured (Vmax 20C, Sensor-Medics, Yorba Linda, CA, USA) before and after 400 μ g of inhaled salbutamol. Pulmonary diffusing capacity was measured with Vmax 20C, Sensor-Medics.

Fractional exhaled nitric oxide concentration at exhalation flow rate of 50 mL/s (FENO_{0.05}) was measured with a Sievers NOA 280 NO-analyzer (Sievers Instruments, Boulder, CO, USA) as previously described [26].

Airway wall thickness and the extent of emphysema on pulmonary high resolution computed tomography (HRCT) (Siemens Somatom Plus 4, Siemens Medical, Erlangen, Germany) were assessed by two experienced thoracic radiologists (Ritva Järvenpää and Lea Kööbi) as previously described [27].

The subjects filled in the Finnish translation of St. George's Respiratory Questionnaire (SGRQ) containing questions and scoring on three aspects of the disease (symptom frequency and severity, activities that cause or are

TABLE 1: Plasma levels of adipokines and other inflammatory markers in patients with COPD and healthy controls.

	COPD	Controls	P value
N	43	41	
NUCB2/nesfatin-1 (pg/mL)	75.0 [26.2–103.1]	43.1 [17.9–86.6]	0.117
Visfatin (ng/mL)	7.5 ± 1.5	8.9 ± 2.3	0.002
IL-6 (pg/mL)	1.5 ± 1.3	1.5 ± 1.2	0.888
MMP-9 (ng/mL)	40.6 ± 17.3	33.9 ± 12.6	0.048

Values are presented as mean ± SD for normally distributed data and as median [interquartile range] for nonnormally distributed data. IL-6: interleukin 6; MMP-9: matrix metalloproteinase 9.

TABLE 2: Correlations between adipokines and other parameters in patients with COPD ($n = 43$).

	NUCB2/nesfatin-1	Visfatin
FEV ₁ (% pred)	rho = 0.097 P = 0.535	r = 0.127 P = 0.422
FENO _{0.05} (ppb)	rho = -0.035 P = 0.823	r = 0.040 P = 0.802
Hb-D _{L,CO} /V _A (% pred)	rho = -0.103 P = 0.512	r = -0.369 P = 0.016
Emphysema percentage (%)	rho = 0.076 P = 0.626	r = 0.204 P = 0.194
IL-6 (pg/mL)	rho = 0.401 P = 0.008	r = 0.341 P = 0.027
IL-8 (pg/mL)	rho = 0.321 P = 0.036	r = 0.121 P = 0.443
TNF-α (pg/mL)	rho = 0.329 P = 0.033	r = 0.305 P = 0.052

The distribution of nesfatin-1 was skewed while visfatin was normally distributed; therefore Spearman's rho and Pearson's r were used to analyze the correlations of NUCB2/nesfatin-1 and visfatin, respectively, to the other parameters presented.

FEV₁: forced expiratory volume in 1 second; FENO_{0.05}: fractional exhaled nitric oxide concentration at exhalation flow rate of 50 mL/s; Hb-D_{L,CO}/V_A: diffusing capacity for carbon monoxide per unit of alveolar volume standardized for haemoglobin; IL: interleukin; TNF: tumor necrosis factor.

limited by breathlessness, and the impact of the disease on social functioning including psychological disturbances resulting from the disease) to obtain a total score. The scale has a range from 0 to 100, with higher scores representing more severe disease.

2.4. Statistics. Visfatin was normally distributed, while the distribution of NUCB2/nesfatin-1 was skewed and could not be normalised after log-transformation. t -test or Mann-Whitney test was used to examine differences between healthy controls and patients with COPD, where appropriate. Pearson's r or Spearman's rho was used to analyse the correlations between adipokines and other inflammatory markers or markers of disease severity, where appropriate. Changes in plasma levels of adipokines and other measures during fluticasone treatment were analysed with paired t -test or Wilcoxon's test, where appropriate. The results are presented as mean ± SD for normally distributed data and as median (interquartile range) for nonnormally distributed data. SPSS 19 software (SPSS Inc., Chicago, Illinois, USA) was used in the statistical analysis.

2.5. Ethics. The study was approved by the Ethics Committee of Tampere University Hospital, Tampere, Finland, and complies with the declaration of Helsinki. All subjects provided their written informed consent.

3. Results

3.1. Characteristics of the Subjects. In the patients with COPD, the mean age was 59.5 ± 7.8 (mean ± SD) years and the mean forced expiratory volume in 1 second (FEV₁) was 53 ± 14% of the predicted. The age- and sex-matched healthy controls had normal lung function and there were no significant differences in BMI between the patients with COPD and the controls (25.8 ± 4.2 versus 26.7 ± 3.9 kg/m², resp., $P = 0.332$). Plasma levels of adipokines NUCB2/nesfatin-1 and visfatin in the patients with COPD and controls are given in Table 1. Visfatin levels were lower in COPD, but plasma NUCB2/nesfatin-1 levels did not differ from controls. Neither of the adipokines measured differed between ex-smokers ($n = 10$) and current smokers ($n = 33$) or between the patients with or without hypertension ($n = 10$) or between the patients with or without hypercholesterolemia ($n = 5$) in the COPD group.

3.2. Correlations between Adipokines and Other Parameters. Correlations between adipokines and other parameters in patients with COPD are given in Table 2. Both NUCB2/nesfatin-1 and visfatin correlated with IL-6 in the patients with COPD but not in controls. Therefore, two other proinflammatory cytokines, that is, TNF-α and IL-8, were also measured in patients with COPD. Interestingly,

NUCB2/nesfatin-1 and visfatin correlated positively with TNF- α and NUCB2/nesfatin-1 also with IL-8. A negative correlation was seen between visfatin and pulmonary diffusing capacity ($Hb-D_{L,CO}/V_A$) suggesting that visfatin is associated with parenchymal impairment. The adipokines did not correlate with BMI, radiological changes, levels of MMP-9, exhaled nitric oxide, or symptoms score.

3.3. The Treatment Responses. Four weeks of treatment with inhaled fluticasone caused no significant changes in plasma levels of NUCB2/nesfatin-1 (before: 75.0 (18.3–119.4) pg/mL, after: 61.1 (17.5–116.1) pg/mL, $P = 0.399$) or visfatin (before: 7.9 ± 1.5 ng/mL, after: 8.0 ± 1.4 ng/mL, $P = 0.804$). As expected, the treatment decreased St. George's Respiratory Questionnaire total score describing the impact of the disease (before: 36.4 ± 15.6 , after: 30.8 ± 16.0 , $P = 0.015$) and symptom score (before: 55.2 ± 22.1 , after: 38.0 ± 23.2 , $P < 0.001$). The baseline plasma levels of NUCB2/nesfatin-1 or visfatin did not correlate with the degree of fluticasone induced changes in either symptoms or lung function in COPD (data not shown).

4. Discussion

The main findings in the present study were that NUCB2/nesfatin-1 and visfatin correlated positively with systemic inflammation and, further, visfatin was associated with parenchymal impairment in patients with emphysematous COPD. This suggests that NUCB2/nesfatin-1 and visfatin may have a role in the inflammatory processes in COPD.

Originally, adipose-tissue-derived adipokines were found to regulate energy metabolism and to be associated with the chronic low-grade inflammation present in obesity-related metabolic disturbances and inflammatory diseases [28, 29]. Later adipokines have also been linked to inflammatory lung diseases like asthma and COPD [8] and the majority of the studies have concentrated on the role of leptin and adiponectin in these diseases. It has been suggested that high circulating leptin and low adiponectin predict asthma independent of obesity and that low leptin and high adiponectin are associated with stable COPD [30]. The results on the association between adipokines and COPD are, however, still conflicting and the studies are not covering all adipokines such as visfatin, which is known to be associated with other chronic inflammatory and destructive syndromes such as rheumatic diseases [31].

We found that NUCB2/nesfatin-1 and visfatin concentrations in plasma correlated with circulating levels of IL-6 and TNF- α and NUCB2/nesfatin-1 also with IL-8 suggesting that these adipokines may have a role in the systemic inflammation in COPD. Neutrophils and macrophages are significant cell types in the pathophysiology of COPD [32]. Macrophages are also major sources of IL-6 and TNF- α [32] and neutrophils of IL-8 [33]. As activated macrophages also secrete adipokines [6], the association between adipocytokines nesfatin-1 and visfatin and proinflammatory cytokines IL-6 and TNF- α in COPD may be linked to the activation of macrophages. Further, IL-6 has been shown to induce

the expression of both visfatin [18] and nesfatin-1 [10], and additionally it has been reported that TNF- α is able to provoke both nesfatin-1 [10] and visfatin secretions [19]. Moreover, it has been presented that visfatin itself can induce the production of TNF- α and especially IL-6 [22]. Also, previous reports have shown that visfatin correlates positively with IL-6 [34] and with CRP and TNF- α without association with BMI in patients with COPD [35], but the current study is the first report on NUCB2/nesfatin-1 in COPD.

Interestingly, we also found a negative correlation between visfatin and pulmonary diffusing capacity suggesting that visfatin is associated with parenchymal impairment in emphysematous COPD. In COPD, diffusing capacity may be decreased due to loss of alveolar surface and due to impaired diffusivity because of inflammation and oedema in the alveolar walls. As visfatin levels were not associated with the degree of emphysema visible on HRCT, we suggest that visfatin is related to the inflammatory activity impairing pulmonary diffusing capacity. This is supported by the previous findings that inflamed endothelium as well as lung epithelial cells can produce visfatin [17, 36] which may promote and amplify lung inflammation and parenchymal vascular damage present in emphysema [37].

Compared to controls, both higher [35] and lower [38] plasma visfatin levels have been reported in COPD. In the current study, plasma visfatin levels were lower in the slightly overweight men with COPD compared to healthy controls with similar BMI. Consistent with our result, significantly lower visfatin levels in normal weight or slightly overweight men with COPD [38] have been reported, while underweight men with COPD had increased visfatin levels [35]. Visfatin is expressed in visceral adipose tissue and higher [20], lower [39], and unchanged [40] circulating visfatin levels have been reported in obese compared to normal weight persons. Accordingly, we and others [35] have not found any correlation between visfatin and BMI in patients with COPD. The higher visfatin in underweight COPD patients reported by Liu and coworkers [35] might be explained by the fact that COPD patients with lower BMI have usually more severe systemic inflammation [41] and that visfatin itself can inhibit neutrophil apoptosis [42] and thus enhance lung inflammation in COPD patients. Liu et al. also hypothesized that hypoxemia present in severe COPD may contribute to increased visfatin [35].

Neither visfatin nor NUCB2/nesfatin-1 concentrations were changed during inhaled fluticasone treatment in the current study. This may be explained by the fact that a short term treatment with inhaled fluticasone likely has no significant systemic anti-inflammatory effect, and this is also supported by the present finding that neither IL-6 nor MMP-9 levels changed during the treatment. As far as we know, there are no other studies on the effect of inhaled glucocorticoids on the levels of NUCB2/nesfatin-1 and visfatin, but in previous studies systemic glucocorticoid treatment did not alter circulating visfatin levels in humans [43, 44]. Further, in the current study neither of these adipokines was associated with the treatment responses assessed by lung function or symptoms. However, in a previous study we

found that high levels of adiponectin were associated with steroid-responsiveness in COPD [27].

5. Conclusions

The present results introduce adipocytokines NUCB2/nesfatin-1 and visfatin as novel inflammatory factors in stable emphysematous COPD. Furthermore, visfatin was also associated with reduced pulmonary diffusing capacity. The findings suggest that NUCB2/nesfatin-1 and visfatin have a proinflammatory role in the pathogenesis of emphysematous COPD, but further studies are needed to evaluate if adipokines could be used as biomarkers for phenotyping or subgrouping patients with COPD or as anti-inflammatory drug targets.

Conflict of Interests

The authors declare that there is no conflict of interests.

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