Obesity-related risk factors for impaired lung function

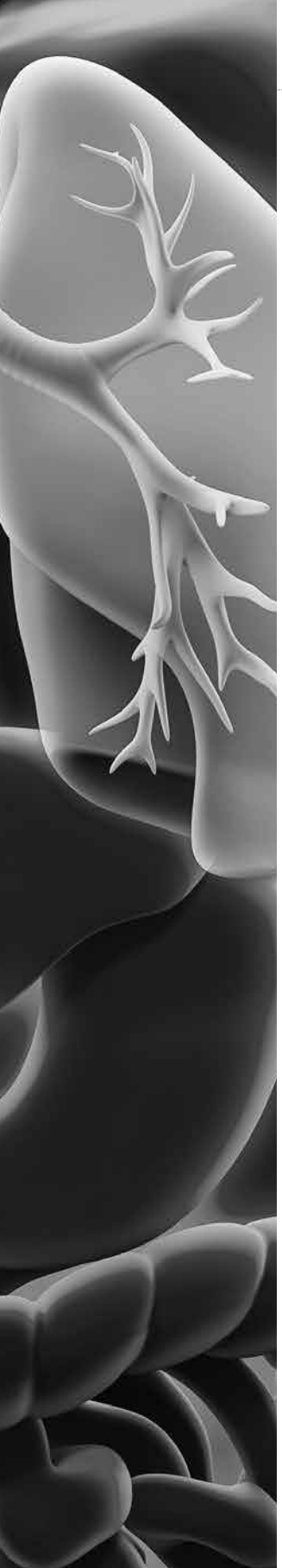
Thijs, W.

Obesity-related risk factors for impaired lung function

Willemien Thijs

Obesity-related risk factors for impaired lung function

Willemien Thijs



COLOPHON

Obesity-related risk factors for impaired lung function
Willemien Thijs
Thesis Leiden University Medical Center

ISBN: 978-90-9030771-8

Photo cover: Shutterstock.com

Concept and design: Meneer E. illustratie en vormgeving Amsterdam & Margreet van Roest organiseert en regelt v.o.f.
Printing: Digiforce, Vianen

This PhD project was financially supported by an unrestricted research grant from AstraZeneca.

Obesity-related risk factors for impaired lung function

Proefschrift

ter verkrijging van de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 7 maart 2018
klokke 16.15 uur

door

Willemien Thijs

geboren te Loenen aan de Vecht in 1977

PROMOTIECOMMISSIE

Promotores:	Prof. dr. P.S. Hiemstra Prof. dr. S. Middeldorp, Universiteit van Amsterdam
Co-promotor:	Dr. ir. R. de Mutsert
Promotiecommissie:	Prof. dr. F.R. Rosendaal Prof. dr. J. Gussekloo Prof. dr. M. den Heijer, Vrije Universiteit Dr. G.J. Braunstahl, Sint Franciscus Gasthuis, Rotterdam

Voor mijn moeder

CONTENTS

CHAPTER

1

General introduction

GENERAL INTRODUCTION

Asthma and COPD are chronic respiratory diseases, which are a major public health problem in many countries. The global prevalence of asthma ranges from 1 to 18% of the population in different countries [1] and in a worldwide study the prevalence of COPD stage II or higher was 10% [2].

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by a history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation [3]. In 2013, the World Health Organization (WHO) estimated that every year 25 billion disability-adjusted life years are lost because of asthma [4]. Asthma is often accompanied with allergic airways disease and this increases susceptibility for infections[5;6]. The main risk factors associated with childhood-onset asthma are genetic predisposition, a family history of allergy and asthma, infections, allergic sensitization, and tobacco exposure [7]. Risk factors for adult onset asthma are irritant exposure in the work place, environmental pollutants, upper airway disease, infections, obesity [8] and the metabolic syndrome [9].

Chronic obstructive pulmonary disease (COPD) is another chronic respiratory disease and it is typically associated with tobacco smoking, is usually present in persons older than forty years of age, and is characterized by progressive and irreversible airway obstruction [10]. COPD is the sixth leading cause of death since 2000 [11]. Although COPD is mainly a smoking-related disorder there are other risk factors for this disease. External risk factors are second hand smoking, occupational exposure, indoor air pollution from the burning of biomass fuels and outdoor air pollution; intrinsic risk factors are genetic predisposition, damaged airways due to prenatal maternal smoke exposure or childhood infection [12]. In addition, the metabolic syndrome and type 2 diabetes have been associated with COPD [13].

Obesity contributes to the overall burden of disease worldwide [14;15], and its prevalence remains increasing due to the abundant availability of energy-dense (fast) food and a sedentary life-style. Body mass index (BMI) is widely used to classify obesity and is expressed as body weight in kilograms divided by height in meters squared. According to the WHO classification over-weight is defined as a BMI of 25 kg/m^2 or higher, obesity as a BMI of 30 kg/m^2 or higher and a BMI of 40 kg/m^2 or higher is considered morbid obesity [16]. In the Netherlands, 60 percent of adult men and 44 percent of adult women have a BMI ≥ 25 kg/m^2 and 13 percent of the men and 14 percent of the women have a BMI ≥ 30 kg/m^2 [17].

It is increasingly recognized that there is a relationship between obesity and asthma, although the cause of this association remains largely unknown [9;18;19]. In a meta-analysis of prospective epidemiological studies the risk of incident asthma in obese persons was 2-fold increased, compared with persons with a normal weight[19]. Obesity also appeared to worsen asthma control [20;21] and in several studies obesity was associated with the severity of asthma [22], whereas weight loss after bariatric surgery in obese asthmatic patients decreased the severity of asthma [23;24]. Although severe COPD is often accompanied by weight loss, in mild to moderate COPD patients obesity is more prevalent than in the normal population [25;26].

Historically, the loss of lung function associated with obesity is ascribed to altered mechanics [27], however more recently it is recognised that systemic effects of obesity and the metabolic syndrome may play a role. It is established that obesity is associated with a modest reduction in total lung capacity and a larger reduction in functional residual capacity [28] and that it increases the work of breathing [29]. Several studies have directly measured the impact of BMI on respiratory mechanics and demonstrated a reduced respiratory system compliance [30;31]. This reduction in respiratory compliance may be due to a reduction in chest wall compliance, a reduction in lung compliance or a combination of both and might vary depending on body fat distribution [30;31].

Although most studies use BMI to define obesity, it is not an ideal measure to define obesity, because it does not distinguish body fat from fat free mass. Moreover, BMI provides no information on the distribution of body fat, which is an important contributing factor to morbidity and mortality [32;33]. Abdominal obesity appeared even more important than overall obesity in relation to diabetes and cardiovascular disease [34;35] and is an important component of the metabolic syndrome.

The metabolic syndrome and lung function

The metabolic syndrome is defined as a cluster of symptoms that occur together and increase the risk of obesity-related diseases. Several definitions exist, and according to the International Diabetes Federation the metabolic syndrome is defined by abdominal obesity based on a high waist circumference plus any two of the following four parameters: hyperglycemia, hypertriglyceridemia, low high-density lipoprotein cholesterol concentrations, and hypertension [36]. More than one-fifth of the adult population and roughly 60% of obese individuals in the United States is affected by the metabolic syndrome [37].

In a prospective cohort study, the metabolic syndrome and in particular high waist circumference and hyperglycemia or diabetes were associated with an increased risk of asthma [38]. All features of the metabolic syndrome may influence lung function. In various studies type 2 diabetes mellitus was related to impaired lung function[39-43]. In patients with diabetes, glycosylation of extracellular matrix proteins in the chest wall and bronchial tree by high circulating glucose might explain in part this association. Glycosylation leads to irreversible collagen cross-linking, which causes collagen to be stiffer and less susceptible to proteolysis, resulting in accumulation of collagen in lung connective tissue [44-46].
Before patients develop overt diabetes, insulin resistance may already be present. Insulin plays a central role in glucose uptake and intracellular glucose metabolism [47], and high insulin concentrations - as observed in patients with insulin resistance - may also impair lung function.

Elevations in insulin promote net muscle protein accumulation primarily by inhibiting protein breakdown, rather than by stimulating protein synthesis [48] and insulin resistance is associated with poor muscle strength [49]. This may link insulin resistance to lung function impairment, because a decrease in skeletal muscle strength is associated with decreases in spirometric pulmonary function [50]. Furthermore patients who inhale insulin have more dyspnea, cough and a reduced lung function [51]. This could be explained by an increased

calcium response to insulin by airway smooth muscle cells, and increased insulin-induced collagen release in these cells. These mechanisms could explain the increased contractility and remodeling of airway smooth muscle cells that was observed in an in vitro study [52]. Hyperlipidemia is also associated with lung function impairment [53]. In patients with the metabolic syndrome, often an excess of triglycerides and free fatty acids is observed in the circulation. Plasma saturated fatty acids are positively associated with sputum neutrophil percentage [54]. In addition, sputum neutrophil percentage increased after a high fat meal in asthmatics [55]. This could be due to the activation of innate immune responses via several inflammatory mechanisms by free fatty acids [56].

Adipose tissue stores energy in the form of lipids, acts as an insulating layer, and it provides mechanical protection and support for some major organs. Nowadays it is recognized that adipose tissue is also an endocrine organ. It secretes several pro-inflammatory cytokines and hormones that may result in a low grade systemic inflammatory state that could contribute to several obesity-related diseases [57;58]. Visceral adipose tissue has a high secretion rate of pro-inflammatory cytokines [59] and other markers of inflammation [60], and it is hypothesized that the excess cardiometabolic risk associated with abdominal obesity is due to increased amounts of visceral adipose tissue [61-63]. In large population-based studies the positive relationships between features of the metabolic syndrome and lung function were predominantly attributed to abdominal obesity as measured by waist circumference [53;64;65]. Leptin and adiponectin are two of the multiple hormones produced by adipose tissue that may exert metabolic effects on the lung. Leptin is not only secreted by adipocytes but also by bronchial epithelial cells [66;67]. A study in patients with heart failure showed that circulating leptin was associated with lung function impairment also after adjusting for percentage of body fat [68]. This may be due to a leptin-induced pro-inflammatory response [69]. In contrast to leptin, adiponectin concentrations decrease with increasing BMI and adiponectin has predominantly anti-inflammatory effects. A lower leptin/adiponectin ratio was associated with lung function decline in patients with COPD [70]. Furthermore, low adiponectin is associated with a future risk of asthma [71]. In a study in which obese patients with asthma were compared with an obese control group without asthma, assessment of several inflammation parameters in bronchial biopsies revealed no difference between these groups, but a subgroup analysis showed lower adiponectin concentrations in uncontrolled asthmatics [72]. After bariatric surgery in this study group, systemic inflammation decreased in all patients (asthmatic and non-asthmatics) and mast cells decreased in bronchial biopsies of the patients with asthma [23]. In the same study group morbidly obese patients with the metabolic syndrome had a higher proportion of blood monocytes and eosinophils, and their lung function was slightly more obstructed than that of obese patients without the metabolic syndrome [73]. Therefore, these components of systemic inflammation might contribute to the lung function impairment in obese asthmatics.

Vitamin D, lung function and infections

Several studies have suggested that vitamin D, lung function and (respiratory) infections are interconnected. First, obesity is associated with lower vitamin D concentrations [74;75;76]. The mechanism underlying this inverse association has not yet been fully elucidated. One possible explanation for the observed relation is that plasma vitamin D is reduced in obesity due to an increased uptake in subcutaneous adipose tissue [77;78] Precursors of vitamin D

synthesized in the skin under the influence of sunlight, might not easily reach the circulation because of subcutaneous fat [79]. Furthermore, there is a positive association between vitamin D status and lung function in the general population [80-84]. This association may be explained in part by the impact of (respiratory) infections or airway inflammation on lung function, because of the regulatory role of vitamin D in immunity and infection [85;86].

Several observational studies showed that serum vitamin D status is inversely associated with the number of respiratory tract infections [87-90]. Especially in asthmatics and COPD patients, respiratory tract infections play an important role as they are associated with exacerbations [91-93], which are the main cause of disease progression, decreased lung function and mortality in these patients [94-96]. Vitamin D treatment in patients with vitamin D deficiency reduced exacerbations in those COPD patients with severe vitamin D deficiency [97;98].
A recent meta-analyses of placebo controlled trials in asthmatic patients showed that vitamin D treatment reduces the number of exacerbations [99]. If high vitamin D concentrations prevent respiratory infections one of the explanations could be that vitamin D increases production of antimicrobial peptides in lung tissue [100], and thereby decreasing the number of exacerbations in asthma and COPD patients. In that case vitamin D suppletion may be an attractive preventive strategy for progressive lung function impairment in these patient groups.

Asthma is often characterized by allergic airways disease and this is accompanied by increased susceptibility to infections [5;6]. Allergic inflammation and especially the T helper 2 (Th2) cytokines produced during allergic inflammation could decrease local host defense against infections by reducing the expression of antimicrobial peptides and proteins [101;102]. Furthermore, this Th2 cytokine-mediated inflammation has also been shown to impair epithelial anti-viral defenses [103] and epithelial cells from asthmatics have decreased anti-rhinovirus activity [104]. Antimicrobial peptides and proteins form an essential element of innate immunity and eliminate a wide range of bacteria, fungi, and viruses [105]. Various studies revealed deficiencies of selected antimicrobial peptides and proteins in airway secretions of patients with allergic rhinitis, sinusitis and asthma [101;106;107]. Vitamin D is an important regulator of the production of antimicrobial peptides and proteins [108-110], and vitamin D administration has been shown to increase antimicrobial peptide expression in neonates and patients with atopic dermatitis [111;112]. These data suggest that allergic inflammation contributes to impaired host defense against infections, and that vitamin D could improve this by stimulating antimicrobial peptide production. Therefore, it is important to establish the effect of vitamin D on antimicrobial peptides in allergic asthma patients by appropriately designed studies.

Outline and aims of the thesis

The aim of the research presented in this thesis was to unravel effects of obesity-related risk factors on lung function. Historically it is thought that lung function impairment is a consequence of abdominal obesity on lung mechanics. As described above metabolic effects of visceral fat on systemic inflammation and insulin resistance could cause airway inflammation and therefore also influence lung function. Furthermore obesity and especially subcutaneous fat is associated with low serum vitamin D concentration. Low vitamin D may influence lung function and the susceptibility to airway infections. A possible mechanism explaining a protective effect of vitamin D against respiratory tract infections is not yet elucidated.

If vitamin D increases the production and expression of antimicrobial peptides this could influence airway inflammation and protect against respiratory infections. This thesis consists of two parts. First, we investigated to what extent components of the metabolic syndrome are associated with lung function. This is described in the first part of the thesis.
In **chapter 2** we explored the association of components of the metabolic syndrome and measurement of visceral fat with lung function as assessed with forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and exhaled nitric oxide in men with the metabolic syndrome. In **chapter 3** we investigated the association between insulin resistance and lung function (FEV_1, FVC) in a population-based cohort. In **chapter 4** the reproducibility of exhaled nitric oxide measurements in overweight and obese participants was established, and based on these findings we used a single exhaled nitric oxide measurement for the studies described in chapter 5 and 6. To examine whether exhaled nitric oxide could be used as a marker of adipose tissue-associated pulmonary inflammation, we investigated the association between visceral fat and exhaled nitric oxide in a population-based cohort study in **chapter 5**.
In the second part of this thesis we aimed to investigate associations between vitamin D, lung function, exhaled nitric oxide and symptoms of the common cold. Therefore in **chapter 6** we examined
the associations of vitamin D status with lung function (FEV_1, FVC), exhaled nitric oxide, and symptoms of the common cold in a population-based cohort. Furthermore, we hypothesized that antimicrobial peptide levels in nasal secretions are lower in allergic asthmatic patients and that vitamin D could increase these levels. Therefore, in **chapter 7** we first examined the expression of antimicrobial peptide levels in nasal secretions from patients with allergic asthma and in healthy controls in a case-control design. Secondly, we assessed if vitamin D administration increased antimicrobial levels in both asthma patients and healthy controls in a placebo-controlled cross-over study. And finally, in **chapter 8** we summarize the results of this thesis and discuss its strengths, limitations and implications.

Study designs and data used in this thesis

The Rubens study

For the research question in **chapter 2** we used the data from the "Rosiglitazone versUs placeBo on the prevENtion of progression of atherosclerosis" (RUBENS) trial [113]. This is a double-blind placebo controlled randomized trial, testing the hypothesis that Rosiglitazone prevents progression of atherosclerosis in 110 participants aged between 50–70 years with the metabolic syndrome. All participants had a waist circumference > 94 cm and at least two other metabolic syndrome criteria: high triglycerides (TG) ≥ 1.7 mmol/L, high density lipoprotein (HDL) <1.03 mmol/l, blood pressure ≥ 130 / ≥ 85mm Hg. Exclusion criteria were presence of type 2 diabetes, overt cardiovascular disease, use of statins or fibrates, and BMI>40 kg/m^2. In **chapter 2** we used the data from the 98 participants who underwent lung function testing after the conclusion of this trial.

The NEO study

To answer the research questions in **chapters 3 to 6**, we used the baseline measurements of the "Netherlands Epidemiology of Obesity" (NEO) study. The NEO study is a population-based prospective cohort study in 6,671 individuals aged between 45 and 65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher, who were recruited in the greater area of Leiden between September 2008 and October 2012. All participants underwent an

extensive physical examination including anthropometric measurements, blood sampling and lung function tests. In a random subset of 2,580 participants without contraindications for undergoing Magnetic Resonance Imaging (MRI), abdominal subcutaneous and visceral adipose tissue was assessed by MRI. Detailed information about the study design and data collection has been described previously [114].

The AVID study

To answer the research question in **chapter 7** we performed the "Asthma and vitamin D" (AVID) study. We designed this study to measure levels of antimicrobial peptides in nasal secretions in asthmatics and healthy controls and to establish the effect of vitamin D treatment on these levels. In this trial, all participants were treated with active vitamin D (2 microgram $1,25(OH)_2D3$ or placebo once daily during seven days[115;116]) in a double-blind, placebo-controlled cross-over design that was identical in asthmatics and healthy controls. This study included 19 patients with mild-to-moderate asthma and 23 healthy controls aged between 18 and 45 years recruited by advertisement in the Leiden area of the Netherlands.
All participants underwent vacuum aided suction for collection of nasal secretions and blood sampling at several visits.

REFERENCE LIST

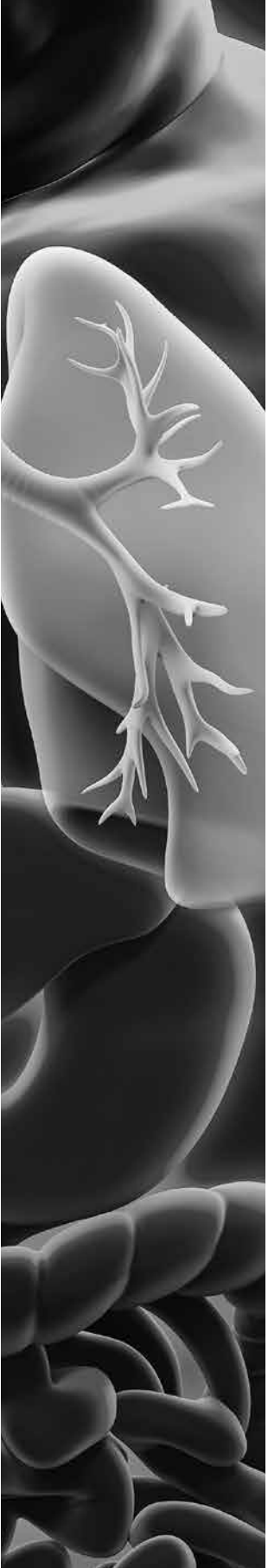
1. Bateman, E. D., S. S. Hurd, P. J. Barnes, J. Bousquet, J. M. Drazen, M. FitzGerald, P. Gibson, K. Ohta, P. O'Byrne, S. E. Pedersen, et al. 2008. Global strategy for asthma management and preven tion: GINA executive summary. *Eur.Respir.J.* 31:143-178.
2. Buist, A. S., M. A. McBurnie, W. M. Vollmer, S. Gillespie, P. Burney, D. M. Mannino, A. M. Menezes, S. D. Sullivan, T. A. Lee, K. B. Weiss, et al. 2007. International variation in the prevalence of COPD(the BOLD Study): a population-based prevalence study. *Lancet* 370:741-750.
3. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. Revised 2014 Vancouver, GINA, 2014.
4. World Health Organization. Chronic respiratory diseases. *Asthma*. 2013.
5. Talbot, T. R., T. V. Hartert, E. Mitchel, N. B. Halasa, P. G. Arbogast, K. A. Poehling, W. Schaffner, A. S. Craig, and M. R. Griffin. 2005. Asthma as a risk factor for invasive pneumococcal disease. *N.Engl.J.Med.* 352:2082-2090.
6. Zambrano, J. C., H. T. Carper, G. P. Rakes, J. Patrie, D. D. Murphy, T. A. Platts-Mills, F. G. Hayden, J. M. Gwaltney, Jr., T. K. Hatley, A. M. Owens, et al. 2003. Experimental rhinovirus challenges in adults with mild asthma: response to infection in relation to IgE. *J.Allergy Clin.Immunol.* 111:1008-1016.
7. Bisgaard, H. and K. Bonnelykke. 2010. Longterm studies of the natural history of asthma in childhood. *J.Allergy Clin.Immunol.* 126:187-197.
8. de Nijs, S. B., L. N. Venekamp, and E. H. Bel. 2013. Adult-onset asthma: is it really different? *Eur. Respir.Rev.* 22:44-52.
9. Assad, N., C. Qualls, L. J. Smith, A. Arynchyn, B. Thyagarajan, M. Schuyler, D. R. Jacobs, Jr., and A. Sood. 2013. Body mass index is a stronger predictor than the metabolic syndrome for future asthma in women. The longitudinal CARDIA study. *Am.J.Respir.Crit Care Med.* 188:319-326.
10. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for Diagnosis, Management and Prevention of COPD. 2015.
11. Murray, C. J., A. D. Lopez, R. Black, C. D. Mathers, K. Shibuya, M. Ezzati, J. A. Salomon, C. M. Michaud, N. Walker, and T. Vos. 2007. Global burden of disease 2005: call for collaborators. *Lancet* 370:109-110.
12. Postma, D. S., A. Bush, and M. van den Berge. 2015. Risk factors and early origins of chronic obstructive pulmonary disease. *Lancet* 385: 899-909.
13. Mannino, D. M., D. Thorn, A. Swensen, and F. Holguin. 2008. Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD. *Eur.Respir.J.* 32:962-969.
14. Ng, M., T. Fleming, M. Robinson, B. Thomson, N. Graetz, C. Margono, E. C. Mullany, S. Biryukov, C. Abbafati, S. F. Abera, et al. 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 384:766-781.
15. Kelly, T., W. Yang, C. S. Chen, K. Reynolds, and J. He. 2008. Global burden of obesity in 2005 and projections to 2030. *Int.J.Obes.(Lond)* 32:1431-1437.
16. 2000. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech.Rep.Ser.* 894:i-253.
17. Hoeveel mensen hebben overgewicht? [http://www.rivm.nl/nldemaat]. 2015.
18. Brumpton, B., A. Langhammer, P. Romundstad, Y. Chen, and X. M. Mai. 2013. General and abdominal obesity and incident asthma in adults: the HUNT study. *Eur.Respir.J.* 41:323-329.
19. Beuther, D. A. and E. R. Sutherland. 2007. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. *Am.J.Respir.Crit Care Med.* 175:661-666.
20. Saint-Pierre, P., A. Bourdin, P. Chanez, J. P. Daures, and P. Godard. 2006. Are overweight asthmatics more difficult to control? *Allergy* 61:79-84.
21. Clerisme-Beaty, E. M., S. Karam, C. Rand, C. M. Patino, A. Bilderback, K. A. Riekert, S. O. Okelo, and G. B. Diette. 2009. Does higher body mass index contribute to worse asthma control in an urban population? *J.Allergy Clin.Immunol.* 124: 207-212.
22. Taylor, B., D. Mannino, C. Brown, D. Crocker, N. Twum-Baah, and F. Holguin. 2008. Body mass index and asthma severity in the National Asthma Survey. *Thorax* 63:14-20.
23. van Huisstede, A. Rudolphus, C. M. Castro, L. U. Biter, G. J. van de Geijn, C. Taube, P. S. Hiemstra, and G. J. Braunstahl. 2015. Effect of bariatric surgery on asthma control, lung function and bronchial and systemic inflammation in mor bidly obese subjects with asthma. *Thorax* 70:659-667.
24. Boulet, L. P., H. Turcotte, J. Martin, and P. Poirier. 2012. Effect of bariatric surgery on airway response and lung function in obese subjects with asthma. *Respir.Med.* 106:651-660.
25. Steuten, L. M., E. C. Creutzberg, H. J. Vrijhoef, and E. F. Wouters. 2006. COPD as a multicomponent disease: inventory of dyspnoea, underweight, obesity and fat free mass depletion in primary care. *Prim.Care Respir.J.* 15:84-91.

26. Franssen, F. M., D. E. O'Donnell, G. H. Goossens, E. E. Blaak, and A. M. Schols. 2008. Obesity and the lung: 5. Obesity and COPD. *Thorax* 63:1110-1117.
27. Naimark, A. and R. M. Cherniack. 1960. Compliance of the respiratory system and its components in health and obesity. *J.Appl.Physiol* 15:377-382.
28. Jones, R. L. and M. M. Nzekwu. 2006. The effects of body mass index on lung volumes. *Chest* 130:827-833.
29. Steier, J., A. Lunt, N. Hart, M. I. Polkey, and J. Moxham. 2014. Observational study of the effect of obesity on lung volumes. *Thorax* 69:752-759.
30. Pelosi, P., M. Croci, I. Ravagnan, S. Tredici, A. Pedoto, A. Lissoni, and L. Gattinoni. 1998. The effects of body mass on lung volumes, respiratory mechanics, and gas exchange during general anesthesia. *Anesth.Analg.* 87:654-660.
31. Pelosi, P., M. Croci, I. Ravagnan, P. Vicardi, and L. Gattinoni. 1996. Total respiratory system, lung, and chest wall mechanics in sedated-paralyzed postoperative morbidly obese patients. *Chest* 109:144-151.
32. Lee, C. M., R. R. Huxley, R. P. Wildman, and M. Woodward. 2008. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. *J.Clin.Epidemiol.* 61:646-653.
33. Sun, Q., M. K. Townsend, O. I. Okereke, O. H. Franco, F. B. Hu, and F. Grodstein. 2009. Adiposity and weight change in mid-life in relation to healthy survival after age 70 in women: prospective cohort study. *BMJ* 339:b3796.
34. Wang, Y., E. B. Rimm, M. J. Stampfer, W. C. Willett, and F. B. Hu. 2005. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *Am.J.Clin.Nutr.* 81:555-563.
35. Flint, A. J., K. M. Rexrode, F. B. Hu, R. J. Glynn, H. Caspard, J. E. Manson, W. C. Willett, and E. B. Rimm. 2010. Body mass index, waist circumference, and risk of coronary heart disease: a prospective study among men and women. *Obes. Res.Clin.Pract.* 4:e171-e181.
36. Alberti, K. G., P. Zimmet, and J. Shaw. 2006. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the Interna tional Diabetes Federation. *Diabet.Med.* 23:469-480.
37. Beltran-Sanchez, H., M. O. Harhay, M. M. Harhay, and S. McElligott. 2013. Prevalence and trends of metabolic syndrome in the adult U.S. popula tion, 1999-2010. *J.Am.Coll.Cardiol.* 62:697-703.
38. Brumpton, B. M., C. A. Camargo, Jr., P. R. Romundstad, A. Langhammer, Y. Chen, and X. M. Mai. 2013. Metabolic syndrome and incidence of asthma in adults: the HUNT study. *Eur.Respir.J.* 42:1495-1502.
39. Bolton, C. E., M. Evans, A. A. Ionescu, S. M. Edwards, R. H. Morris, G. Dunseath, S. D. Luzio, D. R. Owens, and D. J. Shale. 2007. Insulin resis tance and inflammation - A further systemic complication of COPD. *COPD.* 4:121-126.
40. Lin, W. Y., C. A. Yao, H. C. Wang, and K. C. Huang. 2006. Impaired lung function is associated with obesity and metabolic syndrome in adults. *Obesity.(Silver.Spring)* 14:1654-1661.
41. McKeever, T. M., P. J. Weston, R. Hubbard, and A. Fogarty. 2005. Lung function and glucose metabolism: an analysis of data from the Third National Health and Nutrition Examination Survey. *Am.J.Epidemiol.* 161:546-556.
42. Yeh, H. C., N. M. Punjabi, N. Y. Wang, J. S. Pankow, B. B. Duncan, C. E. Cox, E. Selvin, and F. L. Brancati. 2008. Cross-sectional and prospective study of lung function in adults with type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care* 31:741-746.
43. Davis, W. A., M. Knuiman, P. Kendall, V. Grange, and T. M. Davis. 2004. Glycemic exposure is associated with reduced pulmonary function in type 2 diabetes: the Fremantle Diabetes Study. *Diabetes Care* 27:752-757.
44. Ofulue, A. F., K. Kida, and W. M. Thurlbeck. 1988. Experimental diabetes and the lung. I. Changes in growth, morphometry, and biochemistry. *Am.Rev.Respir.Dis.* 137:162-166.
45. Ofulue, A. F. and W. M. Thurlbeck. 1988. Experimental diabetes and the lung. II. In vivo connective tissue metabolism. *Am.Rev.Respir.Dis.* 138:284-289.
46. Brownlee, M., H. Vlassara, and A. Cerami. 1984. Nonenzymatic glycosylation and the pathogenesis of diabetic complications. *Ann.Intern.Med.* 101:527-537.
47. Perriott, L. M., T. Kono, R. R. Whitesell, S. M. Knobel, D. W. Piston, D. K. Granner, A. C. Powers, and J. M. May. 2001. Glucose uptake and metabolism by cultured human skeletal muscle cells: rate-limiting steps. *Am.J.Physiol Endocrinol.Metab.* 281:E72-E80.
48. Gelfand, R. A. and E. J. Barrett. 1987. Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J.Clin. Invest* 80:1-6.
49. Abbatecola, A. M., L. Ferrucci, G. Ceda, C. R. Russo, F. Lauretani, S. Bandinelli, M. Barbieri, G. Valenti, and G. Paolisso. 2005. Insulin resistance and muscle strength in older persons. *J.Gerontol.A Biol.Sci.Med.Sci.* 60:1278-1282.
50. Sillanpaa, E., L. Stenroth, A. Y. Bijlsma, T. Rantanen, J. S. McPhee, T. M. Maden-Wilkinson, D. A. Jones, M. V. Narici, H. Gapeyeva, M. Paasuke, et al. 2014. Associations between muscle strength, spirometric pulmonary function and mobility in healthy older adults. *Age (Dordr.)* 36:9667.

51. Rosenstock, J., W. T. Cefalu, P. A. Hollander, S. S. Klioze, J. Reis, and W. T. Duggan. 2009. Safety and efficacy of inhaled human insulin (exubera) during discontinuation and readministration of therapy in adults with type 2 diabetes: a 3-year randomized controlled trial. *Diabetes Technol. Ther.* 11:697-705.
52. Singh, S., M. Bodas, N. K. Bhatraju, B. Pattnaik, A. Gheware, P. P. Kolumam, M. A. Thompson, M. Freeman, U. Mabalirajan, R. Gosens, et al. 2016. Hyperinsulinemia Adversely Affects Lung Structure and Function. *Am.J.Physiol Lung Cell Mol. Physiol*ajplung.
53. Leone, N., D. Courbon, F. Thomas, K. Bean, B. Jego, B. Leynaert, L. Guize, and M. Zureik. 2009. Lung function impairment and metabolic syndrome: the critical role of abdominal obesity. *Am.J.Respir.Crit Care Med.* 179:509-516.
54. Scott, H. A., P. G. Gibson, M. L. Garg, and L. G. Wood. 2011. Airway inflammation is augmented by obesity and fatty acids in asthma. *Eur.Respir.J.* 38:594-602.
55. Wood, L. G., M. L. Garg, and P. G. Gibson. 2011. A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma. *J.Allergy Clin.Immunol.* 127:1133-1140.
56. Ringseis, R., K. Eder, F. C. Mooren, and K. Kruger. 2015. Metabolic signals and innate immune activation in obesity and exercise. *Exerc.Immunol.Rev.* 21:58-68.
57. Ferrante, A. W., Jr. 2007. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. *J.Intern.Med.* 262:408-414.
58. Bastard, J. P., M. Maachi, C. Lagathu, M. J. Kim, M. Caron, H. Vidal, J. Capeau, and B. Feve. 2006. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur.Cytokine Netw.* 17:4-12.
59. Tchernof, A. and J. P. Despres. 2013. Pathophysiology of human visceral obesity: an update. *Physiol Rev.* 93:359-404.
60. Hamdy, O., S. Porramatikul, and E. Al-Ozairi. 2006. Metabolic obesity: the paradox between visceral and subcutaneous fat. *Curr.Diabetes Rev.* 2:367-373.
61. Despres, J. P. and I. Lemieux. 2006. Abdominal obesity and metabolic syndrome. *Nature* 444: 881-887.
62. Fox, C. S., J. M. Massaro, U. Hoffmann, K. M. Pou, P. Maurovich-Horvat, C. Y. Liu, R. S. Vasan, J. M. Murabito, J. B. Meigs, L. A. Cupples, et al. 2007. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation* 116:39-48.
63. Oka, R., J. Kobayashi, A. Inazu, K. Yagi, S. Miyamoto, M. Sakurai, K. Nakamura, K. Miura, H. Nakagawa, and M. Yamagishi. 2010. Contribution of visceral adiposity and insulin resistance to metabolic risk factors in Japanese men. *Metabolism* 59:748-754.
64. Lam, K. B., R. E. Jordan, C. Q. Jiang, G. N. Thomas, M. R. Miller, W. S. Zhang, T. H. Lam, K. K. Cheng, and P. Adab. 2009. Airflow obstruction and the metabolic syndrome: the Guangzhou Biobank Cohort Study. *Eur.Respir.J.*
65. Park, H. Y., S. Y. Lim, J. H. Hwang, J. H. Choi, W. J. Koh, J. Sung, G. Y. Suh, M. P. Chung, H. Kim, Y. H. Choe, et al. 2010. Lung function, coronary artery calcification, and metabolic syndrome in 4905 Korean males. *Respir.Med.*
66. Vernooy, J. H., N. E. Drummen, R. J. van Suylen, R. H. Cloots, G. M. Moller, K. R. Bracke, S. Zuyderduyn, M. A. Dentener, G. G. Brusselle, P. S. Hiemstra, et al. 2009. Enhanced pulmonary leptin expression in patients with severe COPD and asymptomatic smokers. *Thorax* 64:26-32.
67. Bruno, A., P. Chanez, G. Chiappara, L. Siena, S. Giammanco, M. Gjomarkaj, G. Bonsignore, J. Bousquet, and A. M. Vignola. 2005. Does leptin play a cytokine-like role within the airways of COPD patients? *Eur.Respir.J.* 26:398-405.
68. Sierra-Johnson, J., A. Romero-Corral, V. K. Somers, L. J. Olson, and B. D. Johnson. 2008. Leptin, a Novel Predictor of Lung Function in Heart Failure. *Chest*.
69. Lugogo, N. L., J. W. Hollingsworth, D. L. Howell, L. G. Que, D. Francisco, T. D. Church, E. N. Potts-Kant, J. L. Ingram, Y. Wang, S. H. Jung, et al. 2012. Alveolar macrophages from overweight/obese subjects with asthma demonstrate a proinflammatory phenotype. *Am.J.Respir.Crit Care Med.* 186:404-411.
70. Suzuki, M., H. Makita, J. Ostling, L. H. Thomsen, S. Konno, K. Nagai, K. Shimizu, J. H. Pedersen, H. Ashraf, P. L. Bruijnzeel, et al. 2014. Lower leptin/adiponectin ratio and risk of rapid lung function decline in chronic obstructive pulmonary disease. *Ann.Am.Thorac.Soc.* 11:1511-1519.
71. Sood, A., C. Qualls, M. Schuyler, B. Thyagarajan, M. W. Steffes, L. J. Smith, and D. R. Jacobs, Jr. 2012. Low serum adiponectin predicts future risk for asthma in women. *Am.J.Respir.Crit Care Med.* 186:41-47.
72. van Huisstede, A., A. Rudolphus, A. van Schadewijk, M. C. Cabezas, G. H. Mannaerts, C. Taube, P. S. Hiemstra, and G. J. Braunstahl. 2014. Bronchial and systemic inflammation in morbidly obese subjects with asthma: a biopsy study. *Am.J.Respir.Crit Care Med.* 190:951-954.
73. van Huisstede A., M. C. Cabezas, E. Birnie, G. J. van de Geijn, A. Rudolphus, G. Mannaerts, T. L. Njo, P. S. Hiemstra, and G. J. Braunstahl. 2013. Systemic inflammation and lung function impairment in morbidly obese subjects with the metabolic syndrome. *J.Obes.* 2013:131349.

74. Parikh, S. J., M. Edelman, G. I. Uwaifo, R. J. Freedman, M. Semega-Janneh, J. Reynolds, and J. A. Yanovski. 2004. The relationship between obe - sity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J.Clin.Endocrinol. Metab* 89:1196-1199.
75. Lagunova, Z., A. C. Porojnicu, F. Lindberg, S. Hexeberg, and J. Moan. 2009. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res.* 29:3713-3720.
76. Hannemann, A., B. H. Thuesen, N. Friedrich, H. Volzke, A. Steveling, T. Ittermann, K. Hegenscheid, M. Nauck, A. Linneberg, and H. Wallaschofski. 2015. Adiposity measures and vitamin D concentrations in Northeast Germany and Denmark. *Nutr.Metab (Lond)* 12:24.
77. Earthman, C. P., L. M. Beckman, K. Masodkar, and S. D. Sibley. 2012. The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. *Int.J.Obes.(Lond)* 36:387-396.
78. Piccolo, B. D., G. Dolnikowski, E. Seyoum, A. P. Thomas, E. R. Gertz, E. C. Souza, L. R. Woodhouse, J. W. Newman, N. L. Keim, S. H. Adams, et al. 2013. Association between subcutaneous white adipose tissue and serum 25-hydroxyvitamin D in overweight and obese adults. *Nutrients.* 5:3352-3366.
79. Wortsman, J., L. Y. Matsuoka, T. C. Chen, Z. Lu, and M. F. Holick. 2000. Decreased bioavailability of vitamin D in obesity. *Am.J.Clin.Nutr.* 72:690-693.
80. Niruban, S. J., K. Alagiakrishnan, J. Beach, and A. Senthilselvan. 2015. Association between vitamin D and respiratory outcomes in Canadian adolescents and adults. *J.Asthma*1-33.
81. Black, P. N. and R. Scragg. 2005. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. *Chest* 128:3792-3798.
82. Berry, D. J., K. Hesketh, C. Power, and E. Hypponen. 2011. Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br.J.Nutr.* 106:1433-1440.
83. Choi, C. J., M. Seo, W. S. Choi, K. S. Kim, S. A. Youn, T. Lindsey, Y. J. Choi, and C. M. Kim. 2013. Relationship between serum 25-hydroxyvitamin D and lung function among Korean adults in Korea National Health and Nutrition Examination Survey (KNHANES), 2008-2010. *J.Clin.Endocrinol.Metab* 98:1703-1710.
84. Afzal, S., P. Lange, S. E. Bojesen, J. J. Freiberg, and B. G. Nordestgaard. 2014. Plasma 25-hydroxyvitamin D, lung function and risk of chronic obstructive pulmonary disease. *Thorax* 69:24-31.
85. Prietl, B., G. Treiber, T. R. Pieber, and K. Amrein. 2013. Vitamin D and immune function. *Nutrients.* 5:2502-2521.
86. Gunville, C. F., P. M. Mourani, and A. A. Ginde. 2013. The role of vitamin D in prevention and treatment of infection. *Inflamm.Allergy Drug Targets.* 12:239-245.
87. Laaksi, I., J. P. Ruohola, V. Mattila, A. Auvinen, T. Ylikomi, and H. Pihlajamaki. 2010. Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double blinded trial among young Finnish men. *J.Infect.Dis.* 202:809-814.
88. Ginde, A. A., J. M. Mansbach, and C. A. Camargo, Jr. 2009. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch.Intern.Med.* 169:384 390.
89. Monlezun, D. J., E. A. Bittner, K. B. Christopher, C. A. Camargo, and S. A. Quraishi. 2015. Vitamin d status and acute respiratory infection: cross sectional results from the United States national health and nutrition examination survey, 2001-2006. *Nutrients.* 7:1933-1944.
90. Berry, D. J., K. Hesketh, C. Power, and E. Hypponen. 2011. Vitamin D status has a linear associ ation with seasonal infections and lung function in British adults. *Br.J.Nutr.* 106:1433-1440.
91. Nicholson, K. G., J. Kent, and D. C. Ireland. 1993. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 307:982-986.
92. Seemungal, T., R. Harper-Owen, A. Bhowmik, I. Moric, G. Sanderson, S. Message, P. Maccallum, T. W. Meade, D. J. Jeffries, S. L. Johnston, et al. 2001. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am.J.Respir.Crit Care Med.* 164:1618-1623.
93. Papi, A., C. M. Bellettato, F. Braccioni, M. Romagnoli, P. Casolari, G. Caramori, L. M. Fabbri, and S. L. Johnston. 2006. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am.J.Respir.Crit Care Med.* 173:1114-1121.
94. Anthonisen, N. R., E. C. Wright, and J. E. Hodgkin. 1986. Prognosis in chronic obstructive pulmonary disease. *Am.Rev.Respir.Dis.* 133:14-20.
95. Jousilahti, P., E. Vartiainen, J. Tuomilehto, and P. Puska. 1996. Symptoms of chronic bronchitis and the risk of coronary disease. *Lancet* 348:567-572.
96. Krishnan, V., G. B. Diette, C. S. Rand, A. L. Bilderback, B. Merriman, N. N. Hansel, and J. A. Krishnan. 2006. Mortality in patients hospitalized for asthma exacerbations in the United States. *Am.J.Respir.Crit Care Med.* 174:633-638.

97. Lehouck, A., C. Mathieu, C. Carremans, F. Baeke, J. Verhaegen, E. J. Van, B. Decallonne, R. Bouillon, M. Decramer, and W. Janssens. 2012. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. *Ann.Intern.Med.* 156:105-114.
98. Martineau, A. R., W. Y. James, R. L. Hooper, N. C. Barnes, D. A. Jolliffe, C. L. Greiller, K. Islam, D. McLaughlin, A. Bhowmik, P. M. Timms, et al. 2015. Vitamin D3 supplementation in patients with chronic obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial. *Lancet Respir.Med.* 3:120-130.
99. Martineau, A. R., C. J. Cates, M. Urashima, M. Jensen, A. P. Griffiths, U. Nurmatov, A. Sheikh, and C. J. Griffiths. 2016. Vitamin D for the management of asthma. *Cochrane.Database.Syst.Rev.* 9:CD011511.
100. Hiemstra, P. S., G. D. Amatngalim, A. M. van der Does, and C. Taube. 2016. Antimicrobial Peptides and Innate Lung Defenses: Role in Infectious and Noninfectious Lung Diseases and Therapeutic Applications. *Chest* 149:545-551.
101. Beisswenger, C., K. Kandler, C. Hess, H. Garn, K. Felgentreff, M. Wegmann, H. Renz, C. Vogelmeier, and R. Bals. 2006. Allergic airway inflammation inhibits pulmonary antibacterial host defense. *J.Immunol.* 177:1833-1837.
102. Ong, P. Y., T. Ohtake, C. Brandt, I. Strickland, M. Boguniewicz, T. Ganz, R. L. Gallo, and D. Y. Leung. 2002. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N.Engl.J.Med.* 347:1151-1160.
103. Contoli, M., K. Ito, A. Padovani, D. Poletti, B. Marku, M. R. Edwards, L. A. Stanciu, G. Gnesini, A. Pastore, A. Spanevello, et al. 2015. Th2 cytokines impair innate immune responses to rhinovirus in respiratory epithelial cells. *Allergy* 70:910-920.
104. Wark, P. A., S. L. Johnston, F. Bucchieri, R. Powell, S. Puddicombe, V. Laza-Stanca, S. T. Holgate, and D. E. Davies. 2005. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J.Exp.Med.* 201:937-947.
105. Zasloff, M. 2002. Antimicrobial peptides of multicellular organisms. *Nature* 415:389-395.
106. Kalfa, V. C., S. L. Spector, T. Ganz, and A. M. Cole. 2004. Lysozyme levels in the nasal secretions of patients with perennial allergic rhinitis and recurrent sinusitis. *Ann.Allergy Asthma Immunol.* 93:288-292.
107. Xiao, W., Y. P. Hsu, A. Ishizaka, T. Kirikae, and R. B. Moss. 2005. Sputum cathelicidin, urokinase plasminogen activation system components, and cytokines discriminate cystic fibrosis, COPD, and asthma inflammation. *Chest* 128:2316-2326.
108. Wang, T. T., F. P. Nestel, V. Bourdeau, Y. Nagai, Q. Wang, J. Liao, L. Tavera-Mendoza, R. Lin, J. W. Hanrahan, S. Mader, et al. 2004. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J.Immunol.* 173:2909-2912.
109. Gombart, A. F., N. Borregaard, and H. P. Koeffler. 2005. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB J.* 19:1067-1077.
110. Schrumpf, J. A., M. A. van Sterkenburg, R. M. Verhoosel, S. Zuyderduyn, and P. S. Hiemstra. 2012. Interleukin 13 Exposure Enhances Vitamin D-Mediated Expression of the Human Cathelicidin Antimicrobial Peptide 18/LL-37 in Bronchial Epithelial Cells. *Infect.Immun.* 80:4485-4494.
111. Hata, T. R., P. Kotol, M. Jackson, M. Nguyen, A. Paik, D. Udall, K. Kanada, K. Yamasaki, D. Alexandrescu, and R. L. Gallo. 2008. Administration of oral vitamin D induces cathelicidin production in atopic individuals. *J.Allergy Clin.Immunol.* 122:829-831.
112. Misawa, Y., A. Baba, S. Ito, M. Tanaka, and M. Shiohara. 2009. Vitamin D(3) induces expression of human cathelicidin antimicrobial peptide 18 in newborns. *Int.J.Hematol.* 90:561-570.
113. Roes, S. D., R. A. Dehnavi, J. J. Westenberg, H. J. Lamb, B. J. Mertens, J. T. Tamsma, and R. A. de. 2011. Effect of lifestyle intervention plus rosiglitazone or placebo therapy on left ventricular mass assessed with cardiovascular magnetic resonance in the metabolic syndrome. *J.Cardiovasc.Magn Reson.* 13:65.
114. de,Mutsert. R., M. den Heijer, T. J. Rabelink, J. W. Smit, J. A. Romijn, J. W. Jukema, R. A. de, C. M. Cobbaert, M. Kloppenburg, C. S. le, et al. 2013. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur.J.Epidemiol.*
115. Bianchi, M. L., G. L. Ardissino, C. P. Schmitt, V. Dacco, L. Barletta, A. Claris-Appiani, and O. Mehls. 1999. No difference in intestinal strontium absorption after an oral or an intravenous 1,25(OH)2D3 bolus in normal subjects. For the European Study Group on Vitamin D in children with renal failure. *J.Bone Miner.Res.* 14:1789-1795.
116. Papapoulos, S. E., T. L. Clemens, L. M. Sandler, L. J. Fraher, J. Winer, and J. L. O'Riordan. 1982. The effect of renal function on changes in circulating concentrations of 1,25-dihydroxycholecalciferol after an oral dose. *Clin.Sci.(Lond)* 62:427-429.



CHAPTER

2

Association of lung function measurements and visceral fat in men with the metabolic syndrome.

Willemien Thijs [1], Reza Alizadeh Dehnavi[2], Pieter S. Hiemstra[1], Albert de Roos[3], Christian F. Melissant [4], Kirsten Janssen[1], Jouke T. Tamsma [2] and Klaus F. Rabe [5].

Department of Pulmonology[1], Department of General Internal Medicine and Endocrinology[2] and Department of Radiology[3], Leiden University Medical Centre, Leiden, the Netherlands

Department of Pulmonology[4], Spaarne Hospital, Hoofddorp, the Netherlands

Department of Pulmonology and Thoracic Surgery[5], Krankenhaus Grosshansdorf, Grosshansdorf, Germany

Respiratory Medicine. 2014 Feb;108(2):351-7.

ABSTRACT

Background
Several studies have reported a positive relationship between lung function impairment and the metabolic syndrome. This is most usually explained by abdominal adiposity.
We hypothesized that the main determinant of the association between lung function impairment and abdominal obesity is the presence of visceral fat.

Methods
The present study is a cross-sectional analysis of 98 non-diabetic men aged between 50–70 years with the metabolic syndrome. The amount of visceral and subcutaneous adipose tissue was determined by an MRI scan. The association between visceral fat and measures of lung function (FEV_1, FVC, exhaled and NO) was assessed using linear regression.

Results
98 participants were included in this analysis. There was a linear inverse association between visceral fat and both FEV_1 and FVC. None of the other different fat-related measurements (subcutaneous fat, waist circumference and BMI) or features of the metabolic syndrome were found to be associated with these lung function measurements

Conclusion
In non diabetic subjects with the metabolic syndrome and a lung function that is within the normal range, visceral fat is negatively correlated with FEV_1 and FVC.

INTRODUCTION

Obesity is increasing and contributes to the overall burden of disease worldwide[1].
The prevention of this chronic disease is one of the priorities of the World Health Organization [2]. In population studies there is an association between reduced FEV_1 and cardiovascular events [3;4]. In addition, it is established that obesity is associated with reduced lung volumes [5].

In obese patients the distribution of fat appears to be an important contributing factor to morbidity and healthy survival [6;7]. Especially an increase in visceral fat is associated with diabetes and the metabolic syndrome [8;9]. Various studies have found an association between type 2 diabetes mellitus and impaired lung function[10-14]. Furthermore, in large population based studies a positive relationship has been found between lung function impairment and features of the metabolic syndrome, predominantly abdominal adiposity. [15-17]

Since subcutaneous fat and visceral fat differ in composition and function, and both contribute to abdominal obesity[18], it is relevant to establish the contribution of each to the association between abdominal obesity and lung function. Recently, it has become clear that adipocytes present in visceral fat produce more pro-inflammatory mediators than adipocytes present in subcutaneous fat[19]. In view of the major role of inflammation in lung function impairment[20], visceral fat could contribute to decreased lung function in central obesity by a different mechanism than the mechanical factors that have been suggested to explain the association between abdominal obesity and decreased lung volumes.

We therefore hypothesized that in subjects with abdominal obesity the main determinant of lung function impairment is the presence of visceral fat. Therefore, it is important to gain insight in the association between visceral fat and lung function impairment. Since measurement of waist circumference does not allow us to estimate the amount of visceral fat, we[21;22] used Magnetic Resonance Imaging (MRI), an imaging technique that allows the direct measurement of visceral and subcutaneous fat distribution.

We selected a male study group with the metabolic syndrome according to the International Diabetes Federation[23] but without overt diabetes to minimize confounding by diabetes or gender (fat distribution and hormonal differences). The aim of the present study was to examine the association of visceral fat as measured by MRI and lung function in non-diabetic men with the metabolic syndrome.

MATERIALS AND METHODS

We conducted a cross-sectional analysis of 98 male subjects aged between 50–70 years with the metabolic syndrome (defined according to the International Diabetes Federation criteria but without diabetes). This study was an addendum to a trial study. The inclusion criteria for the participants were: a waist circumference > 94 cm and at least two other metabolic syndrome criteria: TG ≥1.7 mmol/L, HDL-chol: < 1.03 mmol/l, blood pressure ≥130 / ≥ 85mm Hg. Exclusion criteria were the presence of type 2 diabetes, overt cardiovascular disease, use of statins or fibrates, and a BMI >40 kg/m^2. For this study we used the measurements taken at the end of the trial.

This study was an addendum to a double-blind placebo controlled randomized trial, testing the hypothesis that rosiglitazone 8 mg (4 mg bd) would prevent progression of atherosclerosis more than placebo in visceral obese male subjects with systemic inflammation. This was defined by hs-CRP levels higher than 1.8 mg/L and in their control patients with the metabolic syndrome and a hs-CRP lower then 1.8 mg/L.
After the placebo-controlled trial was finished 110 patients were invited a visit the lung department and 98 patients agreed to a lung function analysis which was performed after informed consent. The addendum to the protocol was approved by the local review board (LUMC, 14-5-2007 Protocol P04.232).

Measurements

Clinical assessments

Clinical history, physical examination including blood pressure measurements, and anthropometry, and laboratory assessments were performed at the clinical research unit at the end of the trial. Blood pressure was recorded in supine position after 15 minutes rest. Blood pressure was defined as the mean value of 3 measurements taken with intervals of at least 2 minutes. Body weight and body length were measured. Waist circumference was measured in a horizontal plane between the lowest costal margin and the upper pelvic rim. Hip circumference is measured at the level of the major trochanters. Circumferences were recorded in centimetres.

Laboratory measurements included fasting glucose, triglyceride, total- and HDL-cholesterol and hs-CRP levels, measured at the department of clinical chemistry.

MRI measurements

Subjects were positioned in the magnet in a supine position. The body coil was used for obtaining the images. A sagittal single shot gradient echo sequence survey scan was used for the imaging of the vertebral column in the lumbar region. Subsequently, a second single shot gradient echo sequence in the transversal plane was used for obtaining three contiguous slices of 10 mm without angulations with the following parameters: echo time 3.7 ms (TE), repetition time7.5 ms (TR), pulse angle 45°. Two signal averages were performed.
The slices were centred at the intervertebral disk level between the fourth and fifth lumbar vertebra. The images were obtained with three breath holds of 6 s. The field of view was 500 mm. A voxel size of 1 mm × 1.3 mm × 10 mm was obtained. The measurements were taken at the end of the trial and images were assessed using the MASS software package allowing a semi-automated detection of subcutaneous and visceral adipose tissue area.

Flow volume curve and reversibility

Flow-volume curves were recorded, after the placebo controlled Rosiglitazone trial ended, by pneumotachograph to obtain the vital capacity (VC), expiratory rest volume (ERV), forced expiratory volume in 1 second (FEV_1) and the forced vital capacity (FVC). To test whether the obstruction is reversible to bronchodilators, FEV_1 and FVC (absolute values and percentage of predicted values) were measured before and 15 minutes after four single inhalations of 100 mcg albuterol administered through a large volume spacer.

Exhaled NO

Exhaled NO measurements were measured with a chemiluminescence analyzer (Aerocrine AB, Niox, Solna, Sweden) according to a standardized procedure. After inhaling NO-free air, the subjects performed a slow expiratory vital capacity maneuver with a constant expiratory flow of 50ml/s against a resistance of 10 cm H_2O using online visual monitoring. Exhaled NO concentrations were determined at a 3 second-plateau and expressed as parts per billion (ppb). If the chemiluminescence analyzer was not available exhaled nitric oxide was measured using a portable analyzer, the NIOX MINO (Aerocrine AB, Solna, Sweden) following the manufacturer's instructions.

Subjects performed a 10 s slow steady exhalation, which was assisted by visual and audio biofeedback systems located on the device. The two methods used give comparable results [24]. Three successive recordings at 1-minute intervals were made and the mean exhaled NO was used. Exhaled NO was considered to be elevated above 25 ppb[25]

Statistical analyses

The data are presented as median and 25th and 75th percentile, or percentage. Triglycerides, exhaled nitric oxide and hs-CRP values were log transformed for statistical analysis due to their non-normal distribution. Linear regression was used to asses the association between lung function and the different features of fat and metabolic syndrome. Adjustments were made for age, pack years, hs-CRP, rosiglitazone, glucose, cholesterol and blood pressure.

A restriction analysis that excluded participants with decreased lung function was performed. Results were considered significant at $p<0.05$ and the data were analyzed using the Statistical Package of Social Science (SPSS) version 20.0.

RESULTS

All analyses were conducted on 98 participants. We included 81 men with metabolic syndrome and CRP levels higher than 1.8 mg/L (of which 40 participants used Rosiglitazone) and 17 control patients with the metabolic syndrome and hs-CRP levels lower than 1.8 mg/L
The characteristics of the study population after the trial are presented in Table 2.1.
Most patients were found to have no significant airway obstruction and no or little airway inflammation (as assessed by exhaled nitric oxide measurement), data presented in Table 2.2.

Table 2.1 Baseline characteristics

	All participants	Participants in Rosiglitazone arm trial	Participants in Placebo arm trial	Participants in control arm with lower Hs-CRP
Age (years)	62 (57-64)	62 (57-64)	62 (59-65)	61 (57-64)
PY (years)	15 (0.3-29)	16 (2-30)	15 (1,5-27)	7 (0-21)
BMI (kg/m^2)	28.9 (26.4-30.7)	28.1 (26.5-30.7)	28.1 (26.1-30.8)	27.8 (26.3-30.8)
BP Systolic (mmHg)	139 (129 -152)	137 (127-144)	134 (125-149)	149 (137-164)
BP Diastolic (mmHg)	82 (74 -88)	79 (63-68)	80 (74-84)	92 (84-97)
Waist circumference (cm)	102 (96-109)	100 (94-112)	100 (96-108)	104 (101-109)
Glucose (mmol/l)	5.0 (4.6-5.3)	4.9 (4.4-5.3)	4.9 (4.6-5.4)	5.2 (4.7-5.4)
Triglyceride (mmol/l)	1.7 (0.8-2.1)	1.2 (0.8-1.8)	1.2 (0.8-2.1)	2.6 (1.5-3.5)
HDL-Cholesterol (mmol/l)	1.4 (1.1-1.6)	1.5 (1.2-1.7)	1.3 (1.1-1.5)	1.1 (1.0-1.2)
Hs-CRP (mg/l)	1.4 (0.7-2.6)	1 (0.6-2.5)	2.2 (1.4-3.1)	0.9 (0.6-1.3)
Waist visceral fat (cm^2)	343 (288-486)	338 (272-575)	328 (287-436)	386 (300-502)
Waist subcutaneous fat (cm^2)	700 (610-885)	736 (623-911)	739 (596-914)	649 (520-807)

Values are median (25th, 75th percentiles). PY is pack years, BMI is body mass index and BP is blood pressure

Table 2.2 Pulmonary function

	All participants	Participants in Rosiglitazone arm trial	Participants in Placebo arm trial	Participants in control arm with lower Hs-CRP
FEV_1 post pred (%)	105 (96-118)	104 (92-114)	106 (97-127)	109 (91-124)
FVC post pred (%)	108 (95-118)	106 (94-113)	111 (98-121)	109 (93-117)
FEV_1/FVC (%)	79 (73- 84)	78 (73- 83)	82 (73- 85)	79 (73- 83)
VC (%)	109 (96-118)	108 (96-115)	109 (100-121)	110 (97-123)
ERV (%)	88 (61-124)	85 (56-115	97 (68-128)	94 (62-129)
NO (ppb)	16 (11- 24)	15 (9- 28)	17 (11- 22)	15 (12- 27)

Values are means, median (25th, 75th percentiles). FEV_1 is forced expiratory volume in 1 s, FVC is forced vital capacity post predicted, VC is vital capacity post predicted, ERV is expiratory reserve volume and NO is exhaled Nitric oxide.

In the linear regression no association was found between any of the features of the metabolic syndrome and FEV_1, FVC and exhaled nitric oxide. Rosiglitazone use was also not correlated with any of these lung function measurements. However, whereas BMI and features of the metabolic syndrome, including waist circumference, were not associated with FEV_1 and FVC there was a significant association between waist visceral fat, and FEV_1 (beta -0.023; 95% CI -0.041, -0.006); this means that an additional 200 cm^2 of visceral fat (the IQR within this study) is associated with a 4.6 % decrease in FEV_1 predicted. There was also an association with FVC (beta -0.024; 95% CI -0.040, -0.008), but not between subcutaneous fat and FEV_1 and FVC (Figure 2.1 and 2.2).
After adjustment for waist subcutaneous fat, age, height, waist circumference, pack years, rosiglitazone use, hs-CRP, glucose, cholesterol, triglyceride and blood pressure the association between visceral fat and FEV_1 (beta -0.055; 95% CI -0.085, -0.025) and FVC (beta -0.040; 95% CI -0.067, -0.014) remained.
There was also a significant association between waist visceral fat and ERV (beta -0.108; 95% CI -0.157, -0.058) and waist subcutaneous fat and ERV (beta -0.070; 95% CI -0.108, -0.032). After adjustment for age, pack years, rosiglitazone use, hs-CRP, glucose, cholesterol and blood pressure the association between visceral fat and ERV (beta -0.116; 95% CI -0.181, -0.052) and subcutaneous fat and ERV (beta -0.059; 95% CI -0.105, -0.014) remained.
To asses whether the association between visceral fat and FEV_1 and FVC is explained by ERV we explored the association. ERV was associated with FEV_1 (beta 1.1; 95% CI 0.6, 1.6) and FVC (beta 1.4; 95% CI 0.8, 1.9). Eight participants had a FEV_1 below 80% excluding these participants did not alter these results.

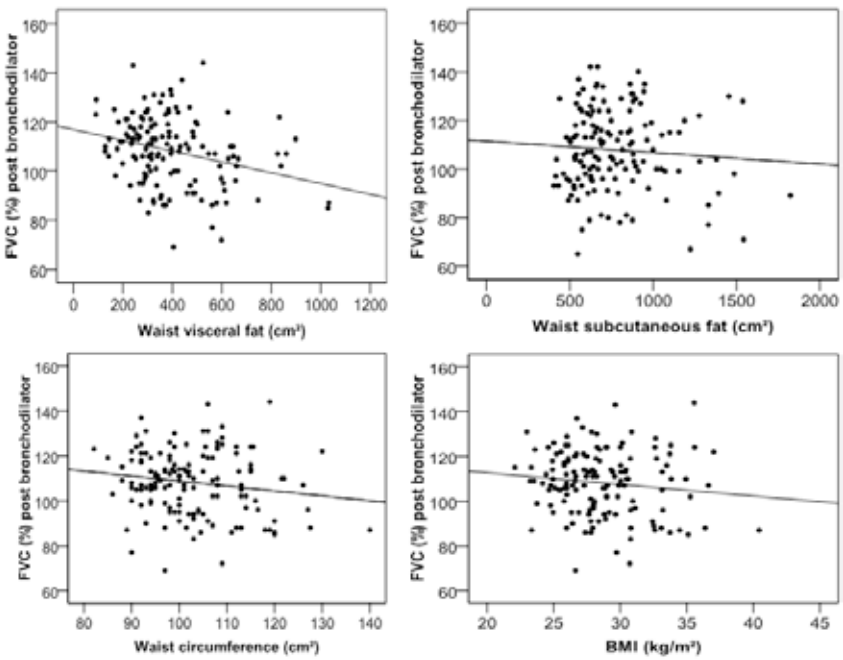


Figure 2.1: The correlation be 428 tween visceral fat (cm^2), subcutaneous fat (cm^2), waist circumference (cm), BMI (kg/m^2) and FEV_1 (%).
The Pearson correlation: visceral fat (cm^2) and FEV_1 (%) r= -0.180 p= 0.038, subcutaneous fat (cm^2) and FEV_1 (%) r= 0.009 p= 0.463, waist circumference (cm) and FEV_1 (%) r= 0.037 p= 0.357, BMI (kg/m^2) and FEV_1 (%) r= -0.027 p= 0.401.

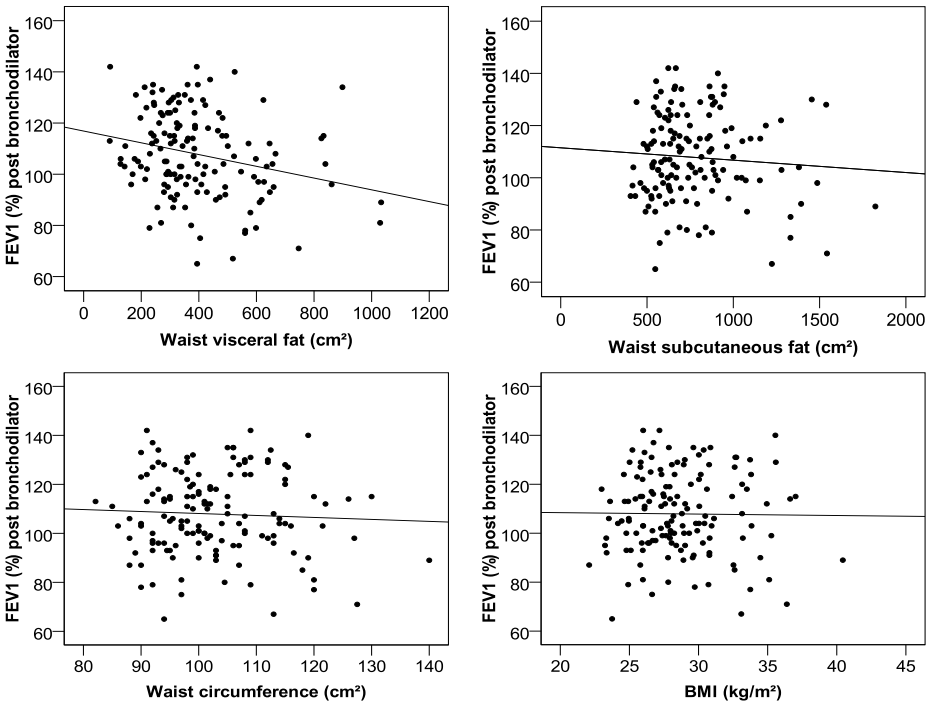


Figure. 2.2 The correlation be 428 tween visceral fat (cm^2), subcutaneous fat (cm^2), waist circumfere The correlation between visceral fat (cm^2), subcutaneous fat (cm^2), waist circumference (cm), BMI (kg/m^2) and FVC (%).
The Pearson correlation between: visceral fat (cm^2) and FVC (%) r= -0.200 p= 0.025, subcutaneous fat (cm^2) and FVC (%) r= -0.069 p= 0.251, waist circumference (cm) and FVC (%) r= -0.038 p= 0.355, BMI (kg/m^2) and FVC (%) r= -0.031 p= 0.387.

Waist subcutaneous fat had a stronger association (beta 19.6; 95% CI 16.1, 23.2) with waist circumference than visceral fat (beta 13.6; 95% CI 11.1, 16.2). Whereas hs-CRP showed a weak negative association with FEV_1, after correction for waist visceral fat this was no longer significant. No association was found between log hs-CRP and FVC and exhaled nitric oxide.

DISCUSSION

The results of this study show that in men at an early stage of the metabolic syndrome (without overt diabetes) and a normal lung function there is a significant linear inverse relation between visceral, but not subcutaneous fat, and FEV_1 and FVC. In contrast, there was no correlation between subcutaneous fat (or other fat measurements) and FEV_1 and FVC. Exhaled nitric oxide was not correlated with the metabolic syndrome and the fat measurements. This indicates that in non diabetic subjects with the metabolic syndrome, visceral fat appears to be a more sensitive parameter to assess the association between obesity and lung function impairment than subcutaneous fat or waist circumference.
Our results are in line with those published by Leone et al [16]and by Lam et al [15], who reported a positive relationship between lung function impairment and metabolic syndrome, which was explained mainly by abdominal obesity and was independent of the body mass index.

We did not find such an association with abdominal obesity, which might be because our study focused on subjects with a high waist circumference and the other studies were population-based studies. We did find a significant association with visceral fat, which may be explained by the fact that waist circumference is predominantly an index of abdominal subcutaneous fat, and not of visceral fat [21]. Indeed, in our study the relation between subcutaneous fat and waist circumference was stronger than the association between visceral fat and waist circumference.

How do we interpret the present findings? Obesity may limit lung expansion due to the mechanical pressure of the abdomen and cause restriction. Although we can not formally exclude that the association between visceral fat and lung function impairment is also explained by mechanical factors, it is important to note that in this small selected group no relation with waist circumference or BMI was found. Therefore, visceral fat appears to be a more selective marker in the relationship of abdominal obesity and lung function impairment.
This higher selectivity may be explained by the observation that adipocytes present in visceral fat are a more important source of pro-inflammatory mediators than adipocytes present in subcutaneous fat[19]. In some studies, visceral fat was found to be correlated with levels of CRP, which is mainly liver derived [26;27]. Although this indicates that increased inflammation resulting from an increase in visceral fat may contribute to lung function impairment, it needs to be noted that we did not find a correlation between markers of inflammation (exhaled NO and hs-CRP) and visceral fat. However, we did not measure other (adipocyte-derived) pro-inflammatory mediators such as leptin that may be related to visceral fact.

Our study was subject to some limitations. Firstly, the majority of subjects were recruited after finishing a randomized controlled trial with Rosiglitazone, and therefore half of the subjects received Rosiglitazone 8 mg during a year previous to our lung function measurements. However, it is unlikely that this treatment affected the outcome of the present study, since similar results were found after statistical adjustment for use of Rosiglitazone. Secondly, whereas the patients that participated in the Rosaglitazone trial all were characterized by a Hs-CRP > 1.8 mg/L, an additional group was studied with metabolic syndrome but a Hs-CRP < 1.8 mg/L. Furthermore, we used the measurements taken after the trial which were closer to each other. This resulted in a study group with heterogeneous CRP levels. This is unlikely to have influenced our results because these were identical after adjustment for Hs-CRP. Finally, due to the cross-sectional design of our study no follow-up lung-function data or parameters of the metabolic syndrome were available.

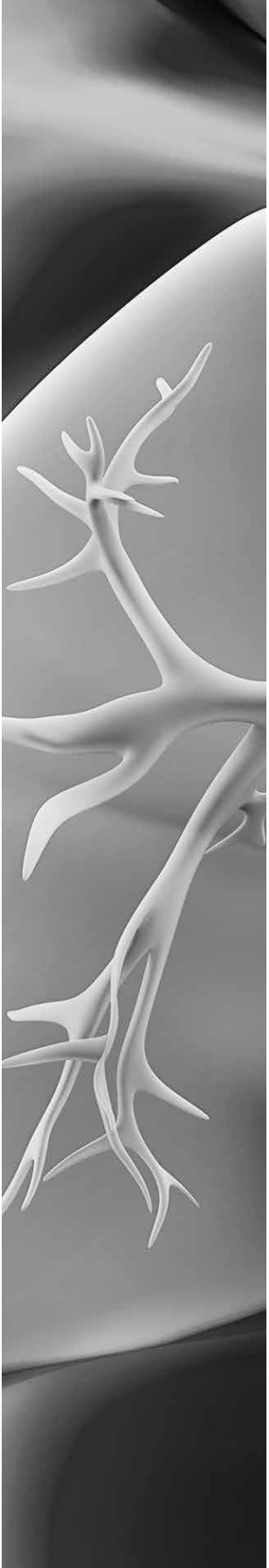
The observed association between visceral fat and lung function measurement impairment was found despite the fact that the study subjects all had increased waist circumference (and therefore likely increased visceral fat) and a lung function that was within a normal range. Further studies are needed to clarify the link between visceral fat, inflammation and lung function impairment and should include study groups with a larger range of visceral fat and lung function. We hypothesize that in such groups the observed association may even be stronger. In addition, measurements of a range of adipocyte-derived pro-inflammatory mediators such as leptin would contribute to our understanding.

In conclusion, we have shown an association between lung function impairment and visceral fat in non diabetic men with the metabolic syndrome and a normal range of lung function. The observation that such an association with lung function impairment was not found with other parameters of obesity indicates that visceral fat is a more sensitive parameter to assess the association between abdominal obesity (a component of the metabolic syndrome) and lung function impairment.

REFERENCE LIST

1. Field, A. E., E. H. Coakley, A. Must, J. L. Spadano, N. Laird, W. H. Dietz, E. Rimm, and G. A. Colditz. 2001. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. *Arch.Intern.Med.* 161:1581-1586.
2. World Health Organization and WHO global report. Preventing chronic diseases: a vital investment. 2010. Ref Type: Generic
3. Hole, D. J., G. C. Watt, G. Davey-Smith, C. L. Hart, C. R. Gillis, and V. M. Hawthorne. 1996. Impaired lung function and mortality risk in men and women: findings from the Renfrew and Paisley prospective population study. *BMJ* 313:711-715.
4. Schroeder, E. B., V. L. Welch, D. Couper, F. J. Nieto, D. Liao, W. D. Rosamond, and G. Heiss. 2003. Lung function and incident coronary heart disease: the Atherosclerosis Risk in Communities Study. *Am.J.Epidemiol*. 158:1171-1181.
5. Jones, R. L. and M. M. Nzekwu. 2006. The effects of body mass index on lung volumes. *Chest* 130:827-833.
6. Lee, C. M., R. R. Huxley, R. P. Wildman, and M. Woodward. 2008. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. *J.Clin.Epidemiol*. 61:646-653.
7. Sun, Q., M. K. Townsend, O. I. Okereke, O. H. Franco, F. B. Hu, and F. Grodstein. 2009. Adiposity and weight change in mid-life in relation to healthy survival after age 70 in women: prospective cohort study. *BMJ* 339:b3796.
8. Fox, C. S., J. M. Massaro, U. Hoffmann, K. M. Pou, P. Maurovich-Horvat, C. Y. Liu, R. S. Vasan, J. M. Murabito, J. B. Meigs, L. A. Cupples, et al. 2007. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation* 116:39-48.
9. Oka, R., J. Kobayashi, A. Inazu, K. Yagi, S. Miyamoto, M. Sakurai, K. Nakamura, K. Miura, H. Nakagawa, and M. Yamagishi. 2010. Contribution of visceral adiposity and insulin resistance to metabolic risk factors in Japanese men. *Metabolism* 59:748 -754.
10. Bolton, C. E., M. Evans, A. A. Ionescu, S. M. Edwards, R. H. Morris, G. Dunseath, S. D. Luzio, D. R. Owens, and D. J. Shale. 2007. Insulin resistance and inflammation - A further systemic complication of COPD. *COPD*. 4:121-126.
11. Lin, W. Y., C. A. Yao, H. C. Wang, and K. C. Huang. 2006. Impaired lung function is associated with obesity and metabolic syndrome in adults. *Obesity*.(Silver.Spring) 14:1654-1661.
12. McKeever, T. M., P. J. Weston, R. Hubbard, and A. Fogarty. 2005. Lung function and glucose metabolism: an analysis of data from the Third National Health and Nutrition Examination Survey. *Am.J.Epidemiol*. 161:546-556.
13. Yeh, H. C., N. M. Punjabi, N. Y. Wang, J. S. Pankow, B. B. Duncan, C. E. Cox, E. Selvin, and F. L. Brancati. 2008. Cross-sectional and prospective study of lung function in adults with type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care* 31:741-746.
14. Davis, W. A., M. Knuiman, P. Kendall, V. Grange, and T. M. Davis. 2004. Glycemic exposure is associated with reduced pulmonary function in type 2 diabetes: the Fremantle Diabetes Study. *Diabetes Care* 27:752-757.
15. Lam, K. B., R. E. Jordan, C. Q. Jiang, G. N. Thomas, M. R. Miller, W. S. Zhang, T. H. Lam, K. K. Cheng, and P. Adab. 2009. Airflow obstruction and the metabolic syndrome: the Guangzhou Biobank Cohort Study. *Eur.Respir.J.*
16. Leone, N., D. Courbon, F. Thomas, K. Bean, B. Jego, B. Leynaert, L. Guize, and M. Zureik. 2009. Lung function impairment and metabolic syndrome: the critical role of abdominal obesity. *Am.J.Respir.Crit Care Med*. 179:509-516.
17. Park, H. Y., S. Y. Lim, J. H. Hwang, J. H. Choi, W. J. Koh, J. Sung, G. Y. Suh, M. P. Chung, H. Kim, Y. H. Choe, et al. 2010. Lung function, coronary artery calcification, and metabolic syndrome in 4905 Korean males. *Respir.Med.*
18. Hajer, G. R., T. W. van Haeften, and F. L. Visseren. 2008. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur.Heart J.* 29:2959-2971.
19. Lafontan, M. and J. Girard. 2008. Impact of visceral adipose tissue on liver metabolism. Part I: heterogeneity of adipose tissue and functional properties of visceral adipose tissue. *Diabetes Metab* 34:317-327.
20. Broekhuizen, R., E. F. Wouters, E. C. Creutzberg, and A. M. Schols. 2006. Raised CRP levels mark metabolic and functional impairment in advanced COPD. *Thorax* 61:17-22.
21. Bosy-Westphal, A., C. A. Booke, T. Blocker, E. Kossel, K. Goele, W. Later, B. Hitze, M. Heller, C. C. Gluer, and M. J. Muller. 2010. Measurement site for waist circumference affects its accuracy as an index of visceral and abdominal subcutaneous fat in a Caucasian population. *J.Nutr.* 140:954-961.
22. Berker, D., S. Koparal, S. Isik, L. Pasaoglu, Y. Aydin, K. Erol, T. Delibasi, and S. Guler. 2010. Compatibility of different methods for the measurement of visceral fat in different body mass index strata. *Diagn.Interv.Radiol.*

23. Alberti, K. G., P. Zimmet, and J. Shaw. 2006. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet.Med.* 23:469-480.
24. Alving, K., C. Janson, and L. Nordvall. 2006. Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respir.Res.* 7:67.
25. Taylor, D. R., M. W. Pijnenburg, A. D. Smith, and J. C. De Jongste. 2006. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 61:817-827.
26. Faber, D. R., Y. van der Graaf, J. Westerink, and F. L. Visseren. 2010. Increased visceral adipose tissue mass is associated with increased C-reactive protein in patients with manifest vascular diseases. *Atherosclerosis*.
27. Cartier, A., M. Cote, I. Lemieux, L. Perusse, A. Tremblay, C. Bouchard, and J. P. Despres. 2009. Sex differences in inflammatory markers: what is the contribution of visceral adiposity? Am.J. *Clin.Nutr.* 89:1307-1314.



CHAPTER

3

Insulin resistance and lung function in the general population: the NEO study

W. Thijs [1], R.A. Prein [1], S. le Cessie [2,3], M. den Heijer [2,4], E. de Koning [5], S. Middeldorp [6], K.F. Rabe[1,7], F.R. Rosendaal [2], P.S. Hiemstra [1], R. de Mutsert [2].

Department of Pulmonology[1], Leiden University Medical Centre, Leiden, The Netherlands
Department of Clinical Epidemiology[2], Leiden University Medical Center, Leiden, The Netherlands
Department of Medical Statistics and Bioinformatics[3], Leiden University Medical Center, Leiden, The Netherlands
Department of Endocrinology[4], VU Medical Center, Amsterdam, The Netherlands
Department of Nephrology[5], Leiden University Medical Center, Leiden, The Netherlands
Department of Vascular Medicine[6], Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
LungenClinic Grosshansdorf[7], Pulmonary Medicine, Grosshansdorf, Germany

Submitted

ABSTRACT

Background

It remains unclear whether insulin resistance and impaired lung function are causally related or merely common consequences of obesity.

Objective

Our objective was to examine the association between insulin resistance and lung function, while adjusting for confounding including confounding by body fat and systemic inflammation.

Design

This is a cross-sectional analysis of the baseline measurements of the Netherlands Epidemiology of Obesity (NEO) study, a population-based cohort of 6,671 participants aged 45 to 65 years. Baseline insulin and glucose concentrations were used to calculate the Homeostastis Model Assessment of Insulin Resistance (HOMA-IR). Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were assessed by spirometry and expressed as percentage predicted (%). We performed linear regression analyses with FEV_1 and FVC, as dependent variables against ^{10}log HOMA-IR adjusted for age, sex, waist circumference, total body fat, body mass index (BMI), smoking, obstructive lung diseases, ethnicity, education, use of bronchodilator and C-reactive protein.

Results

After exclusion of participants who used systemic corticosteroids (n=45), inhaled corticosteroids (n=444), or glucose lowering therapy (n=316), and those with missing data (n=619), in total 5,247 participants (44% men) were analysed, with a mean (SD) age of 56 (6) years, and BMI of 26.1 (4.2) kg/m^2. In the crude model, insulin resistance was associated with lung function (a 10-fold higher HOMA-IR corresponded to a decrease in FEV 1 (change of -7.5 % [95% CI: -9.6, -5.4] and a decrease in FVC % (change -11.6 % [-13.7, -9.5).
After adjustment for confounding factors, a small non-clinically relevant association remained (FEV_1: -2.2 % [-5.1, 0.6]; FVC: -3.3 % [-5.9, -0.8]). In particular adjustment for measures of adiposity attenuated the associations.

Conclusion

In this population-based study, the observed association between insulin resistance and lung function was mainly explained by adiposity. Our results suggest that insulin resistance and impaired lung function are merely a common consequences of obesity.

INTRODUCTION

Obesity is a world-wide public health problem and well-established risk factor for major chronic diseases such as cardiovascular disease, certain cancers,[1-4] insulin resistance[5] and type 2 diabetes.[6] In addition, several studies have reported associations between obesity and lung function.[7-12]

Traditionally, a lower lung function in persons with obesity has been considered as a purely mechanical consequence.[13-15] Possible explanations for this decrease in lung function are decreased pulmonary muscle strength,13 pressure of abdominal fat on the diaphragm and restricted expansion of the thorax cavity due to excessive adipose tissue.[14;15] More recent hypotheses suggest the involvement of adipose tissue-mediated inflammation through release of inflammatory cytokines such as TNF-α and IL-6, and the activity of NF-kappa-B.[16] C-reactive protein (CRP) is a marker of chronic inflammation produced by the liver in response to inflammatory meditators including those secreted by adipose tissue, and CRP levels have been associated with a decrease in lung function.[17;18] In addition, the production of CRP has been linked to decreased insulin sensitivity.[19-22]

Several studies indicate that both insulin resistance[23;24] and type 2 diabetes[25-27] are associated with a decreased lung function. It has been hypothesized that such a decrease is the result of loss in muscle strength resulting from insulin resistance.[28] Insulin plays a role in glucose uptake, and is involved in muscle contraction[29] and protein catabolism, necessary for liberating amino acids for muscle synthesis.[30] A study performed in 1,429 young adults in the National Health and Nutrition Examination Survey observed a more pronounced inverse association between insulin resistance and lung function in individuals with overweight or obesity than in individuals with normal weight.[31]

It remains unclear whether insulin resistance and impaired lung function are merely unrelated consequences of excess body fat, or whether they are causally related. Therefore, the aim of this study was to investigate the association between insulin resistance and lung function in a general population, and to what extent this association is explained by body fat and systemic inflammation.

MATERIALS AND METHODS

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in 6,671 men and women aged 45 to 65 years, with an oversampling of persons with a body mass index (BMI) of 27 kg/m^2 or higher. Between September 2008 and September 2012 the study included individuals from the region of Leiden (in the West of The Netherlands) with a self-reported BMI of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI.

At the baseline of the study, information on demography, lifestyle and medical history has been collected by questionnaires. Participants were asked to bring all their current medication that they used in the month preceding the study visit to the study centre, which was recorded by research nurses. Participants underwent an extensive physical examination, including anthropometry, blood sampling and spirometry. More detailed information about the study design and data collection has been described previously.[32]

The present study is a cross-sectional analysis of the baseline measurements of the NEO study. From the 6,671 participants, we excluded all participants who were using systemic corticosteroids (n= 45), inhaled corticosteroids (n=145), a combination preparation of corticosteroids (n=299) or medication for diabetes (oral hypoglycemic agents or insulin) (n=316). We furthermore excluded participants if they had missing data on one or more of the following: lung function (n=182), blood insulin concentration (n=40), fasting glucose concentration (n=22), waist circumference (n=1), total body fat (n=27), number of packyears smoked (n=111), ethnicity (n=7), educational level (51), diabetes status (n=174), or self-reported asthma and COPD (n=4), leaving 5,247 participants for the analyses.

The study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and all participants gave written informed consent.

Data collection

On the baseline questionnaire, participants reported ethnicity by self-identification in eight categories which we grouped into Caucasian and other. Tobacco smoking was reported and pack-years were calculated as the number of packs of cigarettes smoked per day multiplied by the number of years as a smoker. In addition, participants were subdivided into three categories: current smoker, former smoker and never smoker. Highest level of education was reported in 10 categories according to the Dutch education system and grouped into high versus low education. Participants reported their medical history of asthma, chronic obstructive pulmonary disease (COPD) and diabetes. At the study site, height and weight were measured without shoes and one kilogram was subtracted to correct for the weight of clothing. BMI was calculated by dividing the weight in kilograms by the height in meters squared. Waist circumference was measured between the border of the lower costal margin and the iliac crest with the precision of 0.1 cm. Total body fat was estimated with a bio-impedance device (TBF-310, Tanita International Division, United Kingdom).

Blood sampling

After participants rested for 5 minutes, fasting blood samples were drawn from the antecubal vein. Fasting plasma glucose concentrations were determined by enzymatic and colorimetric methods (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany; CV < 5%) and serum insulin concentrations were determined by an immunoassay (Siemens Immulite 2500, Siemens Healthcare Diagnostics, Breda, The Netherlands; CV < 5%). All analyses were performed in the clinical chemistry laboratory of the LUMC [32].

As a measure of insulin resistance we calculated the homeostasis model assessment of insulin resistance (HOMA-IR) as fasting glucose (mg/dl) * fasting insulin (μU/mL) / 22.5.[33]

Serum CRP concentrations at baseline were determined at the same laboratory using a commercial immunoturbidimetric assay with a detection limit of 3 mg/l. The between-assay coefficient of variation (CV) was 1.8%. The within-run CV was 1.8%, run-to-run CV 1.7% and day-to-day CV 2.8%.

Lung function

All participants underwent spirometry to determine forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC). Participants were required to perform at least three forced expiratory manoeuvers. The highest FEV_1 value with acceptable curves was used in the analyses. FEV_1 and FVC were expressed in litres and as a percentage of the predicted values (%pred) of individuals with similar characteristics (height, age, sex).[34] If no proper curve could be produced (i.e. because of a missing peak at exhalation, lack of extended exhalation or continuous inhalation during the test), lung function was defined as missing.

Statistical analyses

In the NEO study there is an oversampling of persons with a BMI of 27 kg/m^2 or higher. To correctly represent associations in the general population,[35] adjustments for the over sampling of individuals with a BMI ≥ 27 kg/m^2 were made. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality,[36] whose BMI distribution was similar to the BMI distribution of the general Dutch population.[37] All results were based on weighted analyses. Consequently, the results apply to a population -based study without oversampling of participants with a BMI ≥ 27 kg/m^2.

Baseline characteristics of the weighted study population were expressed as mean (SD), or as percentage, stratified by quartiles of HOMA-IR. Linear regressions were performed with FEV_1% and FVC% as dependent variables. Because of a skewed distribution HOMA-IR was log transformed and ^{10}log HOMA-IR was used as a continuous independent variable. Regression coefficients and corresponding 95% confidence intervals (CI) can be interpreted as change in FEV_1% or FVC% for a tenfold increase in HOMA-IR.

First, the crude association between HOMA-IR and the measurements of lung function was examined. Second, this association was adjusted for age and sex. Third, the model was additionally adjusted for waist circumference and total body fat. Finally, the analyses were also adjusted for smoking (in packyears), asthma, COPD, use of bronchodilators, ethnicity, level of education, BMI and CRP.

To explore whether associations differ between persons with or without obesity, we tested the presence of an interaction between ^{10}log HOMA-IR and BMI by including an interaction term between ^{10}log HOMA-IR (continuous and in quartiles) and BMI (continuous and < or ≥ 30 kg/m^2) in the models. Subsequently we stratified the analyses by BMI according to the WHO BMI cut-offs for being normal (<25), overweight (25-30), obese (30-35), or morbidly obese (≥35).38 We repeated all analyses after exclusion of participants with obstructive airway disease and participants using glucose lowering therapy. Analyses were performed with STATA Statistical Software (Statacorp, College Station, Texas, USA), version 12.1.

RESULTS

Baseline characteristics

The present analysis included 5,247 participants (44% men) with a mean (SD) age of 56 (6) years, BMI of 26.1 (4.2) kg/m^2, FEV_1 of 108 (16) %pred, and FVC of 117 (16) %pred. Table 3.1 shows the characteristics of our study population by quartiles of HOMA-IR. There were more men in the higher quartiles of HOMA-IR. Mean BMI, waist circumference

and total body fat were higher in higher quartiles of HOMA-IR in both men and women. Both mean FEV_1 and FVC in percentage predicted were lower in higher quartiles of HOMA-IR, whereas mean FEV_1 and FVC in litres were equal among groups. Individuals in the higher quartiles of HOMA-IR more likely had smoked in the past. Current smokers were equally divided over HOMA-IR quartiles. In addition, the mean number of packyears smoked and prevalence of diabetes was higher in the higher HOMA-IR quartiles.

Table 3.1 Characteristics of participants of the Netherlands Epidemiology of Obesity study for the total population aged 45 to 65 years by quartiles of HOMA-IR.

Characteristics		**<1.58**	
Age (median)		55	(50, 63)
Sex (% Men)		36	
BMI		24	(3)
Waist circumference	Men	92	(92)
	Women	81	(9)
Total body fat (%)	Men	21	(5)
	Women	34	(6)
Ethnicity (% White)		96	
Serum insulin (µU/mL)		4.7	(1.5)
Plasma glucose (mg/dl)		5.1	(.5)
HOMA-IR		1.2	(0.9, 1.4)
Self reported asthma (%)		2	
Self reported COPD (%)		2	
FEV_1 (% predicted)		111	(15)
FVC (% predicted)		120	(15)
FEV_1 (in Litres)		3.3	(.8)
FVC (in Litres)		3.3	(1.0)
Smoking behavior	Never	41	
	Former	41	
	Current	17	
Smoking packyears		8	(12)

Results were based on analyses weighted towards the BMI distribution of the general population. Total population n=5247; 2308 men and 2938 women. Results are shown as mean (SD), percentage or median (25th, 75th percentile). HOMA-IR: Homeostastis Model Assessment of Insulin Resistance; BMI: Body mass index; FEV1: forced expiratory volume in one second; FVC: forced vital capacity

Association between HOMA-IR and lung function measurements

First, the association between insulin resistance and lung function was examined (Figure 3.1). The crude association between ^{10}log HOMA-IR and FEV_1 was -7.5 %pred (95% CI: -9.6, -5.4), meaning that per 10-fold higher HOMA-IR the FEV_1 decreased with 7.5 %pred. This association was -7.2 %pred (95% CI: -9.4, -5.1) after adjustment for age and sex, but attenuated after adjustment for waist circumference and total body fat (-2.0 %pred, 95% CI: -4.6, 0.5). After additional adjustment for BMI, smoking, obstructive lung diseases, ethnicity, education, use of bronchodilator and CRP, the association was -2.2 %pred (95% CI: -5.1, 0.6). The crude association between 10log HOMA-IR and FVC (-11.6 %pred; 95% CI: -13.7, -9.5) attenuated after adjustment for age and sex (-9.4 %pred; -11.5, -7.4) and further attenuated after adjustment for waist circumference and total body fat (-3.4 %pred; -5.7, -1.1). This association did not further change after adjustment for BMI, smoking, obstructive lung diseases, ethnicity, education, use of bronchodilator and CRP (-3.3 %pred; -5.9, -0.8). Thus a tenfold higher HOMA-IR was associated with a 3.3 %pred lower lung function.

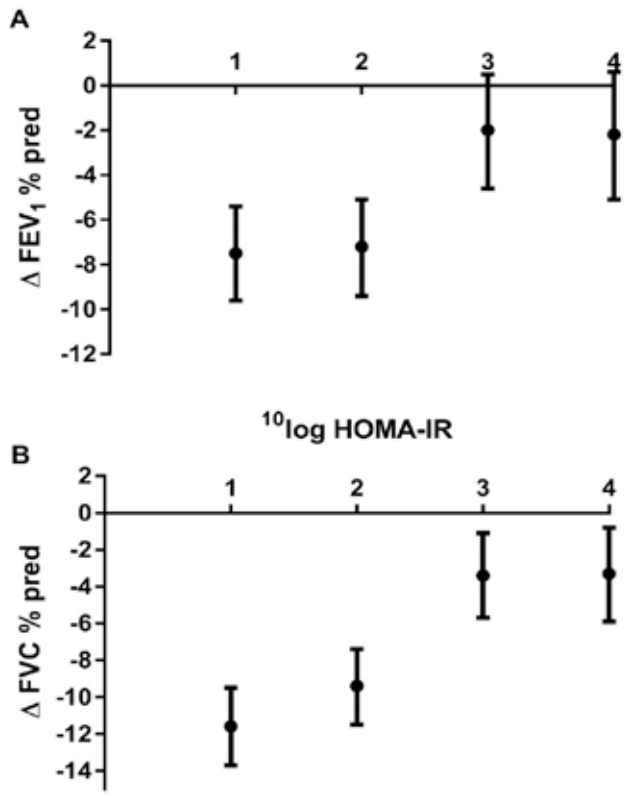


Figure 3.1: The association between ^{10}log HOMA-IR and FEV_1 (3.1.a) and FVC (3.1.b) in Netherlands Epidemiology of Obesity study for the total population aged 45 to 65

Results were based on analyses weighted towards the BMI distribution of the general population. Total population n=5247; 2308 men and 2938 women. HOMA-IR: Homeostastis Model Assessment of Insulin Resistance; BMI: Body mass index; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity

Association between HOMA-IR and lung function stratified by BMI

When considering the interaction between obesity and insulin resistance in the models, the interaction term between BMI and HOMA-IR was significant in all models (P=0.000). Subsequent analyses were stratified by four BMI groups according to the WHO classification[38]: BMI below 25 kg/m^2(43.5% of participants), BMI between 25 and 30 kg/m^2 (42.1%), BMI between 30 and 35 kg/m^2 (10.5%), and BMI of 35 kg/m^2 or higher (3.9% of participants) (Table 3.2 and 3.3).

Table 3.2 The association between ^{10}log HOMA-IR and FEV1 in Netherlands Epidemiology of Obesity study for the total population aged 45 to 65 in four BMI categories

	BMI <25 kg/m²			**BMI 25 – 30 kg/m²**		
Model	Δ% in FEV_1 % pred	95% confidence interval		Δ% in FEV_1 % pred	95% confidence interval	
Crude	-3.4	-7.4	.7	-6.5	-9.6	-3.3
Adjusted *	-3.6	-7.8	.6	-6.1	-9.2	-2.9
Adjusted **	-1.5	-5.9	2.9	-2.3	-5.6	.9
Adjusted ***	-2.3	-7.0	2.4	-1.7	-4.9	1.5
	BMI 30 – 35 kg/m²			**BMI >35 kg/m²**		
Model	Δ% in FEV_1 % pred	95% confidence interval		Δ% in FEV_1 % pred	95% confidence interval	
Crude	-8.6	-11.5	-5.7	-13.6	-18.3	-8.9
Adjusted *	-7.5	-10.4	-4.5	-11.9	-16.8	-7.0
Adjusted **	-4.0	-7.0	-1.0	-9.2	-14.2	-4.2
Adjusted ***	-2.9	-5.8	-.1	-7.4	-12.4	-2.4

* Adjusted for age and sex

** Adjusted for age, sex, waist circumference and total body fat

*** Adjusted for age, sex, waist circumference, total body fat, BMI, smoking, obstructive lung diseases, ethnicity, education, use of bronchodilator and CRP

Results were based on analyses weighted towards the BMI distribution of the general population. Total population n=5247; 2308 men and 2938 women. Results are shown as delta in FEV_1 % predicted. HOMA-IR: Homeostastis Model Assessment of Insulin Resistance; BMI: Body mass index; FEV_1: forced expiratory volume in one second; CRP: C-reactive protein

Table 3.3 The association between ^{10}log HOMA-IR and FVC in four BMI categories

	BMI <25 kg/m²			**BMI 25 – 30 kg/m²**		
Model	Δ% in FVC % pred	95% confidence interval		Δ% in FVC % pred	95% confidence interval	
Crude	-4.8	-8.6	-1.1	-10.2	-13.4	-7.0
Adjusted *	-3.7	-7.4	.0	-7.7	-10.8	-4.6
Adjusted **	-2.5	-6.4	1.3	-3.8	-7.0	-.6
Adjusted ***	-3.0	-7.0	1.1	-3.0	-6.1	.1
	BMI 30 – 35 kg/m²			**BMI >35 kg/m²**		
Model	Δ% in FVC % pred	95% confidence interval		Δ% in FVC % pred	95% confidence interval	
Crude	-12.7	-15.5	-9.9	-16.1	-20.3	-12.0
Adjusted *	-9.3	-12.1	-6.6	-12.1	-16.5	-7.6
Adjusted **	-6.7	-9.5	-3.9	-10.0	-14.6	-5.4
Adjusted ***	-5.9	-8.6	-3.1	-8.6	-13.1	-4.1

* Adjusted for age and sex

** Adjusted for age, sex, waist circumference and total body fat

*** Adjusted for age, sex, waist circumference, total body fat, BMI, smoking, obstructive lung diseases, ethnicity, education, use of bronchodilator and CRP

Results were based on analyses weighted towards the BMI distribution of the general population. Total population n=5247; 2308 men and 2938 women. Results are shown as delta in FVC % predicted. HOMA-IR: Homeostastis Model Assessment of Insulin Resistance; BMI: Body mass index; FVC: forced vital capacity; CRP: C-reactive protein

Mean BMI of these four BMI categories was respectively 22.6 (SD: 1.6), 27.1 (1.4), 31.9 (1.3), and 38.8 (3.7) kg/m^2. Mean HOMA-IR was respectively 1.6 (1.1), 2.5 (1.6), 3.5 (2.3) and 5.3 (5.4). Lung function was lower in higher BMI groups: FEV_1 was respectively 110.5 (15.1), 108.2 (15.8), 105.3 (15.5) and 100.7 (16.1) %pred, while FVC was respectively 120.8 (15.4), 108.2 (15.8), 105.3 (15.5) and 100.7 (16.1) %pred. After adjustment for all known confounding factors, the association between HOMA-IR and FEV_1 and FVC was weakest in participants with a BMI below 25 (FEV_1: -2.3% predicted, 95% CI: -7.0, 2.4; FVC: -3.0% predicted, 95% CI: -7.0, 1.1 per 10-fold higher HOMA-IR) and was stronger in individuals with a BMI between 30 and 35 (FEV_1: -2.9% predicted, 95% CI: -5.8, -0.1; FVC: -5.9% predicted, 95% CI: -8.6, -3.1 per 10-fold higher HOMA-IR) and in individuals with a BMI above 35 (FEV_1: -7.4% predicted, 95% CI: -12.4, -2.4; FVC: -8.6% predicted, 95% CI: -13.1, -4.1 per 10-fold higher HOMA-IR) (Table 3.2 and 3.3). The association between a tenfold higher HOMA-IR and FEV_1 and FVC in persons with a BMI between 25 and 30 were for FEV_1: -1.7% predicted (95% CI: -4.9, 1.5), and for FVC: -3.0% predicted (95% CI: -6.1, 0.1) after adjustment for all confounding factors.

DISCUSSION

We hypothesized that insulin resistance might lead to lung function impairment. We therefore investigated the association between insulin resistance and lung function in a general population aged 45 to 65 years, and explored to what extent this association could be explained by obesity. We observed a weak association between insulin resistance and lung function, but this was mainly explained by adiposity. Insulin resistance seemed to interact with BMI, with somewhat stronger associations in higher BMI groups. However, even in the group with a BMI of 35 or higher the association was not clinically relevant (7.4 percent predicted lower lung function per 10-fold higher HOMA-IR). We therefore conclude that our study does not provide evidence for a clinically relevant association between insulin resistance and lung function.

Various explanations have been proposed for the potential association between insulin resistance and lung function. First of all, insulin resistance has been associated with poor muscle strength,[28] as defined by handgrip strength. Insulin plays a role in glucose uptake and promotes intracellular glucose metabolism, both required for adequate muscle contraction.[29] As skeletal muscles are actively used in the manoeuvers used in obtaining a FEV_1 and FVC, a decline in muscle strength negatively influences lung function. Insulin also prevents breakdown of proteins, decreasing free amino acids availability which is essential for protein synthesis in muscle tissue.[30]

Multiple studies have associated insulin resistance with a small decrease in lung function [23;24;31;39-44] while in our study there was no clinically relevant association. An explanation for this difference could be the differences in study group, different lung function outcome measurements and different ways of adjusting for adiposity. The fact that the observed association between insulin resistance and lung function in our study was mainly explained by adiposity suggests that insulin resistance and impaired lung function are merely separate consequences of obesity.

Strengths of our study are the population size and extensive phenotyping of the population, allowing adjustment for the most important confounding factors. The present study also has a few limitations that should be considered. Firstly, insulin resistance was assessed using the HOMA index of insulin resistance instead of the hyperinsulinemic euglycemic clamp. The HOMA index is strongly correlated with the hyperinsulinemic euglycemic glucose clamp in large cohorts[45] and is more practical to assess in large epidemiologic studies, but it should be noted that this is a surrogate measure of insulin resistance and may therefore not account for the total effect of insulin resistance.[33] Although we studied insulin resistance in relation to lung function and multiple cross-sectional studies have used insulin resistance as determinant and lung function as outcome, some follow-up studies showed that lower baseline lung function is a risk factor for both insulin resistance[24;40-42] and type 2 diabetes.[46]

We cannot exclude that after stratification and adjustment for BMI, BMI could still have influenced our results. The small non-clinically relevant negative association between insulin resistance and lung function follows the same pattern as the negative association between BMI and lung function, which is also more pronounced at higher BMI.[47-53] In fact, two studies even showed that BMI is negatively associated with lung function in overweight and obese individuals, but positively associated in lean individuals.[7;54] The authors speculated that a higher BMI in lean individuals indicates more muscle mass instead of adipose tissue,[55] while in overweight and obese individuals the excessive fat tissue, especially abdominal fat, obstructs normal breathing.[13-15] Also, overweight results in loss of muscle strength[56] required for optimal FEV_1 and FVC curves. In addition to the mechanical factors of adiposity, adipose tissue also secretes various cytokines and hormones, such as IL-6 57, TNF-α [58], leptin[59], and adiponectin[54;60] that may affect lung function. Moreover, CRP[21;22] and TNF-α have also been negatively associated with insulin resistance[19;20.] After adjustment of our models for CRP (measured using a high-sensitivity CRP test), the weak associations remained. Nevertheless, our results suggest that insulin resistance and impaired lung function are merely separate consequences of obesity.

In conclusion, in this study we observed a small but non-clinically relevant association between insulin resistance and lung function that was mainly explained by adiposity. The influence of overweight could be the result of mechanical and endocrine factors affecting lung function. Future prospective studies are needed to explore the association between insulin resistance and lung function. In addition, it should be noted that the presently observed lung functions were within the normal range as expected in a general population. It will therefore be relevant to investigate these relationships also in patients with obstructive lung disease, to determine whether these relationships are possibly more pronounced, and to begin to understand how high BMI contributes to the development of obstructive lung disease.

ACKNOWLEDGEMENTS

We express our gratitude to all individuals who participate in the Netherlands Epidemiology in Obesity study. We are grateful to all participating general practitioners for inviting eligible participants. We furthermore thank P.R. van Beelen and all research nurses for collecting the data, P.J. Noordijk and her team for sampling handling, and I. de Jonge, MSc for data management of the NEO study.

The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Centre, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'.

REFERENCE LIST

1. Stevens GA, Singh GM, Lu Y et al. National, regional, and global trends in adult overweight and obesity prevalences. *Popul Health Metr* 2012;10:22-10.
2. Bray GA. Medical consequences of obesity. *J Clin Endocrinol Metab* 2004;89:2583-2589.
3 Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008;371: 569-578.
4. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of comorbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health* 2009;9:88. doi: 10.1186 /1471-2458-9-88.:88-89.
5. Guillet C, Masgrau A, Walrand S, Boirie Y. Impaired protein metabolism: interlinks between obesity, insulin resistance and inflammation. *Obes Rev* 2012;13 Suppl 2:51-7. doi: 10.1111/ j.1467- 789X.2012.01037.x.:51-57.
6. Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. *J Clin Endocrinol Metab* 2008;93:S9-30.
7. Chen Y, Rennie D, Cormier YF, Dosman J. Waist circumference is associated with pulmonary function in normal-weight, overweight, and obese subjects. *Am J Clin Nutr* 2007;85:35-39.
8. Morgan WK, Reger RB. Rise and fall of the $FEV_{(1)}$. *Chest* 2000;118:1639-1644.
9. Chinn DJ, Cotes JE, Reed JW. Longitudinal effects of change in body mass on measurements of ventilatory capacity. *Thorax* 1996;51:699-704.
10. Santana H, Zoico E, Turcato E et al. Relation between body composition, fat distribution, and lung function in elderly men. *Am J Clin Nutr* 2001; 73:827-831.
11. Steier J, Lunt A, Hart N, Polkey MI, Moxham J. Observational study of the effect of obesity on lung volumes. *Thorax* 2014;69:752-759.
12. Salome CM, King GG, Berend N. Physiology of obesity and effects on lung function. *J Appl Physiol* (1985) 2010;108:206-211.
13. Koenig SM. Pulmonary complications of obesity. *Am J Med Sci* 2001;321:249-279.
14. Pedoto A. Lung physiology and obesity: anesthetic implications for thoracic procedures. *Anesthesiol Res Pract* 2012;2012:154208. doi: 10.1155/2012/154208. Epub@2012 Feb 26.: 154208.
15. Pelosi P, Luecke T, Rocco PR. Chest wall mechanics and abdominal pressure during general anaesthesia in normal and obese individuals and in acute lung injury. *Curr Opin Crit Care* 2011;17: 72-79.
16. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005;115:1111-1119.
17. Ahmadi-Abhari S, Kaptoge S, Luben RN, Wareham NJ, Khaw KT. Longitudinal association of C-reactive protein and lung function over 13 years: The EPIC-Norfolk study. *Am J Epidemiol* 2014;179:48-56.
18. Gaughan DM, Christiani DC, Hughes MD et al. High hsCRP is associated with reduced lung function in structural firefighters. *Am J Ind Med* 2014;57:31-37.
19. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995;95:2409-2415.
20. Uysal KT, Wiesbrock SM, Hotamisligil GS. Functional analysis of tumor necrosis factor (TNF) receptors in TNF-alpha-mediated insulin resistance in genetic obesity. *Endocrinology* 1998;139:4832-4838.
21. Gelaye B, Revilla L, Lopez T et al. Association between insulin resistance and c-reactive protein among Peruvian adults. *Diabetol Metab Syndr* 2010;2:30-32.

22. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999;19:972-978.
23. Lawlor DA, Ebrahim S, Smith GD. Associations of measures of lung function with insulin resistance and Type 2 diabetes: findings from the British Women's Heart and Health Study. *Diabetologia* 2004;47:195-203.
24. Engstrom G, Hedblad B, Nilsson P, Wollmer P, Berglund G, Janzon L. Lung function, insulin resistance and incidence of cardiovascular disease: a longitudinal cohort study. *J Intern Med* 2003;253: 574-581.
25. Barrett-Connor E, Frette C. NIDDM, impaired glucose tolerance, and pulmonary function in older adults. The Rancho Bernardo Study. *Diabetes Care* 1996;19:1441-1444.
26. Yeh HC, Punjabi NM, Wang NY et al. Cross-sectional and prospective study of lung function in adults with type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetes Care* 2008;31:741-746.
27. Davis TM, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: the Fremantle Diabetes Study. *Diabetes Res Clin Pract* 2000;50:153-159.
28 Abbatecola AM, Ferrucci L, Ceda G et al. Insulin resistance and muscle strength in older persons. *J Gerontol A Biol Sci Med Sci* 2005;60:1278-1282.
29 Perriott LM, Kono T, Whitesell RR et al. Glucose uptake and metabolism by cultured human skeletal muscle cells: rate-limiting steps. *Am J Physiol Endocrinol Metab* 2001;281:E72-E80.
30. Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U. Insulin action and age. European Group for the Study of Insulin Resistance (EGIR). *Diabetes* 1996;45:947-953.
31 Forno E, Han YY, Muzumdar RH, Celedon JC. Insulin resistance, metabolic syndrome, and lung function in US adolescents with and without asthma. *J Allergy Clin Immunol* 2015;136:304-311.
32. R de Mutsert, H den Heijer, Rabelink TJ et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur J Epidemiol* 2013;28:513-523.
33. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487-1495.
34. Miller A, Enright PL. PFT interpretive strategies: American Thoracic Society/ European Respiratory Society 2005 guideline gaps. *Respir Care* 2012;57127-133.
35. Korn EL, Graubard BI. Epidemiologic studies utilizing surveys: accounting for the sampling design. *Am J Public Health* 1991;81:1166-1173.
36. Lumley T. Analysis of complex survey samples. *Journal of Statistical Software* 2004;9.
37. Ministerie van VWS. NdMG. Hoeveel mensen hebben er overgewicht? 2013. 2-3-2013.
38. World Health Organization. 2015. 7-5-2015.
39. Lecube A, Sampol G, Munoz X, Lloberes P, Hernandez C, Simo R. Insulin resistance is related to impaired lung function in morbidly obese women: a case-control study. *Diabetes Metab Res Rev* 2010;26:639-645.
40. Lim SY, Rhee EJ, Sung KC. Metabolic syndrome, insulin resistance and systemic inflammation as risk factors for reduced lung function in Korean nonsmoking males. *J Korean Med Sci* 2010;25 :1480-1486.
41. Lazarus R, Sparrow D, Weiss ST. Baseline ventilatory function predicts the development of higher levels of fasting insulin and fasting insulin resistance index: the Normative Aging Study. *Eur Respir J* 1998;12:641-645.
42. Ford ES, Mannino DM. Prospective association between lung function and the incidence of diabetes: findings from the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *Diabetes Care* 2004;27:2966-2970.
43. Wei YF, Wu HD, Yung-Chieh Yen PD, Huang CK, Tai CM, Hsuan CF. The impact of metabolic parameters on the change of pulmonary function in obese patients. *Surg Obes Relat Dis* 2014;10:23-28.
44. Forno E, Han YY, Muzumdar RH, Celedon JC. Insulin resistance, metabolic syndrome, and lung function in US adolescents with and without asthma. *J Allergy Clin Immunol* 2015;10.
45. Bonora E, Targher G, Alberiche M et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000;23:57-63.
46. Engstrom G, Janzon L. Risk of developing diabetes is inversely related to lung function: a population -based cohort study. *Diabet Med* 2002;19:167-170.
47. Banerjee J, Roy A, Singhamahapatra A, Dey PK, Ghosal A, Das A. Association of Body Mass Index (BMI) with Lung Function Parameters in Non -asthmatics Identified by Spirometric Protocols. *J Clin Diagn Res* 2014;8:12-14.
48 Thijs W, Alizadeh DR, Hiemstra PS et al. Association of lung function measurements and visceral fat in men with metabolic syndrome. *Respir Med* 2014;108:351-357.

49. Jones RL, Nzekwu MM. The effects of body mass index on lung volumes. Chest 2006;130:827-833.
50. N.K.Mungreiphy, Satwanti Kapoor, Sashimi Sinha. Relationship between nutritional status, respiratory performance and age: study among Tangkhul Naga females of Northeast India. *Acta Biologica Szegediensis* 2012;56:31-36.
51. Attaur-Rasool S, Shirwany TA. Body mass index and dynamic lung volumes in office workers. *J Coll Physicians Surg Pak* 2012;22:163-167.
52. Ochs-Balcom HM, Grant BJ, Muti P et al. Pulmonary function and abdominal adiposity in the general population. Chest 2006;129:853-862.
53. Forrester DL, Knox AJ, Smyth AR, Fogarty AW. Measures of body habitus are associated with lung function in adults with cystic fibrosis: a population-based study. *J Cyst Fibros* 2013;12: 284-289.
54. Thyagarajan B, Jacobs DR, Jr., Apostol GG et al. Longitudinal association of body mass index with lung function: the CARDIA study. *Respir Res* 2008;9:31. doi: 10.1186/1465-9921-9-31.:31-39.
55. Chen Y, Horne SL, Dosman JA. Body weight and weight gain related to pulmonary function decline in adults: a six year follow up study. *Thorax* 1993;48:375-380.
56. Lazarus R, Sparrow D, Weiss ST. Handgrip strength and insulin levels: cross-sectional and prospective associations in the Normative Aging Study. *Metabolism* 1997;46:1266-1269.
57. Cui AH, Zhao J, Liu SX, Hao YS. Associations of IL-4, IL-6, and IL-12 levels in peripheral blood with lung function, cellular immune function, and quality of life in children with moderate-to-severe asthma. *Medicine* (Baltimore)2017; 96:e6265.
58. Huang AX, Lu LW, Liu WJ, Huang M. Plasma Inflammatory Cytokine IL-4, IL-8, IL-10, and TNF-alpha Levels Correlate with Pulmonary Function in Patients with Asthma-Chronic Obstructive Pulmonary Disease (COPD) Overlap Syndrome. *Med Sci Monit* 2016;22:2800-2808.
59. Malli F, Papaioannou AI, Gourgoulianis KI, Daniil Z. The role of leptin in the respiratory system: an overview. *Respir Res* 2010;11:152. doi: 10.1186 /1465-9921-11-152.:152-11.
60. Thyagarajan B, Jacobs DR, Jr., Smith LJ, Kalhan R, Gross MD, Sood A. Serum adiponectin is positively associated with lung function in young adults, independent of obesity: the CARDIA study. *Respir Res* 2010;11:176.

CHAPTER

4

Reproducibility of exhaled nitric oxide measurements in overweight and obese adults

Willemien Thijs[1], Renée de Mutsert[2], Saskia le Cessie[2,3], Pieter S Hiemstra[1], Frits R Rosendaal[2], Saskia Middeldorp[4], Klaus F Rabe[5]

Department of Pulmonology[1], Leiden University Medical Center, Leiden, the Netherlands
Department of Clinical Epidemiology[2], Leiden University Medical Center, Leiden, the Netherlands
Department of Medical Statistics[3], Leiden University Medical Center, Leiden, the Netherlands
Department of Vascular Medicine[4], Academic Medical Center, Amsterdam, the Netherlands
LungenClinic Grosshansdorf[5], Grosshansdorf, Germany

BMC Respiratory Notes. 2014 Nov 3;7:775

ABSTRACT

Background

Exhaled nitric oxide is a noninvasive measure of airway inflammation that can be detected by a handheld device. Obesity may influence the reproducibility of exhaled nitric oxide measurements, by - for instance – decreased expiratory reserve volume.

Findings

We analyzed triple exhaled nitric oxide measurements from 553 participants (aged 45 to 65 years with a body mass index ≥27 kg/m^2) of the Netherlands Epidemiology of Obesity Study. The interclass correlation coefficient (single measurement reliability) was 0.965 (95% CI: 0.960, 0.970).

Conclusions

We conclude that for assessment of exhaled nitric oxide in large cohorts of overweight and obese adults a single measurement suffices.

INTRODUCTION

Exhaled nitric oxide (eNO) is a noninvasive marker of inflammation in the airways. The levels of eNO correlate well with other markers of inflammation in the airways of asthmatics, such as sputum eosinophils and airway eosinophilia in bronchial biopsies [1,2]. Measuring eNO with a handheld device is a convenient way to assess airways inflammation and has been used to study e.g. occupational hazards or asthma[3,4]. The prevalence of obesity has risen dramatically in the past decades and an increasing proportion of participants in studies will be overweight or obese[5]. Because eNO measurements take time and generate costs it is important to establish the reproducibility of eNO measurements in overweight and obese adults.

How could obesity influence eNO measurements? Obesity is associated with a loss in expiratory reserve volume [6], which may influence eNO measurement that require a slow and steady exhalation. In addition, obesity is associated with low grade systemic inflammation [7] which may be accompanied by airways inflammation resulting in increased eNO levels. However, studies into the association between obesity and levels of eNO show conflicting results [8-11]. Therefore it is not clear whether putatively increased eNO levels may contribute to decreased reproducibility in obese subjects.

The ATS/ERS recommendations for eNO measurements suggest two measurements of eNO[12]. Because of the time requirement and costs associated with multiple eNO measurements in large scale studies, a single measurement would be preferable. Reproducibility of eNO measured by the handheld NIOX MINO has been evaluated in children [13], adults [14], asthma patients and pregnant women [15], but not in overweight and obese individuals.
Therefore, we used a triplicate measurement to assess the reproducibility of eNO measured by a handheld NIOX MINO in a cohort study of overweight and obese adults, with the aim to assess whether a single measurement may suffice in large scale studies.

MATERIALS AND METHODS

The Netherlands Epidemiology of Obesity (NEO) Study is a population-based cohort study in adults aged 45 to 65 years, with an oversampling of participants with overweight or obesity [16]. The study was approved by the ethical committee of the Leiden University Medical Center and all participants gave written informed consent. The present analysis includes the first 630 participants with a body mass index (BMI) ≥ 27 kg/m^2. Completed multiple questionnaires including self-reported asthma, and anthropometric and maximal flow-volume curves measurements were obtained. Exhaled nitric oxide was measured using a portable analyzer, the NIOX MINO (Aerocrine AB, Solna, Sweden). Participants performed a 10 seconds slow steady exhalation. Three successive recordings at 1-minute intervals, expressed as parts per billion (ppb), were made. The interclass correlation coefficient (ICC) was calculated for the three measurements in all participants, participants with self reported asthmaand separately for participants with a BMI ≥ 35 and for elevated mean eNO levels (>25 ppb and for >50) [17]. The mean intra-participant difference in eNO was calculated and a Bland-Altman plot was constructed. Statistical analyses were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL).

RESULTS

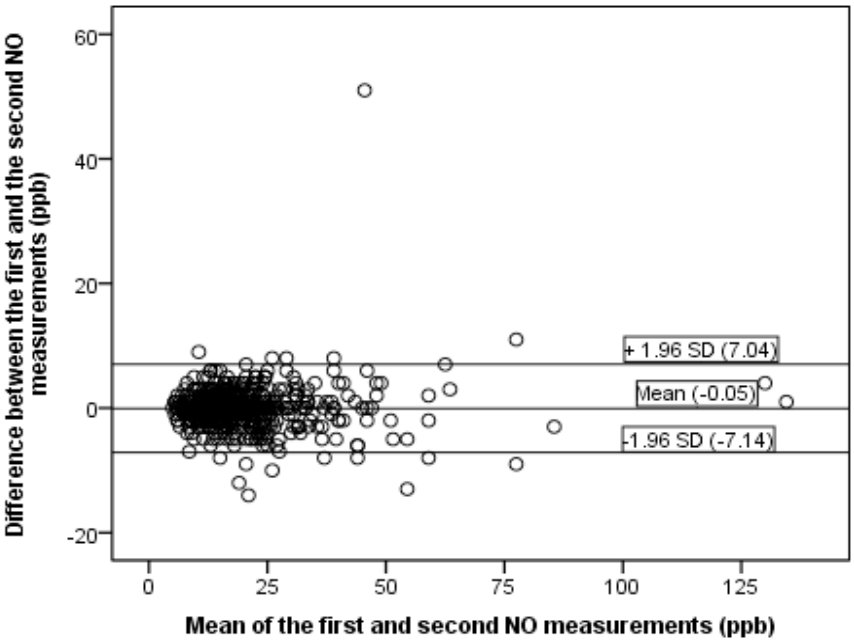
Of the first 630 participants of the NEO study, 46 participants did not perform eNO measurements because they did not visit the lung function department due to logistic problems. In another 31 patients, no measurements were obtained because of inability to perform the technique or because of a technical failure with the nitric oxide machines. As a result, the present analysis includes 553 participants who performed all three eNO measurements. The characteristics of the study population and results of eNO measurements are presented in Table 4.1. The ICC (single measurement reliability) for all participants was 0.965 (95% CI: 0.960, 0.970), whereas it was 0.926 (95% CI: 0.926, 0.965) for the participants with a BMI ≥ 35 (n = 92). The ICC (single measurement reliability) for all participants with asthma (n = 39) was 0.988 (95% CI: 0.979, 0.993), whereas it was 0.932 (95% CI: 0.818, 0.981) for the participants with asthma and a BMI ≥ 35 (n = 10). The ICC for all eNO measurements that exceeded 25 ppb (n = 109) was 0.949 (95% CI: 0.931, 0.963) and for those that exceeded 50 ppb (n = 18) was 0.911 (95% CI: 0.818, 0.963). The mean intra-participant difference in eNO for all participants was for the second and first reading: -0.05 ppb (95% CI: -7.14, 7.04); third and first reading -0.15 ppb (95% CI: -6.8, 7.6); and third and second reading -0.13 ppb (95% CI: - 5.9, 6.5). A Bland-Altman plot was constructed for the first two measurements (Figure 4.1).

Table 4.1 Clinical characteristics and eNO measurements of the study population (n = 553)

Characteristic	**Median or** %	**IQR**
Age (years)	56	(50- 61)
Sex (women %)	47	NA
Self reported asthma (%)	7	NA
BMI (kg/m^2)	30	(28- 33)
FEV_1 % predicted	103	(92-114)
FVC % predicted	105	(96-115)
First nitric oxide (ppb)	17	(12- 23)
Second nitric oxide (ppb)	17	(12- 24)
Third nitric oxide (ppb)	17	(13- 24)

BMI: Body mass index; IQR: Interquartile range; NA: not applicable; FEV_1 %: percent predicted of forced expiratory volume; FVC % percent predicted of forced vital capacity; ppb: parts per billion.

Figure 4.1 Bland- Altman plot for the first two eNO measurements by the NIOX MINO (n = 553).



The dots represent the difference between the first and the second measurement.

DISCUSSION

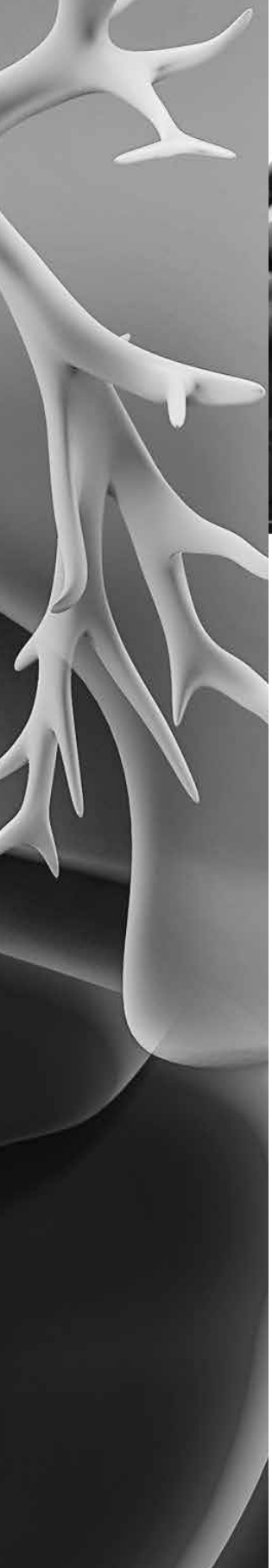
The ICC and mean intra-participant difference in eNO for all 553 participants was in line with previous reproducibility studies performed on the NIOX MINO in other populations [13,14]. The ICC for participants with a BMI ≥ 35 kg/m^2 was slightly lower (but clinically not relevant) than within the whole group, possibly as a result of decreased expiratory reserve volumes. Low grade inflammation associated with obesity appears a less likely explanation for the small loss in reproducibility because only early studies report a positive correlation between BMI and eNO [8,9]; later studies have not been able to reproduce these initial findings [10,11]. The reproducibility in participants with self reported asthma was in line with the results in the whole group but within our study the reproducibility at higher eNO levels was slightly lower. In earlier study by Selby at al [13] it is concluded that for individual absolute levels two measurements are needed. Similarly, we conclude that in clinical practice two eNO measurements are advised but despite small differences of ICC in different analyses, our results demonstrate that in large cohorts of overweight and obese adults a single eNO measurement suffices, which will have significant logistical and financial consequences for cohort studies.

ACKNOWLEDGEMENTS

Supported by an unrestricted research grant from Astra-Zeneca NL

REFERENCE LIST

1. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ: Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998, 53:91-95.
2. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A: Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001, 164:1376-1381.
3. Sordillo JE, Webb T, Kwan D, Kamel J, Hoffman E, Milton DK, Gold DR: Allergen exposure modifies the relation of sensitization to fraction of exhaled nitric oxide levels in children at risk for allergy and asthma. *J Allergy Clin Immunol* 2011, 127: 1165-1172.
4. Tungu AM, Bratveit M, Mamuya SD, Moen BE: Fractional exhaled nitric oxide among cement factory workers: a cross sectional study. *Occup Environ Med* 2013, 70:289-295.
5. WHO: Obesity, Preventing and Managing the Global Epidemic. Report of a WHO Consultation. Geneva: WorldHealth Organization; 2000:894.
6. Littleton SW: Impact of obesity on respiratory function. *Respirology* 2012, 17:43-49.
7. Tilg H, Moschen AR: Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases. *Clin Sci (Lond)* 2008, 114:275-288.
8. Depalo A, Carpagnano GE, Spanevello A, Sabato R, Cagnazzo MG, Gramiccioni C, Foschino-Barbaro MP: Exhaled NO and iNOS expression in sputum cells of healthy, obese and OSA subjects. *J Intern Med* 2008, 263:70–78.
9. Maestrelli P, Ferrazzoni S, Visentin A, Marian E, Dal BD, Accordino R, Fabbri LM: Measurement of exhaled nitric oxide in healthy adults. *Sarcoidosis Vasc Diffuse Lung Dis* 2007, 24:65–69.
10. Kim SH, Kim TH, Lee JS, Koo TY, Lee CB, Yoon HJ, Shin DH, Park SS, Sohn JW: Adiposity, adipokines, and exhaled nitric oxide in healthy adults without asthma. *J Asthma* 2011, 48:177–182.
11. Lombardi C, Gargioni S, Gardinazzi A, Canonica GW, Passalacqua G: Impact of bariatric surgery on pulmonary function and nitric oxide in asthmatic and non-asthmatic obesepatients. *J Asthma* 2011, 48(6):553–557. doi:10.3109/02770903.2011.587581. *Epub* 2011 Jun 28.
12. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005, 171:912–930.
13. Selby A, Clayton B, Grundy J, Pike K, Drew K, Raza A, Kurukulaaratchy R, Arshad SH, Roberts G: Are exhaled nitric oxide measurements using the portable NIOX MINO repeatable? *Respir Res* 2010, 11:43.
14. Alving K, Janson C, Nordvall L: Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respir Res* 2006, 7:67.
15. Tamasi L, Bohacs A, Bikov A, Andorka C, Rigo J Jr, Losonczy G, Horváth I: Exhaled nitric oxide in pregnant healthy and asthmatic women. *J Asthma* 2009, 46:786–791.
16. De MR, Den HM, Rabelink TJ, Smit JW, Romijn JA, Jukema JW, de Roos A, Cobbaert CM, Kloppen burg M, le Cessie S, Middeldorp S, Rosendaal FR: The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur J Epidemiol* 2013, 28(6):513–523. doi: 10.1007/s10654-013-9801-3. *Epub* 2013 Apr 11.
17. Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC: Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006, 61:817–827.



CHAPTER

5

Total body fat and visceral fat are not associated with exhaled nitric oxide in a middle-aged population

W. Thijs[1], P.S. Hiemstra[1], H.J. Lamb[2], A. de Roos[2], F.R. Rosendaal[3,4], C. Taube[1], M. den Heijer[3,5], and R. de Mutsert[3]

Department of Pulmonology[1], Leiden University Medical Center, Leiden, Leiden, the Netherlands
Department Radiology[2], Leiden University Medical Center, Leiden, the Netherlands
Department of Clinical Epidemiology[3], Leiden University Medical Center, Leiden, the Netherlands
Department of Endocrinology[4], VU University Medical Center, Amsterdam, the Netherlands

Submitted

ABSTRACT

Background
Exhaled nitric oxide (FeNO) is used as a non-invasive marker for airway inflammation in asthma. Obesity, in particular abdominal obesity is accompanied by systemic inflammation and is a risk factor for asthma. We hypothesized that body fat is associated with exhaled nitric oxide, as a potential mechanism underlying the link between obesity and asthma. Our objective was to investigate the association between measures of overall and abdominal body fat and especially visceral fat and exhaled nitric oxide.

Methods
In this cross-sectional analysis of the Netherlands Epidemiology of Obesity (NEO) study, a population-based cohort including 6,671 individuals aged 45 to 65 years, total body fat (TBF) was measured by bio-impedance analysis, and FeNO was measured using a portable analyzer. In a random sample of participants, abdominal subcutaneous and visceral adipose tissue (VAT) were assessed by magnetic resonance imaging. We performed linear regression analysis to examine the associations of TBF and VAT with FeNO adjusted for sex, age, ethnicity, smoking habits, a history of allergy or asthma; the model of VAT was additionally adjusted for TBF.

Results
After exclusion of participants with missing data on FeNO (n=379), TBF or waist circumference (WC) (n=33), self-reported allergy (n=24), ethnicity (n=8), self reported asthma (n=5) and smoking (n=3), 6,219 participants were analyzed with a mean (standard deviation, SD) age of 56 (6) years, BMI of 26.3 (4.4) kg/m^2 and TBF of 32% (9); 44% were men and 5% reported to have asthma. The mean (SD) FeNO was 18.9 (12.6) parts per billion (ppb).
Per SD of TBF the difference in FeNO was -2 ppb higher (95% confidence interval: -1.5, -2.5). After adjustment for confounding factors this difference attenuated to -0.9 ppb (-1.5, -0.3). Visceral fat was not associated with FeNO 0.5 ppb (-0.4, 1.3).

Conclusion
In this population-based study, there was no evidence for clinically relevant associations of total body fat and visceral fat with FeNO. These findings suggest that the visceral adipose tissue volume is not accompanied by an increased NO production in the airways.

Keywords
Exhaled nitric oxide, body mass index, waist circumference, total body fat, visceral adipose tissue, asthma, obesity

BACKGROUND

The prevalence of obesity is increasing worldwide and it is a well-established risk factor for diabetes and cardiovascular disease [1,2]. Obesity has also been reported as a risk factor for asthma [3,4]. The global prevalence of asthma ranges 1-18% of the population in different countries [5]. In a prospective cohort study women with a high BMI had an increased risk of developing asthma compared with women with a normal BMI [6]. Furthermore, in several studies the severity of asthma was negatively affected by obesity [7,8], whereas weight loss in obese asthmatic patients decreased asthma severity and airway hyper-responsiveness [9,10]. However, a pathophysiological explanation for this association between obesity and asthma is not yet established.

There are several hypotheses to explain the association between obesity and asthma. First, there are mechanical effects of obesity on the chest and abdomen. Specifically, obesity increases the work of breathing and changes lung mechanics [11]. The increased work of breathing could influence the diagnosis and treatment of asthma and additionally might affect airway hyper-responsiveness [10]. Second, there could be genetic predisposition for, or common lifestyle factors causing both obesity and asthma, resulting in spurious associations [12,13]. Finally, adipose tissue secretes several pro-inflammatory cytokines that may result in a low grade systemic inflammatory state that might contribute to several obesity associated diseases[14,15]. It is yet unclear whether this adipose tissue-associated inflammation also results in local pulmonary inflammation.

Exhaled nitric oxide (FeNO) is a noninvasive measure of airway inflammation and is used to monitor patients with asthma. Levels of FeNO are related with other markers of inflammation in the airways of asthmatics, such as sputum eosinophils and airway eosinophilia in bronchial biopsies [16,17]. If adipose tissue-associated inflammation would lead to pulmonary inflammation this could be an eosinophilic inflammation and cause higher levels of FeNO asthmatic and in non asthmatic patients.

Earlier studies on the relationship between body fat and FeNO have shown inconsistent results. Both positive [18,19] and null associations [20,21] between body mass index (BMI) and FeNO levels are reported. In a recent large cohort study with a wide range of BMI values there was no association between body mass index and FeNO [20]. However, BMI is a crude measure of body fat and provides no information on body fat distribution. In addition to overall obesity, abdominal obesity is important in the development of several metabolic diseases [22] and mortality [23,24]. Intraabdominal visceral adipose tissue has a high secretion rate of pro-inflammatory cytokines [25] and displays other markers of inflammation [26].
If visceral fat causes a local eosinophilic inflammation in the lungs this might explain why obesity is associated with asthma. On the other hand it is important to rule out obesity as a cause for higher FeNO levels. Therefore, our objective was to study the associations of measures of total and abdominal body fat (including visceral fat) withFeNO in a population-based cohort study.

METHODS

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in men and women aged between 45 and 65 years, with an oversampling of persons with a high BMI. The present study is a cross-sectional analysis of the baseline measurements of the 6,671 participants included in the NEO study between September 2008 and September 2012. Detailed information about the study design and data collection has been described previously [27]. Men and women with self-reported BMI ≥27 kg/m^2 living in the greater area of Leiden (in the west of the Netherlands) were eligible to participate in the NEO study. In addition, in one municipality (Leiderdorp) all inhabitants aged 45 to 65 years were invited irrespective of their BMI, allowing for a reference distribution of BMI. All participants completed questionnaires on demographic, lifestyle, and clinical information and visited the NEO study center at the Leiden University Medical Center (LUMC) after an overnight fast.

At the study center, the participants completed a screening form, asking about anything that might create a health risk or interfere with magnetic resonance imaging (MRI) (most notably metal devices, or claustrophobia). A body circumference of more than 1.70 m was an additional contraindication for undergoing MRI at the NEO study center. Of the participants who were eligible for MRI imaging, approximately 35% were randomly selected to undergo direct assessment of abdominal subcutaneous adipose tissue (aSAT) and visceral adipose tissue (VAT). All participants underwent an extensive physical examination, including anthropometric measurements, spirometry and FeNO measurement. For the present analysis, we excluded participants with missing data on FeNO, BMI, waist circumference, total body fat, self-reported asthma, allergy or smoking, and performed the analyses of aSAT and VAT within the subgroup with these measurements. The study was approved by the medical ethics committee of the Leiden University Medical Center and all participants gave written informed consent.

Data collection

On the questionnaire, participants reported ethnicity by self-identification in eight categories which we grouped into white and other. Reported tobacco smoking was categorized in the three categories: current, former, and never smoking. Participants reported their medical history of allergy and asthma. At the study site, height was measured without wearing shoes with a vertically fixed, calibrated tape measure.

Measures of body fat

Body weight and percent of total body fat (TBF) were measured by the Tanita bio impedance balance (TBF-310, Tanita International Division, UK) without shoes and one kilogram was subtracted to correct for the weight of clothing. To assess the reproducibility, repeated measurements were performed after approximately 3 months in a random sample of 72 participants; the calculated intraclass correlation coefficient was 0.98. BMI was calculated by dividing the weight in kilograms by the height in meters squared. Waist circumference was measured between the border of the lower costal margin and the iliac crest with a precision of 0.1 cm. Abdominal visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (aSAT) were quantified by a turbo spin echo imaging protocol, performed on a 1.5 Tesla system (Philips, Medical Systems, Best, the Netherlands). At the level of the fifth

lumbar vertebra, three transverse images with a slice thickness of 10 mm were obtained during a breathhold. Imaging parameters were: TR=300 ms; TE=20 ms; flip angle=90°; slice thickness=10 mm, slice gap=2 mm. Mean VAT and abdominal aSAT areas for each participant were quantified by converting the number of pixels to square cm for all three slides, and then averaging theses values, using in-house-developed software (MASS, Medis, Leiden, the Netherlands).

Exhaled nitric oxide

Exhaled nitric oxide (FeNO) was measured using a portable analyzer, the NIOX MINO (Aerocrine AB, Solna, Sweden). FeNO measurements by the NIOX MINO showed a strong correlation and a high degree of agreement with a standard stationary device [28]. Participants performed a 10 seconds slow steady exhalation maneuver.

Previously we have shown that in large cohorts of overweight and obese adults a single FeNO measurement suffices [29]. Therefore, one recording was made for each participant and expressed in parts per billion (ppb).

Statistical analysis

Data were analyzed using STATA version 12 (StataCorp LP, College Station, TX, USA). In the NEO study there is an oversampling of persons with BMI ≥27 kg/m^2. To correctly represent associations in the general population [30], adjustments were made for the oversampling of individuals with BMI ≥ 27 kg/m^2. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp[31], whose BMI distribution was similar to the BMI distribution in the general Dutch population [32]. Consequently, results apply to a population-based study without oversampling of BMI ≥ 27 kg/m^2.

The data were summarized as mean or percentage and were stratified by sex. We performed linear regression to examine the associations between measures of body fat and FeNO. We adjusted the crude associations for age, ethnicity, smoking, self reported asthma and allergy and in the total population additionally for sex. Because abdominal fat is strongly related to total body fat, for the study of specific effects of abdominal fat it is important to adjust the associations for total body fat [33].Therefore, the models for waist circumference and VAT were additionally adjusted for total body fat. To investigate whether associations were different between men and women, we tested for interaction with sex by including product terms of the measures of body fat and sex all models, and subsequently performed all analyses separately for men and women. Regression coefficients can be interpreted as the difference in FeNO in ppb that is associated with one standard deviation increase in measure of body fat. We repeated all analyses after exclusion of participants with self-reported asthma.

RESULTS

The NEO study included 6,671 participants. After exclusion of participants with missing data on FeNO (n=379), TBF or WC (n=33), self-reported allergy (n=24), ethnicity (n=8), self reported asthma (n=5) and smoking (n=3), 6,219 participants were analyzed 44% were men and 5% reported to have asthma. The characteristics of the study population are presented in table 5.1. In this study population, there were 2,393 participants with aSAT and VAT measurement available. Because there was an interaction of sex with BMI and TBF in the association with FeNO, we also show the results separately for men and women in our tables. Table 5.2 shows the differences in FeNO (in ppb) that are associated with a standard deviation increase in the measures of body fat. The associations of TBF, BMI and waist circumference with FeNO were very weak. For example, with each increase in BMI of 4.4 kg/m^2, FeNO was 0.6 ppb lower. Levels of exhaled nitric oxide are considered elevated >25 ppb [34] and the manufacture of the exhaled nitric oxide device (NIOX MINO Aerocrine AB, Solna, Sweden) reports a accuracy ± 5 ppb therefore we find these observed associations not clinically relevant. Similarly, aSAT and VAT were not associated with FeNO (table 5.3). Excluding participants with asthma did not alter the results.

Table 5.1 Characteristics of participants of the Netherlands Epidemiology of Obesity study for the total population.

Characteristic	Total population	Men	Women
Age (years)	56 [6]	56 [6]	55 [6]
Sex (% men)	44		
Ethnicity (% white)	95	95	95
current smoker (%)	16	18	14
Self-reported asthma (%)	5	4	5
Self-reported allergy (%)	33	29	37
	95	95	95
BMI (kg/m^2)	26.3 [4.4]	26.6 [3.4]	25 [4]
WC (cm)	92.0 [13.4]	97.7 [10.3]	85.6 [12.2]
TBF (%)	32 [9]	25 [6]	36 [7]
aSAT (cm^2)	235 [98]	209 [82]	258 [105]
VAT (cm^2)	89 [56]	114 [58]	67 [43]
FeNO (ppb)	18.9 [12.6]	21.3 [13.0]	16.4 [9.5]

Results were based on analyses weighted towards the BMI distribution of the general population. Total population n=6,219; 2955 men and 3264 women. Population with aSAT and VAT measurements n=2,253; 1,190 men and 1,063 women. Results are shown as mean [SD] or percentage.

BMI: Body mass index; WC: waist circumference; TBF: total body fat; aSAT: abdominal subcutaneous adipose tissue; VAT: visceral adipose tissue; FeNO: exhaled nitric oxide; ppb: parts per billion.

Table 5.2 Differences in FeNO in ppb of body fat measure in the total population.

Difference in FeNO (ppb) [95% CI]			
	Total population	**Men**	**Women**
BMI (SD kg/m²)	4.4	3.4	4
Crude model	-0.3 [-0.7; 0.0]	-0.8 [-1.4; -0.2]	-0.5 [-0.9; 0.1]
Model 1[1]	-0.6 [-0.9; -0.3]	-0.7 [-1.3; -0.1]	-0.6 [-1.0; -0.2]
Multivariate[2]	-0.6 [-0.9; -0.3]	-0.6 [-1.2; -0.1]	-0.6 [-1.0; -0.2]
WC (SD cm)	**13.4**	**10.3**	**12.2**
Crude model	0.4 [-0.0; 0.8]	-0.7 [-1.3; -0.1]	-0.5 [-1.0; -0.0]
Model 1[1]	- 0.6 [-1.0; - 0.2]	-0.6 [-1.2; 0.0]	-0.5 [-1.0; -0.1]
Multivariate[2]	- 0.6 [-1.0; - 0.2]	-0.6 [-1.3; 0.0]	-0.6 [-1.1; -0.1]
TBF (SD %)	**9**	**6**	**7**
Crude analyses	-2.0 [-2.5; -1.5]	-0.9 [-1.6; -0.3]	-0.5 [-1.2; 0.1]
Model 1[1]	-0.9 [-1.5; -0.3]	-0.8 [-1.5; -0.2]	-0.6 [-1.2; 0.0]
Multivariate[2]	-0.9 [-1.5; -0.3]	-0.8 [-1.4; -0.1]	-0.6 [-1.2; 0.0]

Results were based on analyses weighted towards the BMI distribution of the general population. Total population n=6,219; 2955 men and 3264 women. Results are shown as mean (SD) or percentage.

ppb: parts per billion; CI: Confidence interval; FeNO: exhaled nitric oxide BMI: Body mass index; SD standard deviation; WC: waist circumference; TBF: total body fat.

[1] Crude model adjusted for age, ethnicity and smoking and in total population additionally for sex
[2] Model 1 additionally adjusted for allergy and asthma

Table 5.3 Difference in FeNO in ppb with deviation of aSAT and VAT in the total population

Difference in FeNO (ppb) [95% CI]			
	Total population	**Men**	**Women**
aSAT (SD cm²)	**98**	**82**	**105**
Crude model	-0.9 [-1.3 ; -0.4]	-0.2 [-0.9; 0.5]	-0.3 [-1.0; 0.4]
Model 1[1]	-0.3 [-0.8; 0.2]	-0.0 [-0.7; 0.6]	-0.4 [-1.1; 0.3]
+ allergy and asthma	-0.3 [-0.8; 0.2]	-0.0 [-0.7; 0.6]	-0.4 [-1.1; 0.2]
+ total body fat	-0.1 [-1.0; 0.8]	1.1 [0.1; 2.1]	-1.2 [-2.6; 0.2]
VAT (SD cm²)	**56**	**58**	**43**
Crude analyses	1.3 [0.7 ; 1.8]	0.1 [-0.8; 1.0]	0.4 [-0.2; 1.1]
model 1[1]	0.1 [-0.5; 0.6]	-0.0 [-0.9; 0.8]	0.2 [-0.4; 0.2]
+ allergy and asthma	0.0 [-0.6; 0.6]	0.1 [-0.9; 0.8]	0.1 [-0.4; 0.7]
+ total body fat	0.5 [-0.4; 1.3]	0.5 [-0.7; 1.6]	0.5 [-0.4; 1.3]

Results were based on analyses weighted towards the BMI distribution of the general population. Population with aSAT and VAT measurements n=2,253; 1,190 men and 1,063 women. Results are shown as mean (SD) or percentage.

ppb: parts per billion; CI: Confidence interval; FeNO: exhaled NO; SD standard deviation; aSAT: abdominal subcutaneous adipose tissue; VAT:visceral adipose tissue.

[1] *Crude model adjusted for age, ethnicity and smoking and in total population for sex*

DISCUSSION

In this large population-based study, there were no clinically relevant associations between any measures of body fat with FeNO. To our knowledge, this is the first large study to investigate the relation between the visceral fat and FeNO.
Even though visceral adipose tissue is related with markers of inflammation [25,26] and is strongly associated with cardiometabolic diseases [35], we showed that both total body fat and visceral fat were not associated with FeNO. Our results are in line with the National Health and Nutrition Examination Surveys (NHANES) for 2007-2010 in which no relationship between BMI and FeNO was observed. [20] Some earlier studies reported a positive relationship between FeNO and BMI [18,19]; this discrepancy with our study may be due to chance as a result of small sample sizes and different study populations. One study only included 33 participants and also included patients with obstructive sleep apnoea [18], while the other study include 122 participants did not correct for sex [19].

Our findings suggest that the low grade inflammation that is associated with obesity does not influence local airway inflammation in the general population. The association between obesity and asthma might be caused by other underlying mechanisms such as increased work of breathing and changed lung mechanisms.[11]. An alternative explanation for the observed association between obesity and asthma may be common causes such as physical inactivity, leading to non-causal associations or overdiagnosis of asthma in obese patients [36,37].

A different explanation is that adipose tissue does increase inflammation in the airways but does not increase FeNO. FeNO is used as a marker for airway inflammation in asthma because it correlates well with sputum eosinophils and airway eosinophilia in bronchial biopsies [16,17]. Whereas allergic asthma is accompanied by Th2 mediated eosinophilic airway inflammation, asthma is now recognized as a heterogeneous disease with various phenotypes [38]. Asthma in the obese has been suggested to represent a specific phenotype that is associated with a higher sputum neutrophil percentage [39,40]. If visceral fat would cause non-eosinophilic airway inflammation, FeNO may not be a good marker for airway inflammation in obese asthmatics. This is supported by a study in morbidly obese patients, in which FeNO levels did not differ between asthmatics and controls [41]. This would be in line with what is known for COPD patients in whom chronic airway inflammation is mainly characterized by neutrophils, macrophages, and mast cells [42], while in the majority of these patients FeNO is not elevated [43].

Strengths of this study are the direct assessment of abdominal adipose tissue depots with MRI in a large population, and the availability of extensive information on multiple potential confounding factors. Our study also has several limitations that need to be considered.
First, we did not assess total VAT volumes, but cross-sectional images at the level of the fifth lumbar vertebra. Nevertheless, cross-sections at this level are highly correlated to total volumes (correlation coefficients around 0.8) [44,45] and can therefore be considered to represent total VAT[45]. Second, we used only FeNO as a measure of local airway inflammation. Although FeNO correlates well with eosinophils in airways it is not a good marker for more neutrophilic inflammation [16,17]. Third, despite extensive phenotyping of the NEO study, residual confounding may remain due to the observational nature of the study.
Additionally because we only had data on self reported asthma we could not perform a

proper sub group analyses. Finally, our study population primarily consists of white individuals and our findings cannot be extrapolated to other ethnic groups.

CONCLUSIONS

In conclusion, this population-based study showed that total body fat and visceral fat were not associated with FeNO. Therefore a higher FeNO in patients and in research can not be explained by obesity. Future prospective studies should investigate to which extent total body fat and visceral fat are associated with the development of local airway inflammation and asthma and reveal the mechanisms underlying the observed association between obesity and asthma.

REFERENCE LIST

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C et al.: Global, regional, and national prevalence of overweight and obesity inchildren and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014, 384:766-781.
2. Kelly T, Yang W, Chen CS, Reynolds K, He J: Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* 2008, 32: 1431-1437.
3. Beuther DA, Sutherland ER: Overweight, obesity, and incident asthma: a metaanalysis of prospective epidemiologic studies. *Am J Respir Crit CareMed* 200, 175: 661-666.
4. Brumpton B, Langhammer A, Romundstad P, Chen Y, Mai XM: General and abdominal obesity and incident asthma in adults: the HUNT study. *Eur Respir J* 2013, 41: 323-329.
5. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M et al.: Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008, 31: 143-178.
6. Assad N, Qualls C, Smith LJ, Arynchyn A, Thyagarajan B, Schuyler M et al.: Body mass index is a stronger predictor than the metabolic syndrome for future asthma in women. The longitudinal CARDIA study. *Am J Respir Crit Care Med* 2013, 188: 319-326.
7. Taylor B, Mannino D, Brown C, Crocker D, Twum-Baah N, Holguin F: Body mass index and asthma severity in the National Asthma Survey. *Thorax* 2008, 63:14-20.
8. Melero MC, Lopez-Vina A, Garcia-Salmones MM, Cisneros SC, Jareno EJ, Ramirez Prieto MT: Factors related with the higher percentage of hospitalizations due to asthma amongst women: the FRIAM study. *Arch Bronconeumo*l 2012, 48: 234-239.
9. van Huisstede A, Rudolphus A, Castro CM, Biter LU, van de Geijn GJ, Taube C et al.: Effect of bariatric surgery on asthma control, lung function and bronchial and systemic inflammation in morbidly obese subjects with asthma. *Thorax* 2015, 70: 659-667.
10. Boulet LP, Turcotte H, Martin J, Poirier P: Effect of bariatric surgery on airway response and lung function in obese subjects with asthma. *Respir Med* 2012, 106: 651-660.
11. Steier J, Lunt A, Hart N, Polkey MI, Moxham J: Observational study of the effect of obesity on lung volumes. *Thorax* 2014, 69: 752-759.
12. Spector SL, Surette ME: Diet and asthma: has the role of dietary lipids been overlooked in the management of asthma? *Ann Allergy Asthma Immunol* 2003, 90:371-377.
13. Hallstrand TS, Fischer ME, Wurfel MM, Afari N, Buchwald D, Goldberg J: Genetic pleiotropy between asthma and obesity in a communty-based sample of twins. *J Allergy Clin Immunol* 2005, 116: 1235-1241.
14. Ferrante AW, Jr.: Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. *J Intern Med* 2007, 262: 408-414.
15. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H et al.: Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 2006, 17: 4-12.
16. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ: Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998, 53: 91-95.
17. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A: Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001, 164: 1376-1381.
18. Depalo A, Carpagnano GE, Spanevello A, Sabato R, Cagnazzo MG, Gramiccioni C et al.: Exhaled NO and iNOS expression in sputum cells of healthy, obese and OSA subjects. *J Intern Med* 2008, 263: 70-78.
19. Maestrelli P, Ferrazzoni S, Visentin A, Marian E, Dal BD, Accordino R et al.: Measurement of exhaled nitric oxide in healthy adults. Sarcoidosis *Vasc Diffuse Lung Dis* 2007, 24: 65-69.
20. Singleton MD, Sanderson WT, Mannino DM: Body mass index, asthma and exhaled nitric oxide in U.S. adults, 2007-2010. *J Asthma* 2014, 51: 756-761.
21. Kim SH, Kim TH, Lee JS, Koo TY, Lee CB, Yoon HJ et al.: Adiposity, adipokines, and exhaled nitric oxide in healthy adults without asthma. *J Asthma* 2011, 48: 177-182.
22. Chandra A, Neeland IJ, Berry JD, Ayers CR, Rohatgi A, Das SR et al.: The relationship of body mass and fat distribution with incident hypertension: observations from the Dallas Heart Study. *J Am Coll Cardiol* 2014, 64: 997-1002.
23. Sluik D, Boeing H, Montonen J, Pischon T, Kaaks R, Teucher B et al.: Associations between general and abdominal adiposity and mortality in individuals with diabetes mellitus. *Am J Epidemiol* 2011, 174: 22-34.
24. Koster A, Murphy RA, Eiriksdottir G, Aspelund T, Sigurdsson S, Lang TF et al.: Fat distribution and mortality: The AGES-Reykjavik study. *Obesity* (Silver Spring) 2015.
25. Tchernof A, Despres JP: Pathophysiology of human visceral obesity: an update. *Physiol Rev* 2013, 93: 359-404.

26. Hamdy O, Porramatikul S, Al-Ozairi E: Metabolic obesity: the paradox between visceral and subcutaneous fat. *Curr Diabetes Rev* 2006, 2: 367-373.
27. de Mutsert R, den Heijer M, Rabelink TJ, Smit JW, Romijn JA, Jukema JW et al.: The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. *Eur J Epidemiol* 2013.
28. Khalili B, Boggs PB, Bahna SL: Reliability of a new hand-held device for the measurement of exhaled nitric oxide. *Allergy* 2007, 62: 1171-1174.
29. Thijs W, de Mutsert R, le Cessie S, Hiemstra PS, Rosendaal FR, Middeldorp S et al.: Reproducibility of exhaled nitric oxide measurements in overweight and obese adults. *BMC Res Notes* 2014, 7: 775.
30. Korn EL, Graubard BI: Epidemiologic studies utilizing surveys: accounting for the sampling design. *Am J Public Health* 1991, 81: 1166-1173.
31. Analysis of Complex Survey Samples [http://www.jstatsoft.org/v09/i08/paper]. 2015.
32. Hoeveel mensen hebben overgewicht? [http://www.rivm.nl/nldemaat]. 2015.
33. Seidell JC, Bouchard C: Visceral fat in relation to health: is it a major culprit or simply an innocent bystander? *Int J Obes Relat Metab Disord* 1997, 21:626-631.
34. Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC: Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006, 61: 817-827.
35. Despres JP, Lemieux I: Abdominal obesity and metabolic syndrome. *Nature* 2006, 444: 881-887.
36. Scott S, Currie J, Albert P, Calverley P, Wilding JP: Risk of misdiagnosis, health-related quality of life, and BMI in patients who are overweight with doctor-diagnosed asthma. *Chest* 2012, 141: 616-624.
37. Aaron SD, Vandemheen KL, Boulet LP, McIvor RA, Fitzgerald JM, Hernandez P et al.: Overdiagnosis of asthma in obese and nonobese adults. *CMAJ* 2008, 179: 1121-1131.
38. Wenzel SE: Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012, 18: 716-725.
39. Scott HA, Gibson PG, Garg ML, Wood LG: Airway inflammation is augmented by obesity and fatty acids in asthma. *Eur Respir J* 2011, 38:594-602.
40. Telenga ED, Tideman SW, Kerstjens HA, Hacken NH, Timens W, Postma DS et al.: Obesity in asthma: more neutrophilic inflammation as a possible explanation for a reduced treatment response. *Allergy* 2012, 67: 1060-1068.
41. van Huisstede A, Rudolphus A, van SA, Cabezas MC, Mannaerts GH, Taube C et al.: Bronchial and systemic inflammation in morbidly obese subjects with asthma: a biopsy study. *Am J Respir Crit Care Med* 2014, 190: 951-954.
42. Barnes PJ: Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol 2008*, 8: 183-192.
43. Donohue JF, Herje N, Crater G, Rickard K: Characterization of airway inflammation in patients with COPD using fractional exhaled nitric oxide levels: a pilot study. *Int J Chron Obstruct Pulmon Dis* 2014, 9: 745-751.
44. Han TS, Kelly IE, Walsh K, Greene RM, Lean ME: Relationship between volumes and areas from single transverse scans of intra-abdominal fat measured by magnetic resonance imaging. *Int J Obes Relat Metab Disord* 1997, 21: 1161-1166.
45. Shen W, Chen J, Gantz M, Velasquez G, Punyanitya M, Heymsfield SB: A single MRI slice does not accurately predict visceral and subcutaneous adipose tissue changes during weight loss. *Obesity* (Silver Spring) 2012, 20: 2458-24

CHAPTER

6

Associations of Serum 25(OH)D Concentrations with Lung Function, Airway Inflammation and Common Cold in the General Population

Willemien Thijs [1,3], Rachida Rafiq[2], Robert Prein [1], Renate T. de Jongh [2], Christian Taube [1,4,] Pieter S. Hiemstra [1], Renée de Mutsert [5] and Martin den Heijer [2,5]

Department of Pulmonology [1], Leiden University Medical Center, Leiden, The Netherlands
Department of Internal Medicine and Endocrinology [2], VU University Medical Center, Amsterdam Movement Sciences, The Netherlands
Department of Pulmonology[3], Haaglanden Medisch Centrum, Den Haag, The Netherlands
Department of Pulmonary Medicine[4], Ruhrlandklinik, West German Lung Center, University Hospital Essen, Germany
Department of Clinical Epidemiology[5], Leiden University Medical Center, The Netherlands

Nutrients. 2018 Jan 3; 10(1): 35

ABSTRACT

Vitamin D is hypothesized to have a beneficial effect on lung function and respiratory infections. The aim of this study was to assess the relationship of serum 25-hydroxyvitamin D (25(OH)D) concentrations with lung function, airway inflammation and common colds. We performed a cross-sectional analysis in the Netherlands Epidemiology of Obesity (NEO) study, a population-based cohort study. We included participants with measurements of serum 25(OH)D, Forced Expiratory Volume in 1 s (FEV_1), Forced Vital Capacity (FVC), Fractional Exhaled Nitric Oxide (FeNO), and data on self-reported common colds (n = 6138). In crude associations, serum 25(OH)D was positively associated with FEV_1 and FVC, and negatively with FeNO and the occurrence of a common cold.

After adjustment for confounders, however, these associations disappeared. Stratified analyses showed that Body Mass Index (BMI) was an effect modifier in the relationship between serum 25(OH)D and FEV_1, FVC and FeNO. In obese participants (BMI ≥ 30 kg/m^2), 10 nmol/L higher 25(OH)D was associated with 0.46% predicted higher FEV_1 (95% Confidence Interval: 0.17 to 0.75), 0.46% predicted higher FVC (0.18 to 0.74), and 0.24 ppb lower FeNO (−0.43 to −0.04). Thus, in the total study population, 25(OH)D concentrations were not associated with lung function, airway inflammation and common colds. In obese participants, however, higher 25(OH)D concentrations were associated with a better lung function and lower airway inflammation.

INTRODUCTION

The role of vitamin D in bone mineralization and calcium homeostasis is well established [1]. In addition, there is a large amount of evidence supporting the influence of vitamin D on immune function and inflammatory disease [2]. In particular, there has been interest in the role of vitamin D in respiratory outcomes such as lung function and respiratory infections [3–10].

In the past decade, several observational studies examined the relationship of vitamin D status with lung function in the general population, leading to inconsistent results, with some studies finding a relationship [4,5], and others not [6,7]. Vitamin D status has also been studied in patients with Chronic Obstructive Pulmonary Disease (COPD) and asthma specifically, where it has been associated with disease severity [11,12]. The exact mechanisms by which vitamin D affects lung function are unknown, but it has been hypothesized that effects of vitamin D on tissue remodeling, muscle function and/or airway inflammation may play a role [13,14].

The role of vitamin D in the immune system has been extensively studied in vitro and vitamin D has been hypothesized to have a dual effect. First, vitamin D decreases inflammatory reactions through the inhibition of NF-κB-pathways [15]. Second, vitamin D improves antimicrobial defense by inducing the production of antimicrobial peptides, and increasing antibacterial and antiviral defenses [16–18]. In observational studies, it has been shown that low vitamin D status is inversely associated with number of respiratory tract infections [5,8–10]. In particular in asthma and COPD patients, respiratory tract infections play an important role as they are associated with exacerbations [19,20], which are the main cause of disease progression, morbidity and mortality in these patients [21,22]. In addition, vitamin D status has been inversely related to measures of airway inflammation in children with asthma [23]. This may suggest that vitamin D deficiency is a risk factor for respiratory infections and inflammation. In two intervention trials in patients with COPD, vitamin D supplementation was shown to decrease exacerbation rate, but only in participants with vitamin D deficiency at baseline [24,25].

Previous studies have shown that the relationship of vitamin D status with lung function and airway inflammation might be affected by sex and adiposity. In an ageing cohort, vitamin D status was only associated with peak expiratory flow (PEF) in men, but not in women [26]. In another study, associations were stronger in subgroups of participants with a Body Mass Index (BMI) of ≥25 kg/m^2 compared to subgroups with a BMI < 25 kg/m^2 [27]. These differences might explain the inconsistent results of previous studies. Our aim was to study the associations of serum 25-hydroxyvitamin D (25(OH)D) concentrations with lung function, airway inflammation and the occurrence of a common cold, and whether associations differed between men and women, or different BMI groups. We hypothesized that low serum 25(OH)D concentrations are associated with an impaired lung function and increased airway inflammation. Furthermore, we hypothesized that low serum 25(OH)D concentrations are associated with a higher number of recent common colds.

MATERIALS AND METHODS

Study Design and Study Population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study in 6671 men and women aged between 45 and 65 years included between September 2008 and September 2012. The present study is a cross-sectional analysis of the baseline measurements.

Design and data collection of the study has been described in detail previously [28]. Men and women with self-reported BMI ≥ 27 kg/m^2 living in the greater area of Leiden (in the west of the Netherlands) were eligible to participate in the NEO study. In addition, in one municipality (Leiderdorp), all inhabitants aged 45 to 65 years were invited, irrespective of their BMI, allowing for a reference distribution of BMI. Participants were invited for a baseline visit at the NEO study center of the Leiden University Medical Center (LUMC) after an overnight fast. Prior to this study visit, participants completed a general questionnaire at home to report demographic, lifestyle and clinical information. All participants underwent an extensive physical examination, including blood sampling and spirometry. In the present analysis, we excluded subjects with missing data. The study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and all participants gave written informed consent.

Data Collection

On the questionnaires, participants reported their medical history, including asthma and COPD, and use of medication, including pulmonary and anti-inflammatory medicine. Ethnicity was assessed by self-identification in eight categories, which were grouped into 'white' and 'other'. Self-reported education was grouped as low versus high education. Tobacco smoking was reported in three categories: 'current smoker', 'former smoker' and 'never smoker'. In addition, the number of pack-years was calculated. Participants also reported when they had had a common cold for the last time, to which they could answer with: 'within a week', 'between 1 week and 1 month ago', 'between 1 and 6 months ago', 'between 6 months and a year ago' or 'more than 1 year ago'. For the analyses, this was dichotomized into 'within a month' and 'more than a month ago'. Participants reported the frequency and duration of their physical activity during leisure time using the Short Questionnaire to assess health-enhancing physical activity. This was expressed in Metabolic Equivalent of Task-hours (MET-hours) per week [29].

25(OH)D Measurements

At the baseline study visit, a fasting blood sample of venous blood was collected by venipuncture, and immediately sent to the central clinical laboratory of the LUMC for the assessment of serum 25-hydroxyvitamin D (25(OH)D) concentrations. During the inclusion period of the NEO study, quantification of the 25(OH)D concentration in the serum was done by three sequential methods. From 1 September 2008 to 4 October 2010, the radioimmunoassay (RIA) method was used (DiaSorin, Saluggia, Italy). From 5 October 2010 to 29 September 2011, the Chemoluminescent Immunoassay was used (iSYS analyzer, ImmunoDiagnostics Inc., Boldon, UK). Finally, from 30 September 2011 until the end of the study, the 2nd generation Electrochemoluminescence Immunoassay (ECLIA) (Modular Analytics E170 analyzers,

Roche Diagnostics, Mannheim, Germany) was used. Two-level commercial Internal Quality Control (IQC) samples were used in all three methods to monitor performance. Maximum overall CVa was <12%. All methods have stated specificity for both 25-hydroxyvitamin D2 and D3 (25(OH)D2 and 25(OH)D3).

Because three different immunoassays were used during the study period, serum 25(OH)D was calibrated towards the "golden standard" liquid chromatography/tandem mass spectrometry (LC-MS/MS) method (isotope dilution/online solid-phase extraction liquid chromatography/tandem mass spectrometry (ID-XLC-MS/MS)) to minimize possible variations. These LC-MS/MS measurements were performed at the Endocrine Laboratory of the VU University Medical Center (Amsterdam, The Netherlands) as described before [30]. The limit of quantitation (LOQ) was 4.0 nmol/L; intra-assay CV was <6%, and inter-assay CV was <8% for concentrations between 25 and 180 nmol/L. 25(OH)D2 and 25(OH)D3 were measured separately. From measurements of each of the three different 25(OH)D assays used, 50 samples were selected to determine serum 25(OH)D with LC/MS-MS. Previous studies have shown that 50 samples suffice to fit an equation for comparison between the different assays [31]. Samples were selected according to tentiles of serum 25(OH)D within each of the methods used. Within each tentile, 5 samples were selected at intervals during the period in which the method was used. This time-dependent sampling was added to minimize the contribution of inter-assay variation to variability between the different assays. Calibrated serum 25(OH)D concentrations were then calculated using linear regression formulas.

Lung Function Assessments

All participants of the NEO study underwent spirometry at the Pulmonology department of the LUMC. The Forced Expiratory Volume in 1 s (FEV_1) and Forced Vital Capacity (FVC) were determined. Participants were required to perform at least three reproducible forced expiratory maneuvers, with a maximum difference of 5 percent or 150 mL between the highest and lowest measurement. Of these three maneuvers, the one with the highest value of FEV_1 and FVC together was used in the analyses [32].

Fractional Exhaled Nitric Oxide

Fractional Exhaled Nitric Oxide (FeNO) was measured using a portable analyzer, the NIOX MINO (Aerocrine AB, Solna, Sweden). Participants performed a 10-s slow steady exhalation. Our previously published results demonstrate that, in large cohorts of overweight and obese adults, a single exhaled NO measurement suffices, and therefore one recording expressed as parts per billion (ppb) was made [33].

Body Composition Measure

At the study site, height and weight were measured with precision of 0.1 cm/kg. BMI was calculated by dividing the weight in kilograms by the height in squared meters. For stratification, BMI was categorized into three categories: <25, 25–30 and ≥30 kg/m^2 according to WHO criteria [34]. Waist circumference was measured between the border of the lower costal margin and the iliac crest with the precision of 0.1 cm. Total body fat (TBF) was estimated by bio-electrical impedance analysis (BIA) using the Tanita foot-to-foot BIA system TBF-300A Body Composition Analyzer (Tanita Corporation of America, Inc., Arlington Heights, IL, USA) [35].

Statistical Analyses

Data were analyzed using STATA version 13.1 (StataCorp LP, College Station, TX, USA). In the NEO study, there is an oversampling of persons with BMI ≥ 27 kg/m^2. To correctly represent associations in the general population, adjustments were made for the oversampling of individuals with a BMI ≥ 27 kg/m^2 [36]. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality whose BMI distribution was similar to the BMI distribution in the general Dutch population and could serve as a reference population [37]. Using the BMI distribution of this reference population, we calculated weight factors for the NEO population, resulting in a higher weight factor for participants with a lower BMI. Using these weight factors, we weighted all our analyses towards the BMI distribution of the general population [38]. Consequently, results apply to a population-based study without oversampling of persons with a BMI ≥ 27 kg/m^2.

We summarized the baseline characteristics as mean (standard deviation, SD) for normally distributed continuous variables, median (interquartile range) for skewed continuous variables and percentages for categorical variables, stratified by categories of serum 25(OH)D concentrations (serum 25(OH)D <50 nmol/L, 50–75 nmol/L and ≥75 nmol/L [2]). Linear regression analyses were used to examine the associations between serum 25(OH)D and FEV_1, FVC and FeNO. Logistic regression analyses were used to calculate the odds ratio (OR) of the occurrence of a common cold in the preceding month per 10 nmol/L serum 25(OH)D.

The crude regression models were adjusted for age, sex, ethnicity, packyears of smoking, self-reported obstructive pulmonary disease, use of pulmonary and anti-inflammatory medication, educational level, season, physical activity, BMI, waist circumference and total body fat. Because serum 25(OH)D concentrations follow a sinusoidal pattern throughout the year, adjustment for season was performed using a cosinor model[39]. Potential effect modification by sex, age and BMI was examined by performing regression analyses stratified for sex, age and BMI categories. Finally, a sensitivity analysis was performed in participants with 25(OH)D concentrations <50 nmol/L.

RESULTS

After exclusion of participants with missing data, we included 6138 participants in our analyses. The characteristics of the study population stratified by serum 25(OH)D categories are shown in Table 6.1. Of the total study population, 20% had serum 25(OH)D concentrations lower than 50 nmol/L. Participants with lower serum 25(OH)D concentrations (<50 nmol/L) more often had asthma and COPD, a higher BMI, total body fat and waist circumference, compared to participants with higher serum 25(OH)D concentrations. In addition, they had higher FEV_1 and FVC, and more often reported a common cold in the preceding month.

Table 6.1 Characteristics of participants aged 45–65 years of the Netherlands Epidemiology of Obesity study, stratified by serum 25(OH)D (nmol/L) category.

25(OH)D (nmol/L) Category	<50	50–75	≥75	*p*-Value *
Proportion of study population (%)	20	37	43	
25(OH)D (nmol/L)	39.5 (8.6)	62.7 (7.2)	93.6 (14.7)	<0.01
Age (years)	55.0 (6.7)	55.9 (6.1)	55.7 (5.6)	0.05
Sex (% men)	47	48	39	<0.01
White (%)	87	97	98	<0.01
Educational level (%high)	45	47	48	0.31
Smoking (%)				
Current	20	17	13	<0.01
Former	40	45	49	<0.01
Packyears	2.9 (0.0–15.2)	3.6 (0.0–15.2)	2.2 (0.0–14.0)	0.12
Season (% winter)	66	55	38	<0.01
Physical activity (MET/h)	23.0 (11.0–44.3)	28.5 (15.0–46.8)	34.5 (19.5–55.9)	<0.01
BMI (kg/m^2)	27.4 (5.9)	26.5 (4.4)	25.5 (3.6)	<0.01
Total body fat (%)	32.3 (10.4)	31.5 (9.1)	31.3 (7.5)	0.03
Waist circumference (cm)	94.9 (16.6)	93.2 (13.1)	89.8 (11.7)	<0.01
Self-reported asthma (%)	6.1	4.8	3.7	0.02
Self-reported COPD (%)	5.4	4.7	3.3	<0.01
Use of pulmonary and anti-inflammatory medication (%)	15	13	13	0.11
FEV_1 (%predicted)	105.7 (18.8)	107.2 (16.8)	109.3 (14.1)	<0.01
FVC (%predicted)	113.4 (17.7)	115.9 (16.9)	118.8 (14.3)	<0.01
Fe_{NO} (ppb)	19.0 (14.5)	19.0 (13.5)	18.7 (11.0)	0.57
Self-reported common cold in preceding month (%)	28	22	21	<0.01

*Data are presented as mean (SD), percentage or median (interquartile range). Results are based on analyses weighted towards the BMI distribution of the general population (n = 6138). 25(OH)D: 25-hydroxyvitamin D; BMI: Body Mass Index; COPD: Chronic Obstructive Pulmonary Disease; FEV_1: Forced Expiratory Volume in 1 s. FVC: Forced Vital Capacity; FeNO: fractional exhaled nitric oxide; ppb: parts per billion. * p-value for trend.*

In Figure 6.1, we have plotted the mean serum 25(OH)D concentrations and percentage of participants that reported a recent common cold, per month. Both serum 25(OH)D concentrations and the occurrence of a common cold showed a sinusoidal pattern, with an inverse relationship between serum 25 (OH) D concentrations and the occurrence of a common cold.

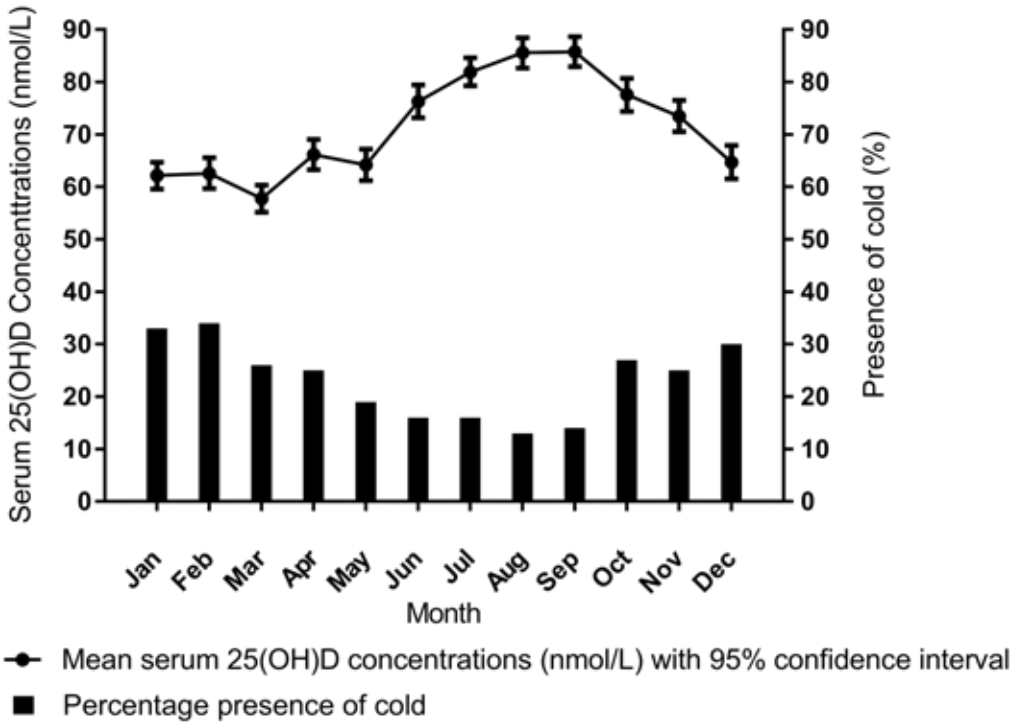


Figure 6.1 Mean serum 25(OH)D concentrations and percentage of participants that reported a recent common cold per month, in men and women participating in the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years.
Data are presented as mean (95% confidence interval) and percentage. Results are based on analyses weighted towards the BMI distribution of the general population (n = 6138). Results are shown per month, combined over different years.

The results of the regression analyses are shown in Table 6.2. In the crude associations, serum 25(OH)D was positively associated with FEV_1 and FVC, and negatively associated with FeNO and the occurrence of a common cold in the preceding month. In addition, 10 nmol/L higher serum 25(OH)D was associated with 0.48% predicted higher FEV_1 (95% CI: 0.23 to 0.73), 0.83% predicted higher FVC (0.58 to 1.07) and 0.18 ppb lower FeNO (−0.39 to 0.03). The OR of the occurrence of a common cold in the preceding month was 0.94 (0.90 to 0.98) per 10 nmol/L serum 25(OH)D. After adjustment for all confounding factors, however, these associations largely disappeared. Adjustment for season attenuated the regression coefficients of FEV_1 with 40%, of FVC with 27%, and of FeNO with 43%. Relationship with common colds was attenuated with 3%. A sensitivity analysis in participants with 25(OH)D concentrations <50 nmol/L did not show an association (Supplementary Table S1). An analysis in

participants using vitamin D and multivitamin supplements only, also did not show different results (Supplementary Table S2).

Table 6.2 Associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and occurrence of a common cold in men and women participating in the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years.

	Crude	Multivariate [1]	+BMI, TBF, WC [2]
	Regression coefficient (95% CI) per 10 nmol/L 25(OH)D		
FEV_1 (% predicted)	0.48 (0.23 to 0.73)	0.23 (−0.05 to 0.51)	0.10 (−0.18 to 0.39)
FVC (% predicted)	0.83 (0.58 to 1.07)	0.51 (0.24 to 0.77)	0.31 (0.04 to 0.57)
Fe_{NO} (ppb)	−0.18 (−0.39 to 0.03)	0.15 (−0.07 to 0.38)	0.16 (−0.06 to 0.36)
	Odds Ratio (95%CI) per 10 nmol/L 25(OH)D		
Common cold	0.94 (0.90 to 0.98)	1.00 (0.95 to 1.04)	1.00 (0.96 to 1.05)

Results were based on analyses weighted towards the BMI distribution of the general population (n = 6138), and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as difference in outcome measure per 10 nmol/L 25(OH)D.
1 Multivariate: Adjusted for age, sex, ethnicity, number of packyears, self-reported obstructive pulmonary disease, use of pulmonary and anti-inflammatory medication, educational level, season and physical activity. 2 Multivariate plus adjustments for BMI, total body fat and waist circumference. CI: confidence interval; BMI: body mass index; FEV_1: Forced Expiratory Volume in 1 s; FVC: Forced Vital Capacity; FeNO: fractional exhaled nitric oxide; ppb: parts per billion; OR: Odds Ratio.

After stratification for BMI categories, the associations of serum 25(OH)D with FEV_1, FVC and FeNO differed per BMI category (Figure 6.2, Supplementary Table S3). Because BMI was an effect modifier in these relationships, we subsequently stratified all models by BMI (BMI < 25: 43%, BMI 25–30: 41% and BMI ≥ 30: 16% of study population). In participants with obesity (BMI ≥ 30), serum 25(OH)D was positively associated with FEV_1 and FVC, and negatively associated with FeNO. In participants with a BMI < 25, serum 25(OH)D was not associated with these outcomes after adjustment for confounding factors. Nevertheless, the stratified results showed a dose–response trend with stronger associations in higher BMI categories. No differences were found between the sexes and different age categories (Supplementary Tables S4 and S5).

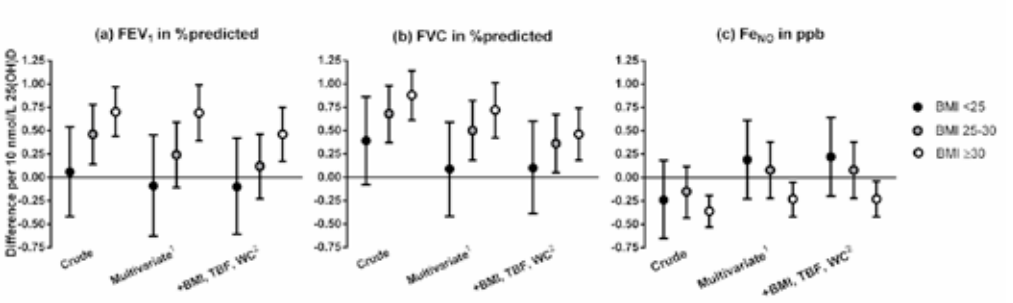


Figure 6.2 Associations of serum 25(OH)D (per 10 nmol/L) with (a) FEV_1, (b) FVC and (c) FeNO stratified by Body Mass Index (BMI) category, in men and women participating in the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years.
Results were based on analyses (n = 6138) weighted towards the BMI distribution of the general population, and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as difference in outcome measure per 10 nmol/L 25(OH)D stratified by BMI category (BMI < 25: 43%, BMI 25–30: 41% and BMI ≥ 30: 16%). 1 Multivariate: Adjusted for age, sex, ethnicity, number of packyears, self-reported obstructive pulmonary disease, use of pulmonary and anti-inflammatory medication, educational level, season and physical activity. 2 Multivariate plus adjustments for BMI, total body fat and waist circumference. FEV_1: Forced Expiratory Volume in 1 s; FVC: Forced Vital Capacity; FeNO: fractional exhaled nitric oxide; ppb: parts per billion; BMI: Body Mass Index; TBF: total body fat; WC: waist circumference.

DISCUSSION

In this study, we assessed the relationship between serum 25(OH)D concentrations and lung function, fractional exhaled nitric oxide and common colds in a population-based cohort study. Whereas there were no associations in the total population, we observed that higher serum 25(OH)D concentrations were associated with a better lung function and lower airway inflammation in participants with a BMI ≥ 30, but not in participants with a BMI < 30. Serum 25(OH)D concentrations were not associated with the occurrence of common colds in the last month.

Several previous observational studies have assessed the relationship between vitamin D and lung function. Results of these studies have been inconsistent. In some studies, a positive association between vitamin D status and lung function was found [4,5,40], while in others this was not confirmed [6,7]. Two studies showed an association between serum 25(OH)D concentrations and lung function in patients with COPD and asthma [11,12]. These differences in study results might be caused by differences in study population and vitamin D status of participants. In our study, we observed an association in participants with obesity, in addition to a dose–response trend after stratification by BMI categories. This is in line with the finding of a previous study that observed an association between 25(OH)D and lung function in participants with obesity, but not in those without obesity [27]. Another recent study in asthmatic children reported an association between vitamin D status and lung function in obese, but not in non-obese children [41].

The explanation for this relationship in obesity only is unclear. It remains difficult to disentangle true causal associations in obesity from the potential confounding effect of obesity as a common cause of both low vitamin D concentrations and impaired lung function. One

explanation might be that a potential relationship of vitamin D status with lung function is only present in participants with vitamin D deficiency. As vitamin D deficiency is more prevalent in individuals with obesity, this might explain why we did find a relationship in obese, but not in non-obese participants. A sensitivity analysis in participants with vitamin D deficiency (serum 25(OH)D concentrations < 50 nmol/L) in our study did not show an association. However, this group was small and might therefore have been underpowered.

Several studies have shown an association between adiposity and lung function [42–45]. A potential effect of obesity on lung function has been explained by effects on metabolic dysregulation, systemic inflammation and mechanical load of truncal fat [46]. Nevertheless, vitamin D deficiency has also been associated with recent-onset obesity [47,48] and it has been hypothesized that adiposity is an intermediate in the relationship between vitamin D and lung function through a direct mechanical effect on the diaphragm [27]. Finally, an explanation for the findings might be that adiposity is indeed an effect modifier. Vitamin D might exert a direct effect on lung function through tissue remodeling, muscle function and/or airway inflammation and the presence of adiposity may affect this relationship, as recently suggested [13].

We also observed an association between serum 25(OH)D concentrations and FeNO in obese, but not in non-obese participants. FeNO is a marker for Th2-mediated allergic inflammation, which is mainly used in the diagnostics of allergic asthma. FeNO is produced by lung epithelial cells mainly by inducible nitric oxide synthase (iNOS/NOS2), of which expression is increased during allergic airway inflammation [49,50]. In our study, we found a negative relationship between serum 25(OH)D concentrations and FeNO levels in obese individuals. This suggests that a higher vitamin D status is associated with lower airway inflammation in these participants. Vitamin D is known to be a potent immunomodulator and has been shown to decrease inflammatory reactions in vitro[15,51]. Few studies, however, have investigated the relationship between vitamin D and airway inflammation. In two cross-sectional studies in (asthmatic) children, serum 25(OH)D concentrations were not associated with FeNO [52,53]. Three intervention trials did also not show an effect of vitamin D supplementation on FeNO in patients with asthma [54–56]. Potential differences with our findings might be caused by the differences in study design. Previous studies did not report on potential effect modification of obesity. In one study, obese individuals were even excluded from the study [53]. In addition, our study was performed in the general adult population. FeNO levels are likely to be lower in the general population than in preselected cohorts of asthma patients. Possibly, vitamin D suppresses the lower levels of iNOS expression in these populations more readily than in patients with eosinophilic asthma. However, this is a hypothesis and exact mechanisms remain to be elucidated in future studies.

In our study, serum 25(OH)D concentrations were not related to the occurrence of a common cold in the preceding month. The relationship between vitamin D and respiratory infections has been extensively studied in observational studies [8,57]. In addition, trials assessing the effect of vitamin D supplementation have remained inconclusive [58–62]. The most recent meta-analysis showed a protective effect of vitamin D supplementation against respiratory infections, with larger effects in patients with vitamin D deficiency at baseline [62].

In our study, only 20% of the study population had serum 25(OH)D concentrations less than 50 nmol/L. This might explain why we did not find an association with the occurrence of a common cold in our study.

Strengths of this study are the large study population and the detailed phenotyping of the population, allowing extensive adjustment for relevant confounding factors. A major limitation of this study is the cross-sectional design. Therefore, no conclusions can be drawn regarding the direction and causality of relationships. Another limitation is that serum 25(OH)D measurements were performed by three different assays during the study period. To minimize possible variations, we calibrated our serum 25(OH)D measurements to the golden standard LC-MS/MS. In addition, the occurrence of a common cold, medical history and medication use were assessed by self-report, which might affect reliability. This may have resulted in an underestimation of the occurrence of common colds, which might have led to a reduced power to detect a potential relationship. We did not have reliable data on vitamin D supplementation use and dietary intake, and therefore could not rule out an effect of vitamin D supplementation. In addition, we did not have data on sunshine exposure. The physical activity questionnaire, however, did contain several outdoor activities such as cycling, walking and gardening, and, therefore, could be used as a proxy for sunshine exposure. Finally, while we did find associations of serum 25(OH)D concentrations with lung function and airway inflammation in obese participants, these differences were very small. This makes it difficult to assess the clinical relevance of our results.

CONCLUSIONS

This study showed that higher serum 25(OH)D concentrations were associated with a better lung function and less airway inflammation in obese, but not in non-obese individuals. Serum 25(OH)D concentrations were not associated with the occurrence of a recent common cold. Further studies are needed to assess the causal pathways and clinical relevance of our findings. Studies investigating the effects of vitamin D supplementation should specifically target persons with obesity and study specific effects stratified by BMI. Finally the underlying mechanisms by which obesity affects the relationship of serum 25(OH)D is still unclear and needs to be further studied.

ACKNOWLEDGMENTS

The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Center, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'. We express our gratitude to all individuals who participate in the NEO study. We are grateful to all participating general practitioners for inviting eligible participants. We furthermore thank Pat R. van Beelen and all research nurses for collecting the data, Petra J. Noordijk and her team for sample handling, and Ingeborg de Jonge for all data management of the NEO study.

REFERENCE LIST

1. Lips, P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implica tions. *Endocr. Rev.* 2001, 22, 477–501.
2. Holick, M.F. Vitamin D deficiency. *N. Engl. J. Med.* 2007, 357, 266–281.
3. Hughes, D.A.; Norton, R. Vitamin D and respiratory health. *Clin. Exp. Immunol.* 2009, 158, 20–25.
4. Black, P.N.; Scragg, R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the third national health and nutrition examination survey. *Chest* 2005, 128, 3792–3798.
5. Berry, D.J.; Hesketh, K.; Power, C.; Hypponen, E. Vitamin D status has a linear association with seasonal infections and lung function in british adults. *Br. J. Nutr.* 2011, 106, 1433–1440.
6. Shaheen, S.O.; Jameson, K.A.; Robinson, S.M.; Boucher, B.J.; Syddall, H.E.; Sayer, A.A.; Cooper, C.; Holloway, J.W.; Dennison, E.M. Relationship of vitamin D status to adult lung function and copd. *Thorax* 2011, 66, 692–698.
7. Kunisaki, K.M.; Niewoehner, D.E.; Singh, R.J.; Connett, J.E. Vitamin D status and longitudinal lung function decline in the lung health study. Eur. Respir. J. 2011, 37, 238–243.
8. Ginde, A.A.; Mansbach, J.M.; Camargo, C.A., Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the third national health and nutrition examination survey. *Arch. Intern. Med*. 2009, 169, 384–390.
9. Monlezun, D.J.; Bittner, E.A.; Christopher, K.B.; Camargo, C.A.; Quraishi, S.A. Vitamin d status and acute respiratory infection: Cross sectional results from the United States national health and nutrition examination survey, 2001–2006. *Nutrients* 2015, 7,1933–1944.
10. Laaksi, I.; Ruohola, J.P.; Tuohimaa, P.; Auvinen, A.; Haataja, R.; Pihlajamaki, H.; Ylikomi, T. An association of serum vitamin D concentrations <40 nmol/L with acute respiratory tract infection in young Finnish men. Am. J. Clin. Nutr. 2007, 86, 714–717.
11. Janssens, W.; Bouillon, R.; Claes, B.; Carremans, C.; Lehouck, A.; Buysschaert, I.; Coolen, J.; Mathieu, C.; Decramer, M.; Lambrechts, D. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. *Thorax* 2010, 65, 215–220.
12. Sutherland, E.R.; Goleva, E.; Jackson, L.P.; Stevens, A.D.; Leung, D.Y. Vitamin d levels, lung function, and steroid response in adult asthma. *Am. J. Respir. Crit. Care Med.* 2010, 181, 699–704.
13. Gilbert, C.R.; Arum, S.M.; Smith, C.M. Vitamin D deficiency and chronic lung disease. *Can. Respir. J.* 2009, 16, 75–80.
14. Janssens, W.; Decramer, M.; Mathieu, C.; Korf, H. Vitamin D and chronic obstructive pulmonary disease: Hype or reality? Lancet Respir. Med. 2013, 1, 804–812.
15. Chen, Y.; Zhang, J.; Ge, X.; Du, J.; Deb, D.K.; Li, Y.C. Vitamin D receptor inhibits nuclear factor kappa b activation by interacting with ikappab kinase beta protein. J. Biol. Chem. 2013, 288, 19450–19458.
16. Wang, T.T.; Nestel, F.P.; Bourdeau, V.; Nagai, Y.; Wang, Q.; Liao, J.; Tavera-Mendoza, L.; Lin, R.; Hanrahan, J.W.; Mader, S.; et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J. Immunol.* 2004, 173, 2909–2912.
17. Brockman-Schneider, R.A.; Pickles, R.J.; Gern, J.E. Effects of vitamin D on airway epithelial cell morphology and rhinovirus replication. *PLoS ONE* 2014, 9, e86755.
18. Liu, P.T.; Stenger, S.; Li, H.; Wenzel, L.; Tan, B.H.; Krutzik, S.R.; Ochoa, M.T.; Schauber, J.; Wu, K.; Meinken, C.; et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006, 311, 1770–1773.
19. Nicholson, K.G.; Kent, J.; Ireland, D.C. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993, 307, 982–986.
20. Seemungal, T.; Harper-Owen, R.; Bhowmik, A.; Moric, I.; Sanderson, G.; Message, S.; Maccallum, P.; Meade, T.W.; Jeffries, D.J.; Johnston, S.L.; et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 2001, 164, 1618–1623.
21. Anthonisen, N.R.; Wright, E.C.; Hodgkin, J.E. Prognosis in chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.* 1986, 133, 14–20.
22. Krishnan, V.; Diette, G.B.; Rand, C.S.; Bilderback, A.L.; Merriman, B.; Hansel, N.N.; Krishnan, J.A. Mortality in patients hospitalized for asthma exacerbations in the united states. *Am. J. Respir. Crit. Care Med.* 2006, 174, 633–638.
23. Brehm, J.M.; Celedon, J.C.; Soto-Quiros, M.E.; Avila, L.; Hunninghake, G.M.; Forno, E.; Laskey, D.; Sylvia, J.S.; Hollis, B.W.; Weiss, S.T.; et al. Serum vitamin D levels and markers of severity of childhood asthma in costa rica. *Am. J. Respir. Crit. Care Med.* 2009, 179, 765–771.
24. Lehouck, A.; Mathieu, C.; Carremans, C.; Baeke, F.; Verhaegen, J.; Van Eldere, J.; Decallonne, B.; Bouillon, R.; Decramer, M.; Janssens, W. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: A randomized trial. *Ann. Intern. Med.* 2012, 156, 105–114.
25. Martineau, A.R.; James, W.Y.; Hooper, R.L.; Barnes, N.C.; Jolliffe, D.A.; Greiller, C.L.; Islam, K.; McLaughlin, D.; Bhowmik, A.; Timms, P.M.; et al. Vitamin D3 supplementation in patients with

chronic obstructive pulmonary disease (vidico): A multicentre, double-blind, randomised controlled trial. *Lancet Respir. Med.* 2015, 3, 120–130.
26. Van Schoor, N.M.; de Jongh, R.T.; Daniels, J.M.A.; Heymans, M.W.; Deeg, D.J.H.; Lips, P. Peak expiratory flow rate shows a gender-specific association with vitamin d deficiency. *J. Clin. Endocrinol. Metab.* 2012, 97, 2164–2171.
27. Khan, S.; Mai, X.M.; Chen, Y. Plasma 25-hydroxyvitamin D associated with pulmonary function in canadian adults with excess adiposity. *Am. J. Clin. Nutr.* 2013, 98, 174–179.
28. De Mutsert, R.; den Heijer, M.; Rabelink, T.J.; Smit, J.W.A.; Romijn, J.A.; Jukema, J.W.; de Roos, A.; Cobbaert, C.M.; Kloppenburg, M.; le Cessie, S.; et al. The Netherlands epidemiology of obesity (neo) study: Study design and data collection. *Eur. J. Epidemiol.* 2013, 28, 513–523.
29. Wendel-Vos, G.C.W.; Schuit, A.J.; Saris, W.H.M.; Kromhout, D. Reproducibility and relative validity of the short questionnaire to assess health -enhacing physical activity. *J. Clin. Epidemiol.* 2003, 56, 1163–1169.
30. Dirks, N.F.; Vesper, H.W.; van Herwaarden, A.E.; van den Ouweland, J.M.; Kema, I.P.; Krabbe, J.G.; Heijboer, A.C. Various calibration procedures result in optimal standardization of routinely used 25(OH)D ID-LC-MS/MS methods. *Clin. Chim. Acta* 2016, 462, 49–54.
31. Heijboer, A.C.; Blankenstein, M.A.; Kema, I.P.; Buijs, M.M. Accuracy of 6 routine 25-hydroxyvitamin D assays: Influence of vitamin d binding protein concentration. *Clin. Chem.* 2012, 58, 543 –548.
32. Miller, M.R.; Hankinson, J.; Brusasco, V.; Burgos, F.; Casaburi, R.; Coates, A.; Crapo, R.; Enright, P.; van der Grinten, C.P.M.; Gustafsson, P.; et al. Standardisation of spirometry. *Eur. Respir. J.* 2005, 26, 319–338.
33. Thijs, W.; de Mutsert, R.; le Cessie, S.; Hiemstra, P.S.; Rosendaal, F.R.; Middeldorp, S.; Rabe, K.F. Reproducibility of exhaled nitric oxide measurements in overweight and obese adults. *BMC Res. Notes* 2014, 7, 775.
34. World Health Organization. Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation; World Health Organization Technical Report Series 894; World Health Organization: Geneva, Switzerland, 2000; pp. 1-253.
35. Ritchie, J.D.; Miller, C.K.; Smiciklas-Wright, H. Tanita foot-to-foot bioelectrical impedance analysis system validated in older adults. *J. Am. Diet. Assoc*. 2005, 105, 1617–1619.
36. Korn, E.L.; Graubard, B.I. Epidemiologic studies utilizing surveys: Accounting for the sampling design. *Am. J. Public Health* 1991, 81, 1166–1173.
37. Ministerie van VWS. Hoeveel Mensen Hebben Overgewicht? Available online: http://www.rivm.nl/nldemaat (accessed on 13 December 2017).
38. Lumley, T. Analysis of Complex Survey Samples. Available online: https://www.jstatsoft.org/article/view/v009i08 (accessed on 13 December 2017).
39. Sachs, M.C.; Shoben, A.; Levin, G.P.; Robinson-Cohen, C.; Hoofnagle, A.N.; Swords-Jenny, N.; Ix, J.H.; Budoff, M.; Lutsey, P.L.; Siscovick, D.S.; et al. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: The multi-ethnic study of atherosclerosis. *Am. J. Clin. Nutr.* 2013, 97, 1243–1251.
40. Afzal, S.; Lange, P.; Bojesen, S.E.; Freiberg, J.J.; Nordestgaard, B.G. Plasma 25-hydroxyvitamin D, lung function and risk of chronic obstructive pulmonary disease.Thorax 2014, 69, 24–31.
41. Lautenbacher, L.A.; Jariwala, S.P.; Markowitz, M.E.; Rastogi, D. Vitamin D and pulmonary function in obese asthmatic children. *Pediatr. Pulmonol.* 2016, 51, 1276–1283.
42. Lin, W.Y.; Yao, C.A.; Wang, H.C.; Huang, K.C. Impaired lung function is associated with obesity and metabolic syndrome in adults. Obesity (Silver Spring) 2006, 14, 1654–1661.
43. Li, A.M.; Chan, D.; Wong, E.; Yin, J.; Nelson, E.A.; Fok, T.F. The effects of obesity on pulmonary function. Arch. Dis. Child. 2003, 88, 361–363.
44. Sutherland, T.J.; Goulding, A.; Grant, A.M.; Cowan, J.O.; Williamson, A.; Williams, S.M.; Skinner, M.A.; Taylor, D.R. The effect of adiposity measured by dual-energy x-ray absorptiometry on lung function. *Eur. Respir. J.* 2008, 32, 85–91.
45. Rastogi, D.; Bhalani, K.; Hall, C.B.; Isasi, C.R. Association of pulmonary function with adiposity and metabolic abnormalities in urban minority adolescents. *Ann. Am. Thorac. Soc.* 2014, 11, 744–752.
46. Dixon, A.E.; Holguin, F.; Sood, A.; Salome, C.M.; Pratley, R.E.; Beuther, D.A.; Celedon, J.C.; Shore, S.A. An official American thoracic society workshop report: Obesity and asthma. *Proc. Am. Thorac. Soc.* 2010, 7, 325–335.
47. Pereira-Santos, M.; Costa, P.R.; Assis, A.M.; Santos, C.A.; Santos, D.B. Obesity and vitamin D deficiency: A systematic review and meta-analysis. *Obes. Rev.* 2015, 16, 341–349.
48. Mai, X.M.; Chen, Y.; Camargo, C.A., Jr.; Langhammer, A. Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin d level and obesity in adults: The hunt study. *Am. J. Epidemiol.* 2012, 175,

1029–1036.
49. Taylor, D.R.; Pijnenburg, M.W.; Smith, A.D.; De Jongste, J.C. Exhaled nitric oxide measurements: Clinical application and interpretation. *Thorax* 2006, 61, 817–827.
50. Roos, A.B.; Mori, M.; Gronneberg, R.; Osterlund, C.; Claesson, H.E.; Wahlstrom, J.; Grunewald, J.; Eklund, A.; Erjefalt, J.S.; Lundberg, J.O.; et al. Elevated exhaled nitric oxide in allergen-provoked asthma is associated with airway epithelial inos. *PLoS ONE* 2014, 9, e90018.
51. Calton, E.K.; Keane, K.N.; Newsholme, P.; Soares, M.J. The impact of vitamin D levels on inflammatory status: A systematic review of immune cell studies. *PLoS ONE* 2015, 10, e0141770.
52. Yao, T.C.; Tu, Y.L.; Chang, S.W.; Tsai, H.J.; Gu, P.W.; Ning, H.C.; Hua, M.C.; Liao, S.L.; Tsai, M.H.; Chiu, C.Y.; et al. Serum 25-hydroxyvitamin D levels in relation to lung function and exhaled nitric oxide in children. *J. Pediatr.* 2014, 165, 1098–1103.
53. Dabbah, H.; Bar Yoseph, R.; Livnat, G.; Hakim, F.; Bentur, L. Bronchial reactivity, inflammatory and allergic parameters, and vitamin D levels in children with asthma. *Respir. Care* 2015, 60, 1157–1163.
54. Bar Yoseph, R.; Livnat, G.; Schnapp, Z.; Hakim, F.; Dabbah, H.; Goldbart, A.; Bentur, L. The effect of vitamin D on airway reactivity and inflammation in asthmatic children: A double-blind placbo-controlled trial. *Pediatr. Pulmonol.* 2015, 50, 747-753.
55. Martineau, A.R.; MacLaughlin, B.D.; Hooper, R.L.; Barnes, N.C.; Jolliffe, D.A.; Greiller, C.L.; Kilpin, K.; McLaughlin, D.; Fletcher, G.; Mein, C.A.; et al. Double-blind randomised placebo-controlled trial of bolus-dose vitamin D3 supplementation in adults with asthma (vidias). *Thorax* 2015, 70, 451–457.
56. de Groot, J.C.; van Roon, E.N.; Storm, H.; Veeger, N.J.; Zwinderman, A.H.; Hiemstra, P.S.; Bel, E.H.; ten Brinke, A. Vitamin D reduces eosinophilic airway inflammation in nonatopic asthma. *J. Allergy Clin. Immunol.* 2015, 135, 670–675.
57. Sabetta, J.R.; DePetrillo, P.; Cipriani, R.J.; Smardin, J.; Burns, L.A.; Landry, M.L. Serum 25-hydroxyvitamin D and the incidence of acute viral respiratory tract infections in healthy adults. *PLoS ONE* 2010, 5, e11088.
58. Charan, J.; Goyal, J.P.; Saxena, D.; Yadav, P. Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis. *J. Pharmacol. Pharmacother.* 2012, 3, 300–303.
59. Vuichard Gysin, D.; Dao, D.; Gysin, C.M.; Lytvyn, L.; Loeb, M. Effect of vitamin D3 supplementation on respiratory tract infections in healthy individuals: A systematic review and meta-analysis of randomized controlled trials. *PLoS ONE* 2016, 11, e0162996.
60. Mao, S.; Huang, S. Vitamin d supplementation and risk of respiratory tract infections: A meta-analysis of randomized controlled trials. Scand. *J. Infect. Dis.* 2013, 45, 696–702.
61. Bergman, P.; Lindh, A.U.; Bjorkhem-Bergman, L.; Lindh, J.D. Vitamin D and respiratory tract infections: A systematic review and meta-analysis of randomized controlled trials. *PLoS ONE* 2013, 8, e65835.
62. Martineau, A.R.; Jolliffe, D.A.; Hooper, R.L.; Greenberg, L.; Aloia, J.F.; Bergman, P.; Dubnov-Raz, G.; Esposito, S.; Ganmaa, D.; Ginde, A.A.; et al. Vitamin D supplementation to prevent acute respiratory tract infections: Systematic review and meta-analysis of individual participant data. *BMJ* 2017, 356, i6583.

SUPPLEMENTARY MATERIALS

Table S1: Associations of serum 25(OH)D with FEV_1, FVC, FeNO and occurrence of a common cold in participants with 25(OH)D concentrations <50 nmol/L; Table S2: Associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold in men and women participating in the Netherlands Epidemiology of Obesity study using vitamin D and multivitamin supplements; Table S3: Associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold stratified by BMI category, in men and women participating in the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years; Table S4: Crude associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold stratified by age, in men and women participating in the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years; Table S5: Crude associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold stratified by sex, in participants of the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years.

Table S1 Associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold in men and women participating in the Netherlands Epidemiology of Obesity study with 25(OH)D levels < 50 nmol/L.

	Crude	Multivariate[1]	+ BMI, TBF, WC[2]
	Regression coefficient (95% CI) per 10 nmol/L 25(OH)D		
FEV_1 (%predicted)	0.98 (-1.02 to	-0.39 (-2.33 to	-0.55 (-2.50 to
FVC (%predicted)	2.12 (0.35 to 3.88)	0.52 (-1.16 to	0.35 (-1.21 to
Fe_{NO} (ppb)	-0.60 (-1.88 to	-0.35 (-1.45 to	-0.08 (-1.19 to
	Odds Ratio's (95%CI) per 10 nmol/L 25(OH D		
Common cold	0.79 (0.64 to 0.98)	0.83 (0.66 to 1.04)	0.83 (0.66 to 1.04)

Results were based on analyses in a subset of participants with 25(OH)D levels<50 nmol/L, weighted towards the BMI distribution of the general population (n=1498), and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as difference in outcome measure per 10 nmol/L 25(OH)D. 1 Multivariate: Adjusted for age, sex, ethnicity, number of packyears, self-reported obstructive pulmonary disease, season, use of pulmonary and anti-inflammatory medication, educational level and physical activity. 2 Multivariate plus adjustments for BMI, total body fat and waist circumference.
BMI: Body mass index; OR: Odds Ratio. FEV_1: Forced Expiratory Volume in 1 s; FVC Forced Vital Capacity; FeNO: fractional exhaled nitric oxide; ppb: parts per billion; OR: Odds Ratio.

Table S2. Associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold in men and women participating in the Netherlands Epidemiology of Obesity study using vitamin D and multivitamin supplements.

	Crude	Multivariate[1]	+ BMI, TBF, WC[2]
	Regression coefficient (95% CI) per 10 nmol/L 25(OH)D		
FEV_1 (%predicted)	0.34 (-0.13 to	0.09 (-0.41 to	0.05 (-0.40 to
FVC (%predicted)	0.73 (0.26 to 1.21)	0.46 (-0.05 to	0.35 (-0.16 to
Fe_{NO} (ppb)	-0.49 (-1.05 to	-0.15 (-0.65 to	-0.14 (-0.64 to
	Odds Ratio's (95%CI) per 10 nmol/L 25(OH D		
Common cold	0.94 (0.87 to 1.01)	0.97 (0.89 to 1.05)	0.98 (0.90 to 1.06)

Results were based on analyses in a subset of participants using vitamin D and multivitamin supplements, weighted towards the BMI distribution of the general population (n=1461), and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as difference in outcome measure per 10 nmol/L 25(OH)D. 1 Multivariate: Adjusted for age, sex, ethnicity, number of packyears, self-reported obstructive pulmonary disease, season, use of pulmonary and anti-inflammatory medication, educational level and physical activity. 2 Multivariate plus adjustments for BMI, total body fat and waist circumference.
BMI: Body mass index; OR: Odds Ratio. FEV_1: Forced Expiratory Volume in 1 s; FVC Forced Vital Capacity; FeNO: fractional exhaled nitric oxide; ppb: parts per billion; OR: Odds Ratio.

Table S3. Associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold stratified by BMI category, in men and women participating in the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years.

		Crude	Multivariate[1]	+ BMI, TBF, WC[2]
		Regression coefficient (95%CI) per 10 nmol/L 25(OH)D		
FEV_1%	BMI <25	0.06 (-0.42 to 0.54)	-0.09 (-0.63 to	-0.10 (-0.61 to
	BMI 25-	0.46 (0.14 to 0.78)	0.24 (-0.11 to 0.59)	0.12 (-0.23 to 0.46)
	BMI ≥30	0.70 (0.44 to 0.97)	0.69 (0.39 to 0.99)	0.46 (0.17 to 0.75)
FVC%	BMI <25	0.39 (-0.08 to 0.86)	0.09 (-0.42 to 0.59)	0.10 (-0.39 to 0.60)
	BMI 25-	0.68 (0.37 to 0.98)	0.50 (0.18 to 0.82)	0.36 (0.05 to 0.67)
	BMI ≥30	0.88 (0.61 to 1.14)	0.72 (0.42 to 1.01)	0.46 (0.18 to 0.74)
Fe_{NO} (ppb)	BMI <25	-0.24 (-0.65 to	0.21 (-0.22 to 0.63)	0.23 (-0.19 to 0.65)
	BMI 25-	-0.15 (-0.43 to	0.14 (-0.16 to 0.44)	0.14 (-0.16 to 0.44)
	BMI ≥30	-0.36 (-0.53 to -	-0.24 (-0.42 to -	-0.24 (-0.43 to -
		Odds Ratio's per 10 nmol/L 25(OH)D		
Common cold	BMI <25	0.95 (0.88 to 1.02)	1.02 (0.94 to 1.10)	1.02 (0.94 to 1.11)
	BMI 25-	0.96 (0.91 to 1.01)	1.00 (0.94 to 1.06)	1.00 (0.94 to 1.06)
	BMI ≥30	0.89 (0.85 to 0.93)	0.96 (0.91 to 1.01)	0.97 (0.92 to 1.01)

Results were based on analyses weighted towards the BMI distribution of the general population (n=6138), and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as difference in outcome measure per 10 nmol/L 25(OH)D stratified by BMI-category (BMI<25: 43%, BMI 25 -30: 41% and BMI ≥30: 16%). 1 Multivariate: Adjusted for age, sex, ethnicity, number of packyears, self-reported obstructive pulmonary disease, use of pulmonary and anti-inflammatory medication, educational level, season and physical activity. 2 Multivariate plus adjustments for BMI, total body fat and waist circumference.
FEV_1: Forced Expiratory Volume in 1 s; FVC: Forced Vital Capacity; FeNO: fractional exhaled nitric oxide; ppb: parts per billion; BMI: Body Mass Index.

Table S4. Crude associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold stratified by age, in men and women participating in the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years.

	<50 years	50-55 years	55-60 years	>60 years
	Regression coefficient (95% CI) per 10 nmol/L 25(OH)D			
FEV_1 (%predicted)	0.55 (0.08 to1.02)	0.69 (0.15 to 1.23)	0.32 (-0.24 to 0.87)	0.38 (-0.06 to 0.82)
FVC (%predicted)	0.84 (0.36 to 1.32)	0.99 (0.46 to 1.52)	1.03 (0.52 to 1.54)	0.57 (0.11 to 1.02)
Fe_{NO} (ppb)	-0.30 (-0.84 to 0.24)	-0.43 (-0.87 to 0.00)	-0.05 (-0.37 to 0.28)	-0.04 (-0.40 to 0.32)
	Odds Ratio's (95%CI) per 10 nmol/L 25(OH)D			
Common cold	0.91 (0.84 to 0.99)	0.90 (0.83 to 0.98)	1.00 (0.93 to 1.07)	0.94 (0.88 to 1.01)

Results were based on analyses weighted towards the BMI distribution of the general population (n=6138), and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as difference in outcome measure per 10 nmol/L 25(OH)D stratified by age-category (age <50: 21%, 50-55:22%, 55-60: 23% and ≥60: 34%).FEV_1: Forced Expiratory Volume in 1 s; FVC: Forced Vital Capacity; FeNO: fractional exhaled nitric oxide; ppb: parts per billion; BMI: Body Mass Index.

Table S5. Crude associations of serum 25(OH)D (per 10 nmol/L) with FEV_1, FVC, FeNO and presence of a common cold stratified by sex, in participants of the Netherlands Epidemiology of Obesity study, aged between 45 and 65 years.

	Men	**Women**
	Regression coefficient (95% CI) per 10 nmol/L 25(OH)D	
FEV_1 (%predicted)	0.54 (0.16 to 0.92)	0.33 (0.01 to 0.65)
FVC (%predicted)	0.56 (0.23 to 0.89)	0.72 (0.39 to 1.04)
Fe_{NO} (ppb)	0.00 (-0.36 to 0.36)	-0.17 (-0.42 to 0.07)
	Regression coefficient (95% CI) per 10 nmol/L 25(OH)D	
Common cold	0.92 (0.87 to 0.97)	0.96 (0.91 to 1.01)

Results were based on analyses weighted towards the BMI distribution of the general population (n=6138), and were derived from regression coefficients with 95% confidence intervals from linear regression analyses and expressed as difference in outcome measure per 10 nmol/L 25(OH)D stratified by sex (men: 44%, women: 56%). FEV_1: Forced Expiratory Volume in 1 s; FVC: Forced Vital Capacity; FeNO: fractional exhaled nitric oxide; ppb: parts per billion; BMI: Body Mass Index

CHAPTER

7

Nasal levels of antimicrobial peptides in allergic asthma patients and healthy controls: differences and effect of a short 1,25(OH)$_2$ vitamin D3 treatment

Willemien Thijs[1,5], Kirsten Janssen[1], Annemarie M. van Schadewijk[1], Socrates E. Papapoulos[2], Saskia le Cessie[3], Saskia Middeldorp[4], Christian F. Melissant[5], Klaus F. Rabe[1,6], and Pieter S. Hiemstra[1].

Department of Pulmonology[1], Leiden University Medical Center, Leiden, The Netherlands
Department of Endocrinology[2], Leiden University Medical Center, Leiden, The Netherlands
Department of Clinical Epidemiology and department of Medical Statistics[3], Leiden University Medical Center, Leiden, The Netherlands
Department of Vascular Medicine[4], Academic Medical Center, Amsterdam, The Netherlands
Department of Pulmonology[5], Spaarne Hospital, Hoofddorp, The Netherlands
Department of Pulmonology and Thoracic Surgery[6], LungenClinic Grosshansdorf, Grosshansdorf, Germany

PLoS One. 2015 Nov 6;10(11):e014098

ABSTRACT

Background

Allergy is often accompanied by infections and lower levels of antimicrobial peptides (AMPs). Vitamin D has been shown to increase expression of selected AMPs. In this study we investigated whether antimicrobial peptide levels in nasal secretions of allergic asthma patients are lower than in healthy controls, and whether administration of the active form of vitamin D (1,25(OH)$_2$D3) affects these antimicrobial peptide levels.

Methods

The levels of antimicrobial peptides in nasal secretions were compared between 19 allergic asthma patients and 23 healthy controls. The effect of seven days daily oral treatment with 2 µg 1,25(OH)$_2$D3 on antimicrobial peptides in nasal secretions was assessed in a placebo-controlled cross-over clinical study.

Results

Levels of neutrophil α-defensins (human neutrophil peptides 1-3; HNP1-3) and lipocalin 2(LCN2; also known as NGAL) were significantly lower in asthmatics, but no differences in LL-37 and SLPI were detected. Treatment with a short-term 1,25(OH)$_2$D3 caused a small increase in HNP1-3, but not when the asthma and control groups were analyzed separately. LL-37, LCN2 and SLPI did not change after treatment with 1,25(OH)$_2$D3.

Conclusion

Levels of the antimicrobial peptides HNP1-3 and LCN2 are lower in nasal secretions in asthmatics, and are not substantially affected by a short-term treatment with active vitamin D.

INTRODUCTION

Exacerbations in asthma are associated with increased airway inflammation, decreased lung function and increased symptoms, and are frequently accompanied by respiratory infections[1,2]. It has been shown that allergic airways disease is accompanied by increased susceptibility to infections[3-5]. This may in part be explained by the observation that allergic inflammation decreases local host defense against infections by reducing the expression of antimicrobial peptides and proteins (AMPs), which is mediated at least in part by Th2 cytokines[6,7].These AMPs form an essential element of innate immunity in most multicellular organisms, are mainly produced by neutrophils and epithelial cells, and kill a wide range of bacteria, fungi, viruses and microbes[8]. Various studies revealed deficiencies of selected AMPs in airway secretions of patients with allergic rhinitis, sinusitis and asthma[6,9,10]. The role of AMPs in allergic asthma is incompletely understood, but in view of the diverse role of these molecules in regulating host defense, immunity and wound repair they are likely important players in allergic asthma.

Vitamin D has been identified as a key regulator of production of AMPs such as the cathelicidin (hCAP18/LL-37) and the neutrophil gelatinase-associated lipocalin (LCN2) in epithelial cells[11,12]. Allergen challenge in allergic asthmatics has been shown to cause an increase in hCAP18/LL-37 and inflammatory markers in bronchoalveolar lavage, which was accompanied by an increase in vitamin D[13]. Furthermore, vitamin D administration has been shown to increase cathelicidin expression in patients with atopic dermatitis and in neonates[14,15]. Collectively, these data suggest that allergic inflammation contributes to impaired host defense against infections, and that vitamin D could improve this by stimulating AMP production. However, there is limited data on AMPs expression in allergic asthmatic patients and to our knowledge there are no data on the effect of vitamin D treatment on AMPs in asthma patients to support a role for vitamin D in reducing exacerbations.

Our study had two objectives: First, we examined the expression of AMP levels in nasal secretions from patients with allergic asthma and in healthy controls in a case control design. To this end we focused on AMPs that are either only expressed in neutrophils (human neutrophil peptides [HNP]1-3; neutrophil α -defensins) or in epithelial cells (secretory leukocyte proteinase inhibitor; SLPI), or in both cell types (LL-37 and LCN2) and compared the levels of these AMP in asthma patients and healthy controls. Secondly, we assessed if vitamin D administration increased AMP levels in both asthma patients and healthy controls in a placebo-controlled crossover design. Since the main circulating form of vitamin D, 25(OH) D3, requires local conversion to the active form (1,25(OH)$_2$D3) by CYP27B1 and since expression of CYP27B1 is regulated by microbial exposure and inflammation[16], we administered the active form[17].

MATERIAL AND METHODS

Participants

For the first case-control part of our study we aimed to include twenty allergic intermittent asthmatic patients and twenty healthy controls. Participants were recruited by the Department of Pulmonology of the Leiden University Medical Center (LUMC) by advertisement in the Leiden area of the Netherlands. The sample size was based on repeatability of AMP immunoassays from our laboratory and on the data on hCAP18/LL-37 levels in sputum from

asthmatics[10]. Exclusion criteria for all participants (asthmatics and healthy controls) were use of vitamin D supplements or antihistamines, smoking or ex-smoking with more than 5 pack-years, pregnancy and a recent (≤2 weeks) upper respiratory tract infection or other relevant diseases. Furthermore participants under 18 and above 45 years were excluded as well as participants who used inhaled corticosteroids during the last 4 weeks or oral corticosteroids within 3 months before the study.

The inclusion criteria for the twenty patients with allergic intermittent asthma were a history of episodic chest tightness or wheezing (but no daily symptoms or symptoms at night), PC20 methacholine less than 9.6 mg/ml and atopy, as determined by a positive skin prick test result (≥3mm wheal) to 1 or more of 10 common aeroallergen extracts (HAL, Haarlem, the Netherlands). Furthermore their baseline FEV_1 had to be above 70% of predicted[18]. The inclusion criteria for the healthy controls were no history of episodic chest tightness or wheezing, PC20 methacholine more than 9.6 mg/ml, a negative skin prick test, and their baseline FEV_1 should be more than 80% of predicted.

All participants, asthmatics and healthy controls, were included in the intervention part with 1,25(OH)$_2$D3 of our study. The protocol was approved by the institutional review board for human studies and before entering the study, the participants gave their written informed consent.

Study design

The levels of AMPs in nasal secretions at baseline in patients with mild-to-moderate allergic asthma were compared to non-allergic controls in an unpaired comparison of participants stratified by disease. The effect of the intervention with 1,25(OH)$_2$D3 was studied in a doubleblind, placebo-controlled cross-over design (Fig 7.1) that was similar in asthmatics and healthy controls. Treatment order was determined by randomization. Samples were collected and measurements performed on 7 separate visits. At the inclusion visit, medical history was taken, atopy was determined and provocative concentration causing a 20% fall in FEV_1 was measured (PC20 metacholine). In four visits before and after 7 days of 1,25 (OH)$_2$D3 or placebo, nasal secretions and venous blood was collected. An additional control visit was made halfway through each of the two treatment weeks, and venous blood was taken to assess calcium levels as a safety measure for the treatment. Between the intervention periods, a two week wash out period was scheduled (Fig 7.1).

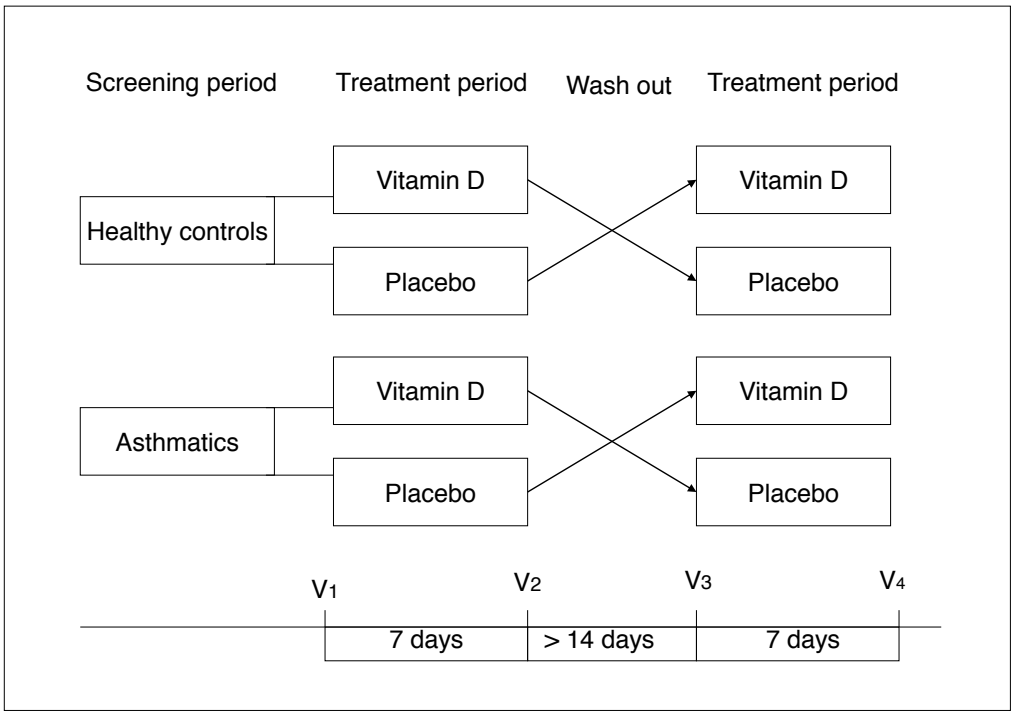


Figure7.1 Single-blind, placebo-controlled cross-over design of the present study

Intervention
All participants received tablets of 2 microgram 1,25(OH)$_2$D3 or placebo once daily during seven days[17,19].

Exhaled nitric oxide
Exhaled nitric oxide (eNO) was measured using a portable analyzer, the NIOX MINO (Aerocrine AB, Solna, Sweden). Participants performed three 10-seconds slow steady exhalation maneuvers, and the mean was used to calculate the levels of eNO.

Spirometry
Flow-volume curves were recorded by pneumotachograph to obtain the forced expiratory volume in 1 second (FEV_1) and the forced vital capacity (FVC).

Nasal secretions
Nasal secretions were collected by vacuum-aided suction[20]. Manipulation with a narrow-tipped vacuum device was used to mildly stimulate nasal secretions. This permits the collection of undiluted whole nasal fluid without the unpredictable effects of dilution in saline or other lavage fluid. Gentle manipulation of a narrow rubber-tipped vacuum device inside the nasal passageways stimulates nasal secretions. Secretions were stored at -80°C for longer-term storage. Nasal secretions were homogenized by brief (3 times 10-second) microtip sonication.

Laboratory analyses

The nasal secretions were analyzed for the presence of antimicrobial peptides and proteins, IL-8 (as a measurement for inflammation) and albumin (as a measurement for vascular leakage). In addition, hCAP18/LL-37 levels were analyzed in plasma. Commercially available ELISA kits were used to detect LCN2 (Bioporto), hCAP18/LL-37 and HNP1-3 (Hycult Biotech), and IL-8 (Sanquin). The SLPI ELISA was developed in our laboratory at the Leiden University Medical Center[21]. The absorbance was measured at 450 nm using a Microplate reader (model 680; Bio- Rad, Hercules, CA) and Microplate Manager software (version 5.2.1, Bio-Rad). The lower limits of detection were: LCN2 10 pg/ml; SLPI 10 ng/ml; HNP1-3 150 ng/ml; LL-37 20 ng/ml and IL-8 200 pg/ml. Albumin levels were determined using nephelometry (Siemens BN Prospec) with a lower limit of detection of 17 µg/ml. Inter and intra-assay variability for all assays was < 10 %.

Serum 1,25(OH)$_2$D3 and 25(OH)D3 were assessed at the central clinical chemistry laboratory of the LUMC. Quantification of the 25(OH)D3 concentration in the serum was done using a DiaSorin 125I RIA Kit (DIASORIN, INC.). Quantification of the 1,25-(OH)D3 concentration in the serum was done using a DiaSorin 125I RIA Kit (DIASORIN, INC.) preceded by extraction and column separation.

Data and statistical analyses

Levels of antimicrobial peptides in mild to moderate asthma versus healthy controls were compared using a Mann-Whitney test. The measurements before and after placebo treatment en the measurements before 1,25(OH)$_2$D3 treatment were used to calculate the mean baseline levels for each participant because these measures are not affected by the 1,25 (OH)$_2$D3 treatment (Fig 7.1). Spearman rank coefficient was used to explore the associations between the AMPs and 25(OH)D3. hCAP18/LL-37 in plasma was log-transformed and a Pearson correlation was used to calculate the association with 25(OH)D3. Pearson correlation was used to explore the relation between albumin, 25(OH)D3, 1,25(OH)$_2$D3, AMPs and IL-8.
The effect of 1,25(OH)$_2$D3 treatment was assessed by calculating the change in outcome measures before and after a treatment or placebo. In this analysis only those patients were included in whom before and after treatment samples were available for a 1,25(OH)$_2$D3 or placebo treatment period. The change after 1,25(OH)$_2$D3 treatment in the whole group was compared to the change after placebo treatment within subjects using the Wilcoxon signed rank test. Statistical analyses were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL). Statistical significance was inferred at $p < 0.05$.

RESULTS

We included a total of 42 participants from the end of 2008 till the spring of 2010 (19 atopic asthma patients and 23 healthy controls). One healthy participant only completed the first treatment period. Participant characteristics are shown in table 7.1, and there were no significant differences between both groups. The mean age of the atopic asthma patients was 27.8 (IQR 22-29) years; the healthy controls were slightly younger and their average age was 23.4 (IQR 20-24) years. There was substantial variability in baseline serum 25(OH)D3 levels (S1 Fig). Only one (asthmatic) participant showed a severe deficiency (<30 nM). The BMI of the asthma patients was 22.9 (IQR 21.6-24.4) and for the healthy controls this was

Table 7.1 Clinical characteristics of the study population according to asthma status.

	Atopic asthma (n=19)				Healthy controls (n=23)				
	Mean	Range	IQR	SD	Mean	Range	IQR	SD	p-value
Age (years)	27.8	19-45	22-29	8.1	23.4	18-45	20-24	6.3	0.06
Sex (male %)	26.3	NA	NA	NA	26.1	NA	NA	NA	0.99
BMI (kg/m^2)	23	19-26	22-24	2.3	22	18-27	20-23	2.1	> 0.05
FEV_1 (%)	97	79-127	88-107	13.3	102	85-121	93.2-110	10.8	0.37
25(OH)D3 (nmol/L)	65	10-127	45-91	31	55	23-113	35-71	23.2	0.22
Exhaled nitric oxide (ppb)	54	19-135	28-71	28	14	7-25	10-17	5	< 0.00

BMI: Body mass index; IQR: Interquartile range; SD standard deviation, NA: not applicable; FEV_1: percent predicted of FEV_1, ppb parts per billion p-values for differences between groups

21.5 (IQR 20.1-22.7). Neither the difference in age nor BMI were statistically significant. When comparing the difference in the AMP and IL-8 levels in nasal secretions between atopic asthmatics and healthy controls, HNP1-3, LCN2 and IL-8 were found to be significantly lower in atopic asthmatics (Table 7.2). LL-37, SLPI and albumin levels did not differ significantly between atopic asthmatics and healthy controls.

Table 7.2 Median baseline AMPs, IL-8 and albumin in nasal secretions according to asthma status.

	Asthma mean (IQR)		Healthy control mean (IQR)		significance
HNP 1-3 (ng/ml)	5194	(1991, 5730)	10618	(3381, 15363)	0.02*
LL-37 (ng/ml)	99	(42, 155)	182	(61, 268)	0.12
LCN2 (ng/ml)	2272	(1104, 3143)	4303	(2150, 6186)	0.01*
SLPI (µg/ml)	674	(112, 398)	1008	(104,471)	0.79
IL-8 (ng/ml)	2	(0.5, 4)	7	(2, 13)	<0.05*
Albumin (µg/ml)	653	(271, 889)	326	(146, 563)	0.088

**statistical significant < 0.05*

No correlation was found in the total group of participants between the baseline levels of antimicrobial peptides and IL-8 in nasal secretion with serum 25(OH)D3. At baseline albumin levels in nasal secretion were not correlated with serum 25(OH)D3 or 1,25(OH)$_2$D3 in the whole group and also not for asthma patients and healthy controls apart. However, there was a significant correlation between albumin levels and HNP 1-3 (r=0.509 p=0.013), IL-8 (r=0.643 p=0.001) and hCAP18/LL-37 (r=0.460 p= 0.029) in healthy controls. In asthma patients albumin was not correlated with any of the AMPs or IL-8. hCAP18/LL-37 plasma levels (log transformed because of their non-normal distribution) and 25(OH)D3 at baseline were significantly correlated (Pearson correlation 0.343, p=0.028) (Fig 7.2) for the whole group; for healthy controls and asthmatics analyzed separately there was no difference.

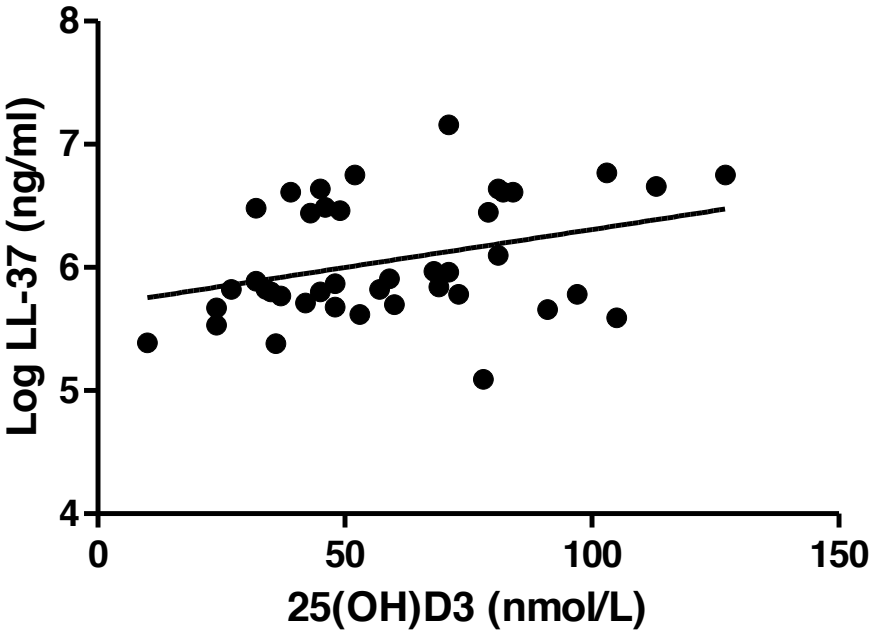


Figure7.2 Correlation between 25(OH)D3 in serum and LL-37 in plasma.
Log-transformed LL-37 levels were significantly correlated to 25(OH)D3 levels (Pearson correlation 0.343, p=0.028).

To evaluate the effect of vitamin D treatment, participants were treated for one week with 1,25(OH)$_2$D3. This treatment duration was selected based on the kinetics of vitamin D-induced expression of antimicrobial peptides in in vitro studies[11,12]. Furthermore, a study by Xystrakis et al that showed that vitamin D3 treatment of steroid resistant asthma patients for 7 days enhanced in vitro responsiveness to dexamethasone of cultured CD4+ T cells[22]. Samples were available for most patients to evaluate the effect of treatment: 39/42 in the placebo period, and 38/42 in the 1,25(OH)$_2$D3 treatment period. Serum levels of 1,25(OH)$_2$D3 increased significantly after intervention (mean difference 86 pg/ml (95% CI 55-116)), but four participants did not show such an increase of 1,25(OH)$_2$D3. All patients had a control visit halfway through the treatment period during which serum calcium and creatinine levels were checked. None of the patients had abnormal levels during treatment and none of the participants reported side affects after treatment. The effect of 1,25(OH)$_2$D3 treatment on serum levels of 1,25(OH)$_2$D3 for asthma and healthy participants are shown in Table 7.3.

Table 7.3 Effect of 1,25(OH)$_2$D3 treatment on serum 1,25(OH)$_2$D3, and nasal AMPs and IL-8 levels according to asthma status.

		Asthma patients (change#)			Healthy controls (change#)		
		Placebo	1,25(OH)$_2$D3	p-value	Placebo	1,25(OH)$_2$D3	p-value
1,25(OH)$_2$D3	(ng/ml)	6	86	0.004	3	97	0.000
HNP1-3	(ng/ml)	-44	1392	0.40	2875	1967	0.07
LL-37	(ng/ml)	-30	5	0.39	-1	22	0.30
LCN2	(ng/ml)	217	448	0.87	635	448	0.40
SLPI	(µg/ml)	-10	-5	0.73	19	30	0.23
IL-8	(ng/ml)	-0.1	0	0.17	0.1	1.7	<0.05

 # change denotes the median difference between the value on day 7 and day 1 of treatment period

The effects of 1,25(OH)$_2$D3 treatment on antimicrobial peptides and IL-8 in nasal secretions for asthma and healthy participants are shown in Table 7.3 and Fig 7.3. In healthy controls, 1,25(OH)$_2$D3 treatment caused a small but significant increase in IL-8 levels. In the group of asthmatics, 1,25(OH)$_2$D3 treatment was associated with an non-significant increase in HNP1-3. In healthy controls, the reverse was observed and there was a trend (p=0.07) for a stronger effect of placebo treatment than 1,25(OH)$_2$D3 treatment on increasing HNP1-3 levels.

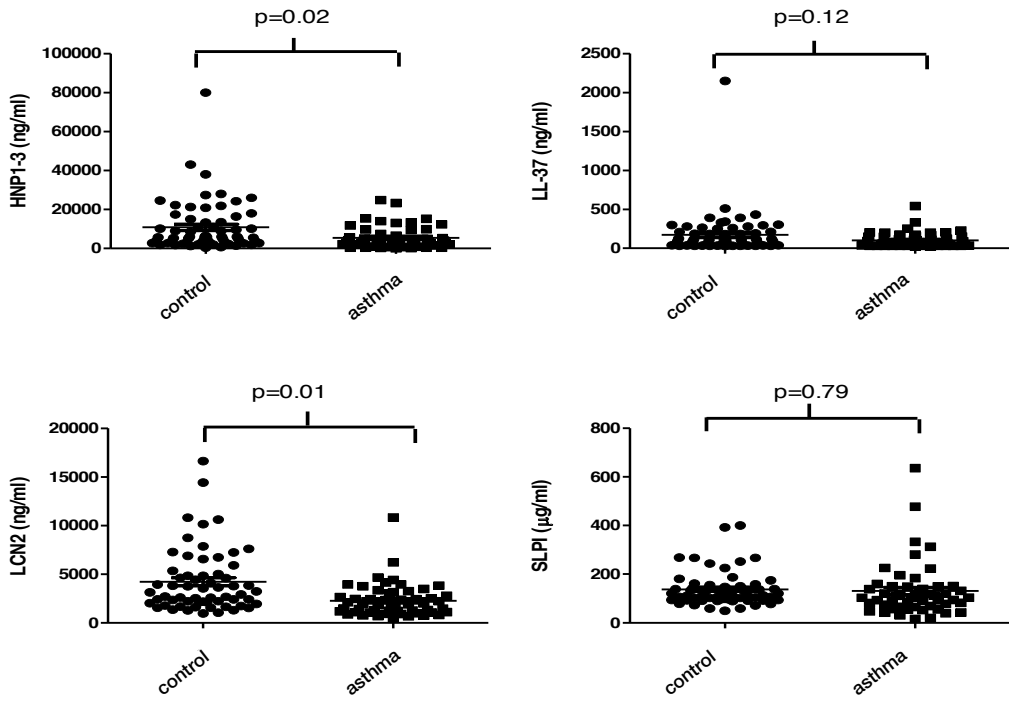


Figure7.3 Baseline AMPs, in nasal secretions.
HNP1-3, LL-37, LCN2 and SLPI in nasal secretions from atopic asthma patients and healthy controls

We next analyzed the effect of 1,25(OH)$_2$D3 on AMP and IL-8 levels in the whole group by pooling the data from the asthma patients and healthy controls. We calculated the median change (difference between the value on day 7 and day 1 of the treatment period) and used a Wilcoxon signed rank test to evaluate if the difference in treatment period was significant. After treatment with active vitamin D, HNP1-3 increased significantly in the whole group (median change after placebo: -151 ng/ml; after 1,25(OH)$_2$D3: 1291 ng/ml; p=0.04) and also IL-8 increased significantly (median change after placebo; -5ng/ml; after 1,25(OH)$_2$D3 9 ng/ml; p=0.02), whereas no significant increases were observed for the levels of the other AMPs shown in Fig 7.4.

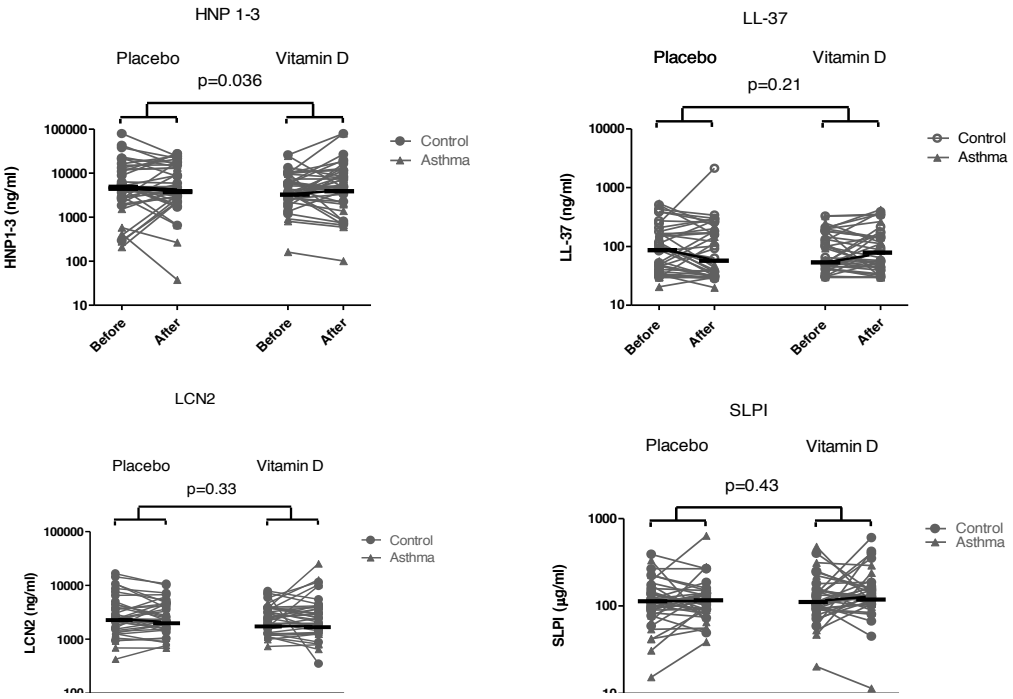


Figure7.4 Effect of 1,25(OH)$_2$D3 treatment on levels of AMPs in nasal secretions.
Change in levels of HNP1-3, LL-37, LCN2 and SLPI in nasal secretions from asthma patients and controls during placebo or 1,25(OH)$_2$D3 treatment. The horizontal bars represent the median AMP level before and after treatment.

DISCUSSION

Analysis of nasal secretions showed that the levels of the antimicrobial peptides (AMPs) HNP1-3 and LCN2 were significantly lower in allergic asthmatics compared to non-allegic controls, whereas hCAP18/LL-37 and SLPI did not differ. Plasma hCAP18/LL-37 was correlated with 25(OH)D3 in the total group of participants at baseline. Furthermore in the total group of participants, treatment with 1,25(OH)$_2$D3 caused a small but significant increase in nasal HNP1-3. When we analyzed asthmatic and healthy participants separately, 1,25(OH)$_2$ vitamin D3 treatment was associated with an non-significant increase in HNP1-3 in the group of asthmatics, whereas in healthy controls the reverse was observed. LCN2 levels also increased to a limited extent, but these effects did not reach statistical significance; hCAP18/LL-37 and SLPI showed no differences. We found IL-8 levels at baseline to be significantly higher in healthy controls. After 1,25(OH)$_2$D3 treatment IL-8 levels significantly increased in the total group of participants. We conclude that levels of HNP1-3 and LCN2 are lower in nasal secretions in asthmatics, and that treatment with active 1,25(OH)$_2$D3 causes only a small increase in HNP1-3.

Our observation that nasal HNP1-3 and LCN2 were significantly lower in the asthma patients is in line with observations in previous studies showing lower AMP levels in allergy[7]. This difference was noted, despite the fact the number of participants was limited. These lower levels may in part be explained by the ability of Th2 cytokines to decrease epithelial expression of AMPs[6,10], but this does not explain the lower levels of neutrophil-derived HNP1-3. Other studies reported higher sputum LCN2 levels in asthma patients compared to controls[23] and higher LCN2 in nasal secretions during the pollen season in asthma compared to controls[24]. Furthermore higher HNP1-3 levels in asthma patients were observed after exposure to rhinovirus[25]. These different results may be explained by different patient groups, disease activity, the analysis of sputum or BAL fluid versus nasal secretions, and the effect of allergen or viral exposure[24,25]. A previous study also showed that sputum LL-37 was lower in asthma compared to controls[10], whereas we did not find significant differences for hCAP18 /LL-37 in nasal secretions. It needs to be recognized that detection errors may also contribute to differences in outcomes between studies, since especially LL-37 is known to stick to anionic substances such as mucins[26] that are abundantly present in e.g. nasal secretions. Although the observed relative deficiency of AMPs was not unexpected, the observed lower IL-8 levels in asthmatics were not in line with previous studies that showed higher levels of this chemokine in asthma[10]. Since patients did not use inhaled or nasal steroids, such treatment is not likely to have interfered with our results.

How can we interpret and explain the effects of vitamin D on AMPs and IL-8 levels in nasal secretions? Vitamin D has been shown to increase expression of LL-37 in vivo [14,15] and epithelial expression of LL-37 and LCN2 in vitro[11,12]. We found a correlation between plasma hCAP18/LL-37 and serum 25(OH)D3 at baseline and thus confirmed previous findings in other patient populations [27]. Furthermore, we found a small, but significant increase of HNP1-3 after 1,25(OH)$_2$D3 treatment in the whole group. The interpretation of the observation that HNP1-3 increases with vitamin D treatment in the whole group is complicated by the observation that vitamin D treatment had opposite effects on HNP1-3 in asthmatics and healthy controls. Whereas treatment appeared to cause a non significant increase in the asthmatics, in healthy controls placebo caused a stronger increase than vitamin D treatment suggesting large variations in HNP1-3 levels. Therefore these results on HNP1-3 need to be interpreted with caution. The vitamin Dinduced increase in LCN2 was not significant and we did not observe an increase of LL-37 and SLPI after vitamin D treatment. Interestingly, there was a significant effect of vitamin D treatment on HNP1-3 which is only expressed by neutrophils, and a non significant increase in LCN2 that is expressed by both neutrophils and epithelial cells. In contrast, SLPI that is mainly expressed by epithelial cells was not affected by treatment. Previous studies have shown that topical application of vitamin D may increase local LL-37 expression in skin[28], and oral administration may be associated with an increased number of hCAP18/LL-37 expressing neutrophils in the circulation[15].
In line with our findings, oral administration of vitamin D during 12 weeks to cystic fibrosis patients did not affect circulating levels of hCAP18/LL-37 and LCN2[29]. Possibly, the number of patients in the present study was too low to observe an effect of vitamin D. Alternatively, despite the observed increases of epithelial LL-37 by vitamin D observed in in vitro studies, the contribution of vitamin D to respiratory epithelial cell-derived hCAP18/LL-37 levels in secretions in vivo may only be limited.

The higher IL-8 levels observed following vitamin D treatment are unexpected. Previous studies have shown that vitamin D decreases poly(I:C)-induced IL-8 release by bronchial epithelial cells[30,31]. Possibly the increased levels of HNP1-3 following vitamin D treatment have contributed to this increased IL-8, since HNP1-3 has been shown to increase IL-8 production in airway epithelial cells [32].

A strength of this study is the comparison of multiple AMPs in airway secretions from allergic asthmatics and healthy controls, and the use of minimally manipulated nasal fluid instead of nasal lavage. Furthermore, the use of active vitamin D treatment for the intervention is another strength of the study, because it prevents the bias introduced by differences in local conversion of 25(OH)D to active vitamin D. The limited number of participants is a weakness of our study. However, because we used a placebo-controlled cross-over design, we were able to analyze within subject treatment effects. Nevertheless, we only found a small but significant increase in HNP1-3 which may be explained by the large range of AMP levels and the small group size.
Furthermore, four of our patients did not show an increase of 1,25(OH)$_2$D3 after treatment. This may indicate a compliance problem, which thus could have weakened our results. However, we did collect the used medication strips to assess compliance, and did not notice a problem. In addition, it needs to be noted that whereas treatment did result in a significant increase in circulating 1,25(OH)$_2$D3 levels, this increase was unexpectedly not correlated with the observed increase in nasal AMPs. At present it is not clear whether this is due to the relatively small subgroups studied, variability in the measurements of AMPs and 1,25(OH)$_2$D3, or to the absence of such a correlation because 1,25(OH)$_2$D3 acts locally. Furthermore, because of the small sample size we did not study polymorphisms in the vitamin D receptor or vitamin D binding protein that may have contributed to our results. Finally, our study does not provide information on AMP expression in the lower airways. The induced sputum samples that were collected during the study were of insufficient quality to allow a reliable analysis.

We conclude that levels of selected AMPs are lower in nasal secretions in asthmatics, and treatment with active vitamin D increases the levels only to a limited extent when analyzed in the combined group of healthy controls and asthmatics. Whether this increase is biologically and clinically relevant is unclear at the present, and requires additional studies in a larger population. Our study suggests that allergic inflammation contributes to impaired antimicrobial peptides levels and vitamin D has only very limited effects on these levels. Possibly the effect of vitamin D is more marked in asthma patients with a severe vitamin D deficiency, as has also been suggested in COPD[33]. To our knowledge this is the first study to evaluate the effect of active vitamin D administration in patients with asthma on AMP expression. These results contribute to our understanding of the effects of allergic asthma on the innate immune system and release of different AMPs, and of how vitamin D can modulate immune functions in these patients.

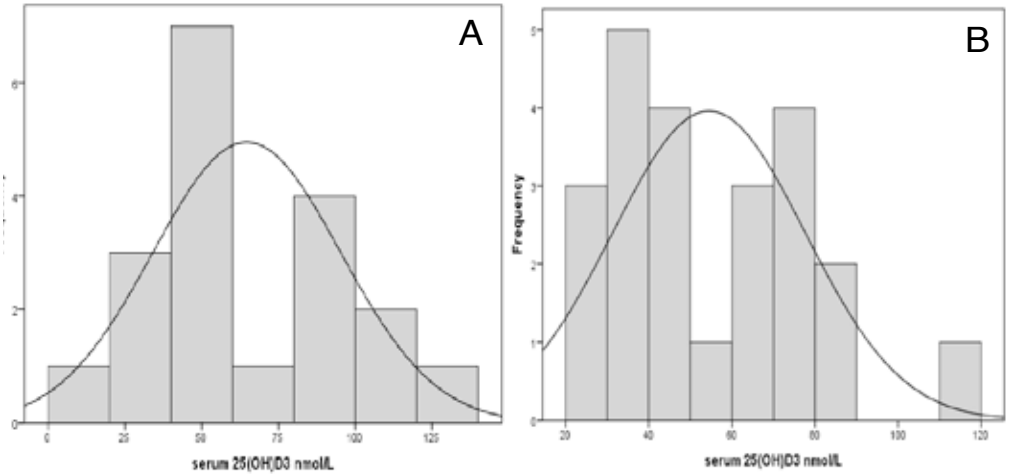
ACKNOWLEDGEMENTS.
The authors thank Dr Alexander Cole (University of Central Florida, Orlando) for helpful suggestions in setting up the collection of undiluted nasal secretions.

REFERENCE LIST

1. ten Brinke A, Sterk PJ, Masclee AA, Spinhoven P, Schmidt JT, Zwinderman AH et al.: Risk factors of frequent exacerbations in difficult-to-treat asthma. *Eur Respir J* 2005, 26: 812-818.
2. McCoy K, Shade DM, Irvin CG, Mastronarde JG, Hanania NA, Castro M et al.: Predicting episodes of poor asthma control in treated patients with asthma. *J Allergy Clin Immunol* 2006, 118: 1226-1233.
3. Jung JA, Kita H, Yawn BP, Boyce TG, Yoo KH, McGree ME et al.: Increased risk of serious pneumococcal disease in patients with atopic conditions other than asthma. *J Allergy Clin Immunol* 2010, 125: 217-221.
4. Talbot TR, Hartert TV, Mitchel E, Halasa NB, Arbogast PG, Poehling KA et al.: Asthma as a risk factor for invasive pneumococcal disease. *N Engl J Med* 2005, 352: 2082-2090.
5. Zambrano JC, Carper HT, Rakes GP, PatrieJ, Murphy DD, Platts-Mills TA et al.: Experimental rhinovirus challenges in adults with mild asthma: response to infection in relation to IgE. *J Allergy Clin Immunol* 2003, 111: 1008-1016.
6. Beisswenger C, Kandler K, Hess C, Garn H, Felgentreff K, Wegmann M et al.: Allergic airway inflammation inhibits pulmonary antibacterial host defense. *J Immunol* 2006, 177: 1833-1837.
7. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T et al.: Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002, 347: 1151-1160.
8. Zasloff M: Antimicrobial peptides of multicellular organisms. *Nature* 2002, 415: 389-395.
9. Kalfa VC, Spector SL, Ganz T, Cole AM: Lysozyme levels in the nasal secretions of patients with perennial allergic rhinitis and recurrent sinusitis. *Ann Allergy Asthma Immunol* 2004, 93: 288- 292.
10. Xiao W, Hsu YP, Ishizaka A, Kirikae T, Moss RB: Sputum cathelicidin, urokinase plasminogen activation system components, and cytokines discriminate cystic fibrosis, COPD, and asthma inflammation. *Chest* 2005, 128:2316-2326.
11. Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J et al.: Cutting edge:1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004, 173: 2909-2912.
12. Gombart AF, Borregaard N, Koeffler HP: Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J2005, 19: 1067-1077.
13. Liu MC, Xiao HQ, Brown AJ, Ritter CS, Schroeder J: Association of vitamin D and antimicrobial peptide production during late-phase allergic responses in the lung. *Clin Exp Allergy* 2012, 42: 383-391.
14. Hata TR, Kotol P, Jackson M, Nguyen M, Paik A, Udall D et al.: Administration of oral vitamin D induces cathelicidin production in atopic individuals. *J Allergy Clin Immunol* 2008, 122: 829-831.
15. Misawa Y, Baba A, Ito S, Tanaka M, Shiohara M: Vitamin D(3) induces expression of human cathelicidin antimicrobial peptide 18 in new 0borns. Int J Hematol 2009, 90: 561-570.
16. Schauber J, Dorschner RA, Coda AB, Buchau AS, Liu PT, Kiken D et al.: Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J Clin Invest* 2007, 117: 803-811.
17. Bianchi ML, Ardissino GL, Schmitt CP, Dacco V, Barletta L, Claris-Appiani A et al.: No difference in intestinal strontium absorption after an oral or an intravenous 1,25(OH)2D3 bolus in normal subjects. For the European Study Group on Vitamin D in children with renal failure. *J Bone Miner Res* 1999, 14:1789-1795.
18. Global Initiative for Asthma (GINA). From the Global Strategy for Asthma Management and Prevention. 1-1-2011. Ref Type: Internet Communication
19. Papapoulos SE, Clemens TL, Sandler LM, Fraher LJ, Winer J, O'Riordan JL: The effect of renal function on changes in circulating concentrations of 1,25-dihydroxycholecalciferol after an oral dose. *Clin Sci* (Lond) 1982, 62: 427-429.
20. Cole AM, Wu M, Kim YH, Ganz T: Microanalysis of antimicrobial properties of human fluids. *J Microbiol Methods* 2000, 41: 135-143.
21. van Wetering S., van der Linden AC, van Sterkenburg MA, Rabe KF, Schalkwijk J, Hiemstra PS: Regulation of secretory leukocyte proteinase inhibitor (SLPI) production by human bronchial epithelial cells: increase of cell-associated SLPI by neutrophil elastase. J Investig Med 2000, 48: 359-366.
22. Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF et al.: Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest* 2006, 116: 146-155.
23. Keatings VM, Barnes PJ: Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med* 1997, 155: 449-453.

24. Kampe M, Stalenheim G, Janson C, Stolt I, Carlson M: Systemic and local eosinophil inflammation during the birch pollen season in allergic patients with predominant rhinitis or asthma. *Clin Mol Allergy* 2007, 5: 4.
25. Rohde G, Message SD, Haas JJ, Kebadze T, Parker H, Laza-Stanca V et al.: Cxc- Chemokines And Antimicrobial Peptides In Rhinovirus-Induced Ex perimental Asthma Exacerbations. *Clin Exp Allergy* 2014.
26. Felgentreff K, Beisswenger C, Griese M, Gulder T, Bringmann G, Bals R: The antimicrobial peptide cathelicidin interacts with airway mucus. *Peptides* 2006, 27: 3100-3106.
27. Jeng L, Yamshchikov AV, Judd SE, Blumberg HM, Martin GS, Ziegler TR et al.: Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis. *J Transl Med* 2009, 7: 28.
28. Vahavihu K, Ala-Houhala M, Peric M, Karisola P, Kautiainen H, Hasan T et al.: Narrowband ultraviolet B treatment improves vitamin D balance and alters antimicrobial peptide expression in skin lesions of psoriasis and atopic dermatitis. *Br J Dermatol* 2010, 163: 321-328.
29. Grossmann RE, Zughaier SM, Liu S, Lyles RH, Tangpricha V: Impact of vitamin D supplementation on markers of inflammation in adults with cystic fibrosis hospitalized for a pulmonary exacerbation. *Eur J Clin Nutr* 2012, 66: 1072-1074.
30. Schrumpf JA, van Sterkenburg MA, Verhoosel RM, Zuyderduyn S, Hiemstra PS: Interleukin 13 Exposure Enhances Vitamin D-Mediated Expression of the Human Cathelicidin Antimicrobial Peptide 18/LL-37 in Bronchial Epithelial Cells. *Infect Immun* 2012, 80: 4485-4494.
31. Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW: Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J Immunol* 2008, 181: 7090-7099.
32. van Wetering S., Mannesse-Lazeroms SP, Dijkman JH, Hiemstra PS: Effect of neutrophil serine proteinases and defensins on lung epithelial cells: modulation of cytotoxicity and IL-8 production. *J Leukoc Biol* 1997, 62: 217-226.
33. Lehouck A, Mathieu C, Carremans C, Baeke F, Verhaegen J, Van EJ et al.: High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. *Ann Intern Med* 2012, 156: 105-114.

SUPPORTING INFORMATION



S1 Fig. Baseline levels of serum 25(OH)D3 in asthma patients (A; n=19) and healthy controls (B;n=23)

CHAPTER

General discussion and summary

The aim of the research presented in this thesis was to unravel effects of obesity-related risk factors on lung function. The current epidemic of obesity has led to several studies suggesting that obesity and the metabolic syndrome affect asthma and COPD [1-6]. In population-based cohorts, the metabolic syndrome and in particular abdominal obesity are associated with impaired lung function [7;8]. It remains unclear whether this is caused by the metabolic effects of visceral fat on systemic inflammation and insulin resistance, or merely is a consequence of abdominal obesity on lung mechanics. Therefore, in the first part of this thesis we investigated if features of the metabolic syndrome influence lung function and we especially focused on the role of visceral fat and insulin resistance. We also assessed whether exhaled nitric oxide could be a simple, noninvasive marker for the suggested airway inflammation caused by obesity-associated systemic inflammation.

A low serum vitamin D concentration is another factor that may influence lung health, and that is also related to obesity. Obesity is associated with lower concentrations of vitamin D [9;10] and several observational studies suggest that vitamin D status influences lung function and the number of respiratory tract infections [11-18]. Despite these well-established associations in observational studies, in controlled clinical trials vitamin D supplementation did not lead to a decrease in respiratory tract infections [19-21]. It is uncertain whether this contradiction is caused by limitations in the design of the intervention trials, or is a consequence of confounding in the observational studies. Therefore, in the second part of this thesis we focused on the association of vitamin D concentrations with lung function and common cold symptoms in a population-based cohort.
A definitive mechanism that could explain a possible protective effect of vitamin D against respiratory tract infections is not yet elucidated. Various studies suggested that vitamin D increases the production and expression of antimicrobial peptides [22;23]. It has also been reported that allergic airway inflammation is associated with low antimicrobial peptide levels in airway epithelial cells and nasal secretions [24;25]. To further explore this potential mechanism, we investigated whether antimicrobial peptide levels are lower in allergic asthmatic patients as compared with healthy controls, and evaluated if supplementation with active vitamin D would increase antimicrobial peptide levels.
In this final chapter, I will provide a summary of the findings, discuss strengths and limitations of the performed studies, and present recommendations for future studies.

SUMMARY OF MAIN FINDINGS

Visceral fat is related to impaired lung function

In **chapter 2** we showed that none of the individual components of the metabolic syndrome, including waist circumference as a measure of abdominal fat, were associated with FEV_1 and FVC or exhaled nitric oxide in non-diabetic men with the metabolic syndrome and with a lung function within the normal range. We did however observe an association of visceral fat assessed by MRI, but not of abdominal subcutaneous fat, with FEV_1 and FVC. The inter quartile range of visceral fat in our study group was 288-488 cm^2. Our results imply that each additional 200 cm^2 of visceral fat is associated with an 11% decrease in FEV_1 predicted. These findings suggest that visceral fat plays a role in the development of lung function impairment. Although we cannot exclude that lung mechanics also explains the observed association, the fact that abdominal subcutaneous fat was not associated with lung function supports the differential contribution of visceral fat in the impairment of lung function. The secretion of

pro-inflammatory cytokines and possibly leptin and adiponectin by visceral fat may underlie this association [26-29]. This adipose tissue-associated inflammation could lead to pulmonary inflammation and result in lung function impairment.

Obesity is responsible for the observed association between insulin resistance and lung function

In **chapter 3** the homeostasis model assessment-estimated insulin resistance (HOMA-IR) index was used as a proxy of insulin resistance in a population-based study. Various previous studies have associated insulin resistance with a decrease in lung function [30-36]. We confirmed such association in our study, but showed that the association between insulin resistance and lung function was mainly explained by adiposity. After adjustment for confounding including total body fat, there was no association between insulin resistance and FEV_1, and a very weak association with FVC. The latter association is not clinically relevant and may easily be explained by residual confounding due to unknown, unmeasured, or inaccurately measured confounding factors. Based on our results it seems more likely that insulin resistance and lung function impairment are both a consequence of excess body fat obesity then that they are causally related.

Visceral fat is not related to exhaled nitric oxide subkop

Exhaled nitric oxide is a routine measurement to monitor pulmonary inflammation in patients with asthma. In routine clinical practice, duplicate measurements are performed. [37]. Since this is time consuming and expensive, we assessed whether two nitric oxide measurements are necessary for large epidemiological studies as well. Therefore, in **chapter 4** we investigated the reliability of a single measurement of exhaled nitric oxide. The interclass correlation coefficient (single measurement reliability) for all participants was 0.97 (95% CI: 0.96, 0.97); this indicates that for assessment of associations with exhaled nitric oxide in large cohorts of overweight and obese adults, a single measurement is sufficient. These results were in line with studies in children, adults, pregnant women and asthma patients [38-40] and made it possible to use a single nitric oxide measurement in the study described in chapter 5.
In the study in **chapter 2** we observed that visceral fat was not associated with exhaled nitric oxide in men with the metabolic syndrome. We hypothesized that by only including men with a high waist circumference the range of visceral fat may not have been sufficiently large to detect a difference in exhaled nitric oxide. However, in **chapter 5** we investigated the association between visceral fat and exhaled nitric oxide in a population-based cohort study with a wide range of waist circumferences, and concluded that there was no clinically relevant association between visceral fat and exhaled nitric oxide. These results are in agreement with those of a study in over 10,000 participants in a general population cohort that observed no relationship between BMI and exhaled nitric oxide [41]. Our findings may indicate that the low-grade inflammation that is associated with obesity does not increase airway inflammation, or that adipose tissue does increase pulmonary inflammation but that this does not translate into increased levels of exhaled nitric oxide.

Vitamin D is related to impaired lung function and the common cold

In **chapter 6** we observed that vitamin D concentrations were positively associated with FEV_1 (beta 0.49 per 10 nmol/L vitamin D) and FVC (beta 0.47 per 10 nmol/L vitamin D) in obese participants. This is in line with earlier (also mostly observational) studies that observed

associations between vitamin D concentrations and lung function [11-14]. In our study this association is not present in non obese participants. Furthermore, we observed an association between vitamin D and exhaled nitric oxide (beta -0.22 per 10 nmol/L vitamin D) in obese participants but not in non obese participants. Other studies in which no association was observed between vitamin D and nitric oxide, [42;43] possibly did not include persons with obesity. In our study there was no association between vitamin D and common cold. Other observational studies did find an association between vitamin D and common cold [15-18] suggesting that people with a high vitamin D concentration have fewer episodes of the common cold. These data are not confirmed in a recent meta-analysis of vitamin supplementation studies in healthy participants where also no effect of vitamin D was shown [44].
In randomized controlled trials in COPD patients, vitamin D supplementation reduced exacerbations in patients with severe vitamin D deficiency, but not in those with normal concentrations [45;46]. Recent meta-analyses of placebo controlled trials showed that vitamin D reduces asthma exacerbations [47]. These data suggest that vitamin D prevents common cold in patients with asthma or COPD but not in the general population.

Active vitamin D treatment does not increase nasal antimicrobial peptides
In **chapter 7** we observed that levels of selected antimicrobial peptides (HNP1-3 and LCN2) are lower in nasal secretions in allergic asthmatics than in healthy controls. This observation confirms observations in previous studies showing lower antimicrobial peptides levels in allergy [24;25]. Interestingly and unexpectedly, we observed that treatment with active vitamin D ($1,25(OH)_2D3$) did not increase nasal antimicrobial peptides.

Strengths and limitations
For this thesis various studies with different designs and measurements have been used. In this section the strengths and limitations are discussed.

The Rubens study
For **chapter 2** we used data from the *"Rosiglitazone versUs placeBo on the prevENtion of progression of atherosclerosis"* (RUBENS) trial [48]. This is a double-blind placebo controlled randomized trial, testing the hypothesis that Rosiglitazone prevents progression of atherosclerosis in participants aged between 50-70 years with the metabolic syndrome. After completion of this trial, we measured lung function in the 98 participants. This enabled us to investigate the contribution of visceral fat to FEV_1 and FVC in non-diabetic men with the metabolic syndrome and a lung function within the normal range. The strength of this study is that all included participants had detailed data on visceral fat assessed by imaging techniques, and extensive measurements of individual metabolic syndrome features and lung function. However, there are several limitations that are of note. First, the participants were recruited after finishing a randomized controlled trial with rosiglitazone. We consider it biologically unlikely that rosiglitazone treatment affected lung function, and results did not change after statistical adjustment for use of rosiglitazone. Second, the patients that participated in the trial were divided for the randomized controlled trial with rosiglitazone in a high and low sensitivity (hs)-CRP group; a majority of the group had a Hs-CRP > 1.8 mg/L and the rest a Hs-CRP < 1.8 mg/L. This could have influenced our results because hs-CRP is a measurement of systemic inflammation and we hypothesized that this could be the underlying mechanism for the association between visceral fat and lung function. Nevertheless

the participants had a wide range of hs-CRP. In addition, this study is based on cross-sectional analyses and although we aimed to adjust for all known and measured confounding variables, residual confounding may still be present by unknown, unmeasured or inaccurately measured confounding factors. In this study we are unable to separate influences of obesity on lung function mechanics from influences of visceral fat. This could have resulted in an overestimation of the association between visceral fat and lung function. Earlier studies used an esophageal balloon technique together with airway occlusion technique to obtain data on lung mechanics, [49;50] which would have been too invasive to measure in our study population. Furthermore obesity is associated with a modest reduction in total lung capacity and a larger reduction in functional residual capacity [51]. In our study we observed an association between expiratory reserve volume and a reduction in FVC and FEV_1. However, if lung mechanics would explain the relationship between visceral fat and FEV_1 then we would also expect a relation between waist circumference and BMI with FEV_1 and this was not present in our study. Therefore, visceral fat appears to be a more selective marker in the relationship of abdominal obesity and lung function impairment. Another limitation is that we only included patients with a high waist circumference, and this could explain why we did not observe an association between waist circumference and lung function while previous studies in a general population did [7;8]. Because this study is based on cross-sectional analyses we cannot exclude the possibility of residual confounding or reverse causation. When the exposure and outcome variables are measured at the same time, the observed exposure may be a consequence of the outcome variable rather than a cause. In this case, we cannot exclude the possibility that impaired lung function has led to accumulation of visceral fat, possibly via physical inactivity as a result of the impaired lunch function. However, the participants had a lung function within the normal range making reverse causation less likely. Finally, the data from the Rubens study cannot be generalized to individuals without metabolic syndrome.

The NEO study

For **chapters 3 to 6**, we used the baseline measurements of the Netherlands Epidemiology of Obesity (NEO) study. The NEO study is a population-based prospective cohort study in 6,671 individuals aged between 45 and 65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. This study enabled us to address the association between insulin resistance and lung function in **chapter 3**, the association between visceral fat and exhaled nitric oxide in **chapter 5**, and the association between vitamin D and lung function and the association between vitamin D and common cold in **chapter 6**.

A major strength of the NEO study is the extensive phenotyping of the study population including data on visceral fat using imaging techniques, and extensive measurements of metabolic syndrome features, lung function and multiple potential confounding factors.

A major limitation is that the analyses are based on cross-sectional observational data. Furthermore, the NEO study may not be representative for the general population.

The participation rate of Leiderdorp was 20.3%. This participation rate is not unusual given the time, blood and extensive measurements (e.g. MRI) that were involved in participation in this study. It is possible that people with a healthy life style decided to participate because of high consciousness about their health. On the other hand, it is also possible that individuals with symptoms or conditions related to obesity have been more likely to participate. Nevertheless, the BMI distribution of the participants from Leiderdorp was comparable with that of the general Dutch population. Finally, the NEO study population consists of

middle-aged, predominantly white individuals. Therefore, our findings need to be confirmed in other age and ethnic groups.

The AVID study

For **chapter 7** we used the data of the Asthma and vitamin D (AVID) study. This study included 19 patients with mild-to-moderate allergic asthma patients and 23 healthy controls aged between 18 and 45 years. In the first part of this study we measured the difference in levels of selected antimicrobial peptides (HNP1-3, LCN2, LL-37 and SLPI) in nasal secretions in asthmatics and healthy controls in a case-control study design. In the second part, all participants were treated with active vitamin D in a double-blind, placebo-controlled cross-over design that was identical in asthmatics and healthy controls. A strength of the first part of the study is the measurement of multiple antimicrobial peptides (HNP 1-3; LCN2, LL-37 and SLPI), which enabled us to compare the levels of antimicrobial peptides between the participants with allergic asthma and healthy controls. The use of active vitamin D treatment for the intervention part is another strength of the study, because it prevents the bias introduced by differences in local conversion of 25(OH)D to active vitamin D. A limitation of the first part of the study, in which levels of nasal secretions in asthmatics and healthy controls were measured, is that the two groups were not fully comparable. The mean age and BMI of the atopic asthma patients were slightly higher than of the healthy controls. Possibly older age and a higher BMI could cause lower levels of antimicrobial peptides, but because of the small differences this seems unlikely. A limitation in the second part of the study is the short treatment period. The seven-day treatment duration was selected based on the kinetics of vitamin D-induced expression of antimicrobial peptides in in vitro studies [52;53]. Furthermore, another study showed that vitamin D3 treatment of steroid resistant asthma patients for 7 days enhanced in vitro responsiveness to dexamethasone of cultured $CD4^+$ T cells [54]. The limited number of participants is another weakness of our study. However, because we used a placebo-controlled cross-over design, we were able to analyze within subject treatment effects.

Assessment of spirometry

Spirometry is the first and most commonly performed lung function test; it is used worldwide and standardized in guidelines [55]. In contrast to chapter 2, in **chapter 3 and 6** FEV_1 and FVC were not measured after bronchodilation, and therefore FEV_1 may have been underestimated. However, we corrected for self-reported asthma and therefore it is unlikely that the lack of post-bronchodilator measurements would have changed the results.

Assessment of visceral fat

Visceral fat was directly assessed by MRI. Three cross-sectional images were made at the level of the fifth lumbar vertebra. Although we did not measure total visceral fat volumes, the cross-sectional images strongly correlate to total volumes (correlation coefficients around 0.8) [56;57] and can therefore be considered representative of total visceral fat [57]. In the Rubens study **(chapter 2)** all participants underwent MRI to assess visceral fat, but in the NEO study **(chapter 5)** approximately 35% of the participants without contraindication to MRI were randomly selected to undergo MRI. Although few participants were excluded our results in **chapter 5** may not be representative for extremely obese persons with a body circumference of more than 1.70 m.

Assessment of insulin resistance
To measure insulin resistance in **chapter 3** we used the HOMA-IR. Although this measurement is not considered the gold standard, it is much more practical to use in large cohort studies. Furthermore, this model correlates well with the gold standard hyperinsulinemic euglycemic glucose clamp [58].

Assessment of exhaled nitric oxide
In **chapter 4 and 5** exhaled nitric oxide was measured using a portable analyzer, the NIOX MINO®. The exhaled nitric oxide measurements taken by this device showed a strong correlation and a high degree of agreement with a standard stationary device [59]. In **chapter 5** we used a single exhaled nitric oxide measurement after we had validated this in **chapter 4** to be sufficient in large epidemiological studies.

Assessment of serum vitamin D
During the recruitment period of the NEO study, serum concentrations of 25(OH)D_3 **(chapter 6)** have been measured on a daily basis by the central chemical laboratory of the LUMC. Quantification of the 25(OH)D concentration in the serum was done using the RIA method (Sept 1st, 2008–Oct 4th, 2010), the Chemoluminescent Immunoassay (Oct 5th, 2010–Sept 29th, 2011) and the LC-MSMS calibrated 2nd generation Electrochemoluminescence Immunoassay (ECLIA) (since Sept 30th, 2011). Because serum 25(OH)D was measured with three different assays during the study period this could have introduced a systematic measurement error, because in the earlier periods of this study we included only overweight participants with a body mass index (BMI) ≥ 27 kg/m^2. Therefore serum 25(OH)D was calibrated towards the "golden standard" LC-MS/MS method (isotope dilution/online solid-phase extraction liquid chromatography/tandem mass spectrometry) to minimize possible variations.

Medical history by self-reporting
In the NEO study participants completed a questionnaire on information about self-reported common cold, ethnicity, tobacco smoking and medical history of allergy and asthma. Smoking may have been underreported and also common cold could be recalled inaccurately. Self-reported asthma could be under- or over-reported. Such misreporting in important confounding factors may have biased our results towards different directions.

Assessment of antimicrobial peptides
A detection error in the ELISA measurements of antimicrobial peptides **(chapter 7)** might have affected our results. Especially LL-37 is known to stick to anionic substances such as mucins [60] that are present in nasal secretions. The level of mucins is influenced by allergic airway inflammation and possibly by vitamin D treatment. If mucin content in the nasal secretions was high it could have underestimated the levels of antimicrobial peptides.
All antimicrobial peptides in nasal secretion showed a great variability and it needs to be recognized that detection errors resulting from measurements in the complex nasal secretion fluids may also have contributed to this wide range.

CONCLUSIONS AND FUTURE PERSPECTIVES

Weighing the strengths and limitations of our studies, we believe our results contribute to the unraveling of causal pathways between obesity and lung function impairment. The main conclusion of the first part of this thesis is that visceral fat is associated with lung function impairment in men with the metabolic syndrome. Furthermore, we conclude that in the general population there is no causal association between insulin resistance and lung function, nor an association between visceral fat and exhaled nitric oxide. The association between visceral fat and lung function as observed in **chapter 2** does not prove causality because longitudinal studies are needed to establish whether visceral fat causes future lung function impairment. The question remains how visceral fat impairs lung function, or in other words, what are the underlying mechanisms? We hypothesized that adipose tissue-related systemic inflammation also leads to inflammation in the lungs. Because individuals with metabolic syndrome have a higher proportion of blood eosinophils than obese persons without metabolic syndrome [61], we hypothesized that this could lead to eosinophilic inflammation in the lung which is accompanied by increased levels of exhaled nitric oxide. Since we did not observe an association between visceral fat and exhaled nitric oxide **(chapter 5),** other research methods that do not only reflect eosinophilic airway inflammation are needed. Bronchial biopsies are a good but very invasive method to measure lung inflammation and in a previous study showed no difference in biopsies before and after weight loss in morbidly obese patients with our without asthma [62]. However, bronchoscopies after weight loss were only performed on 24 participants and therefore the power to detect differences may have been too low. Another less invasive way to measure airway inflammation could be exhaled breath condensate [63] and induced sputum [64]. Because the prevalence of obesity is increasing world-wide and is associated with both the incidence and severity of asthma, it is important to unravel the mechanisms underlying the association between visceral fat and lung function in future research.

In the second part of this thesis we showed that vitamin D is associated with lung function and nitric oxide in obese participants. Longitudinal studies and especially intervention trials with vitamin D supplementation should establish whether the relationship between vitamin D, lung function is causal or not. Several previous studies did not show any effect of vitamin D supplementation [19-21;47]. However, studies with higher doses of vitamin D and longer follow-up time are needed. In the study presented in this thesis antimicrobial peptides levels were lower in allergic asthmatics than in healthy controls. Unfortunately, we were not able to conclude if vitamin D supplementation influences these antimicrobial peptide levels. Larger studies are needed to investigate whether vitamin D also increases antimicrobial peptides in the lung.

In summary, obesity is a global burden that influences lung function. Future research should reveal if obesity, and in particular visceral fat, causes lung inflammation and thereby impairs lung function. This knowledge will help to develop prevention strategies aimed at protecting lung function from declining in obese patients. If research identifies that vitamin D treatment is acting as an anti-infective therapy, then it would be a useful adjuvant therapy in a variety of infections. Large randomized trials are necessary to establish the effect of vitamin D therapy on infections in vitamin deficient patients. This may eventually lead to better treatment of respiratory infections in all patients.

REFERENCE LIST

1. Beuther, D. A. and E. R. Sutherland. 2007. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. *Am.J.Respir.Crit Care Med.* 175:661-666.
2. Brumpton, B., A. Langhammer, P. Romundstad, Y. Chen, and X. M. Mai. 2013. General and abdominal obesity and incident asthma in adults: the HUNT study. *Eur.Respir.J.* 41:323-329.
3. Brumpton, B. M., C. A. Camargo, Jr., P. R. Romundstad, A. Langhammer, Y. Chen, and X. M. Mai. 2013. Metabolic syndrome and incidence of asthma in adults: the HUNT study. *Eur.Respir.J.* 42:1495-1502.
4. Steuten, L. M., E. C. Creutzberg, H. J. Vrijhoef, and E. F. Wouters. 2006. COPD as a multicomponent disease: inventory of dyspnoea, underweight, obesity and fat free mass depletion in primary care. *Prim.Care Respir.J.* 15:84-91.
5. Franssen, F. M., D. E. O'Donnell, G. H. Goossens, E. E. Blaak, and A. M. Schols. 2008. Obesity and the lung: 5. Obesity and COPD. *Thorax* 63:1110-1117.
6. Mannino, D. M., D. Thorn, A. Swensen, and F. Holguin. 2008. Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD. *Eur.Respir.J.* 32:962-969.
7. Leone, N., D. Courbon, F. Thomas, K. Bean, B. Jego, B. Leynaert, L. Guize, and M. Zureik. 2009. Lung function impairment and metabolic syndrome: the critical role of abdominal obesity. *Am.J.Respir.Crit Care Med.* 179:509-516.
8. Lam, K. B., R. E. Jordan, C. Q. Jiang, G. N. Thomas, M. R. Miller, W. S. Zhang, T. H. Lam, K. K. Cheng, and P. Adab. 2009. Airflow obstruction and the metabolic syndrome: the Guangzhou Biobank Cohort Study. *Eur.Respir.J.*
9. Parikh, S. J., M. Edelman, G. I. Uwaifo, R. J. Freedman, M. Semega-Janneh, J. Reynolds, and J.A. Yanovski. 2004. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J.Clin.Endocrinol.Metab* 89:1196-1199.
10. Lagunova, Z., A. C. Porojnicu, F. Lindberg, S. Hexeberg, and J. Moan. 2009. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res.* 29:3713-3720.
11. Niruban, S. J., K. Alagiakrishnan, J. Beach, and A. Senthilselvan. 2015. Association between vitamin D and respiratory outcomes in Canadian adolescents and adults. *J.Asthma*1-33.
12. Black, P. N. and R. Scragg. 2005. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. *Chest* 128:3792-3798.
13. Berry, D. J., K. Hesketh, C. Power, and E. Hypponen. 2011. Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br.J.Nutr.* 106:1433-1440.
14. Choi, C. J., M. Seo, W. S. Choi, K. S. Kim, S. A. Youn, T. Lindsey, Y. J. Choi, and C. M. Kim. 2013. Relationship between serum 25-hydroxyvitamin D and lung function among Korean adults in Korea National Health and Nutrition Examination Survey (KNHANES), 2008-2010. *J.Clin.Endocrinol.Metab* 98:1703-1710.
15. Laaksi, I., J. P. Ruohola, V. Mattila, A. Auvinen, T. Ylikomi, and H. Pihlajamaki. 2010. Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. *J.Infect.Dis.* 202:809-814.
16. Ginde, A. A., J. M. Mansbach, and C. A. Camargo, Jr. 2009. Association between serum 25-hydroxy-vitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. A*rch.Intern.Med.* 169:384-390.
17. Monlezun, D. J., E. A. Bittner, K. B. Christopher, C. A. Camargo, and S. A. Quraishi. 2015. Vitamin d status and acute respiratory infection: cross sectional results from the United States national health and nutrition examination survey, 2001-2006. *Nutrients.* 7:1933-1944.
18. Berry, D. J., K. Hesketh, C. Power, and E. Hypponen. 2011. Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br.J.Nutr.* 106:1433-1440.
19. Li-Ng, M., J. F. Aloia, S. Pollack, B. A. Cunha, M. Mikhail, J. Yeh, and N. Berbari. 2009. A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. *Epidemiol.Infect.* 137:1396-1404.
20. Murdoch, D. R., S. Slow, S. T. Chambers, L. C. Jennings, A. W. Stewart, P. C. Priest, C. M. Florkowski, J. H. Livesey, C. A. Camargo, and R. Scragg. 2012. Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. *JAMA* 308:1333-1339.
21. Rees, J. R., K. Hendricks, E. L. Barry, J. L. Peacock, L. A. Mott, R. S. Sandler, R. S. Bresalier, M. Goodman, R. M. Bostick, and J. A. Baron. 2013. Vitamin D3 supplementation and upper respiratory tract infections in a randomized, controlled trial. Clin.Infect.Dis. 57:1384-1392.
22. Misawa, Y., A. Baba, S. Ito, M. Tanaka, and M. Shiohara. 2009. Vitamin D(3) induces expression of human cathelicidin antimicrobial peptide 18 in newborns. *Int.J.Hematol.* 90:561-570.

23. Hata, T. R., P. Kotol, M. Jackson, M. Nguyen, A. Paik, D. Udall, K. Kanada, K. Yamasaki, D. Alexandrescu, and R. L. Gallo. 2008. Administration of oral vitamin D induces cathelicidin production in atopic individuals. *J.Allergy Clin.Immunol.* 122:829-831.
24. Beisswenger, C., K. Kandler, C. Hess, H. Garn, K. Felgentreff, M. Wegmann, H. Renz, C. Vogelmeier, and R. Bals. 2006. Allergic airway inflammation inhibits pulmonary antibacterial host defense. *J.Immunol.* 177:1833-1837.
25. Kalfa, V. C., S. L. Spector, T. Ganz, and A. M. Cole. 2004. Lysozyme levels in the nasal secretions of patients with perennial allergic rhinitis and recurrent sinusitis. Ann.Allergy Asthma *Immunol.* 93:288-292.
26. Lemieux, I., A. Pascot, D. Prud'homme, N. Almeras, P. Bogaty, A. Nadeau, J. Bergeron, and J. P. Despres. 2001. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. *Arterioscler.Thromb.Vasc.Biol.* 21:961-967.
27. Fried, S. K., D. A. Bunkin, and A. S. Greenberg. 1998. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J.Clin.Endocrinol.Metab* 83:847-850.
28. He, G., S. B. Pedersen, J. M. Bruun, A. S. Lihn, P. F. Jensen, and B. Richelsen. 2003. Differences in plasminogen activator inhibitor 1 in subcutaneous versus omental adipose tissue in non-obese and obese subjects. *Horm.Metab Res.* 35:178-182.
29. Good, M., F. M. Newell, L. M. Haupt, J. P. Whitehead, L. J. Hutley, and J. B. Prins. 2006. TNF and TNF receptor expression and insulin sensitivity in human omental and subcutaneous adipose tissue--influence of BMI and adipose distribution. *Diab.Vasc.Dis.Res.* 3:26-33.
30. Lawlor, D. A., S. Ebrahim, and G. D. Smith. 2004. Associations of measures of lung function with insulin resistance and Type 2 diabetes: findings from the British Women's Heart and Health Study. *Diabetologia.* 47:195-203.
31. Engstrom, G., B. Hedblad, P. Nilsson, P. Wollmer, G. Berglund, and L. Janzon. 2003. Lung function, insulin resistance and incidence of cardiovascular disease: a longitudinal cohort study. *J.Intern.Med*. 253:574-581.
32. Lecube, A., G. Sampol, X. Munoz, P. Lloberes, C. Hernandez, and R. Simo. 2010. Insulin resistance is related to impaired lung function in morbidly obese women: a case-control study. *Diabetes Metab Res.Rev.* 26:639-645.
33. Lim, S. Y., E. J. Rhee, and K. C. Sung. 2010. Metabolic syndrome, insulin resistance and systemic inflammation as risk factors for reduced lung function in Korean nonsmoking males. *J.Korean Med.Sci.* 25:1480-1486.
34. Lazarus, R., D. Sparrow, and S. T. Weiss. 1998. Baseline ventilatory function predicts the development of higher levels of fasting insulin and fasting insulin resistance index: the Normative Aging Study. *Eur.Respir.J.* 12:641-645.
35. Ford, E. S. and D. M. Mannino. 2004. Prospective association between lung function and the incidence of diabetes: findings from the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *Diabetes Care*. 27:2966-2970.
36. Wei, Y. F., H. D. Wu, P. D. Yung-Chieh Yen, C. K. Huang, C. M. Tai, and C. F. Hsuan. 2014. The impact of metabolic parameters on the change of pulmonary function in obese patients. Surg. *Obes.Relat Dis.* 10:23-28.
37. 2005. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am.J.Respir.Crit Care Med.* 171:912-930.
38. Selby, A., B. Clayton, J. Grundy, K. Pike, K. Drew, A. Raza, R. Kurukulaaratchy, S. H. Arshad, and G. Roberts. 2010. Are exhaled nitric oxide measurements using the portable NIOX MINO repeatable? *Respir.Res.* 11:43.
39. Alving, K., C. Janson, and L. Nordvall. 2006. Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respir.Res*. 7:67.
40. Tamasi, L., A. Bohacs, A. Bikov, C. Andorka, J. Rigo, Jr., G. Losonczy, and I. Horvath. 2009. Exhaled nitric oxide in pregnant healthy and asthmatic women. *J.Asthma* 46:786-791.
41. Singleton, M. D., W. T. Sanderson, and D. M. Mannino. 2014. Body mass index, asthma and exhaled nitric oxide in U.S. adults, 2007-2010. *J.Asthma* 51:756-761.
42. Dabbah, H., Y. R. Bar, G. Livnat, F. Hakim, and L. Bentur. 2015. Bronchial Reactivity, Inflammatory and Allergic Parameters, and Vitamin D Levels in Children With Asthma. *Respir.Care* 60:1157-1163.
43. Yao, T. C., Y. L. Tu, S. W. Chang, H. J. Tsai, P. W. Gu, H. C. Ning, M. C. Hua, S. L. Liao, M. H. Tsai, C. Y. Chiu, et al. 2014. Serum 25-hydroxyvitamin D levels in relation to lung function and exhaled nitric oxide in children. *J.Pediatr.* 165:1098-1103.
44. Vuichard, G. D., D. Dao, C. M. Gysin, L. Lytvyn, and M. Loeb. 2016. Effect of Vitamin D3 Supplementation on Respiratory Tract Infections in Healthy Individuals: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *PLoS.One.* 11.

45. Lehouck, A., C. Mathieu, C. Carremans, F. Baeke, J. Verhaegen, E. J. Van, B. Decallonne, R. Bouillon, M. Decramer, and W. Janssens. 2012. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. *Ann.Intern.Med.* 156:105-114.
46. Martineau, A. R., W. Y. James, R. L. Hooper, N. C. Barnes, D. A. Jolliffe, C. L. Greiller, K. Islam, D. McLaughlin, A. Bhowmik, P. M. Timms, et al. 2015. Vitamin D3 supplementation in patients with chronic obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial. *Lancet Respir.Med.* 3:120-130.
47. Martineau, A. R., C. J. Cates, M. Urashima, M. Jensen, A. P. Griffiths, U. Nurmatov, A. Sheikh, and C. J. Griffiths. 2016. Vitamin D for the management of asthma. Cochrane.Database. *Syst.Rev.* 9:CD011511.
48. Roes, S. D., R. A. Dehnavi, J. J. Westenberg, H. J. Lamb, B. J. Mertens, J. T. Tamsma, and R. A. de. 2011. Effect of lifestyle intervention plus rosiglitazone or placebo therapy on left ventricular mass assessed with cardiovascular magnetic resonance in the metabolic syndrome. *J.Cardiovasc.Magn Reson*. 13:65.
49. Pelosi, P., M. Croci, I. Ravagnan, S. Tredici, A. Pedoto, A. Lissoni, and L. Gattinoni. 1998. The effects of body mass on lung volumes, respiratory mechanics, and gas exchange during general anesthesia. *Anesth.Analg.* 87:654-660.
50. Pelosi, P., M. Croci, I. Ravagnan, P. Vicardi, and L. Gattinoni. 1996. Total respiratory system, lung, and chest wall mechanics in sedated-paralyzed postoperative morbidly obese patients. *Chest* 109:144-151.
51. Jones, R. L. and M. M. Nzekwu. 2006. The effects of body mass index on lung volumes. *Chest* 130:827-833.
52. Wang, T. T., F. P. Nestel, V. Bourdeau, Y. Nagai, Q. Wang, J. Liao, L. Tavera-Mendoza, R. Lin, J. W. Hanrahan, S. Mader, et al. 2004. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J.Immunol.* 173:2909-2912.
53. Gombart, A. F., N. Borregaard, and H. P. Koeffler. 2005. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB J.* 19:1067-1077.
54. Xystrakis, E., S. Kusumakar, S. Boswell, E. Peek, Z. Urry, D. F. Richards, T. Adikibi, C. Pridgeon, M. Dallman, T. K. Loke, et al. 2006. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J.Clin.Invest* 116:146-155.
55. Miller, M. R., J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, R. Crapo, P. Enright, C. P. van der Grinten, P. Gustafsson, et al. 2005. Standardisation of spirometry. *Eur.Respir.J.* 26:319-338.
56. Han, T. S., I. E. Kelly, K. Walsh, R. M. Greene, and M. E. Lean. 1997. Relationship between volumes and areas from single transverse scans of intra-abdominal fat measured by magnetic resonance imaging. *Int.J.Obes.Relat Metab Disord.* 21:1161-1166.
57. Shen, W., J. Chen, M. Gantz, G. Velasquez, M. Punyanitya, and S. B. Heymsfield. 2012. A single MRI slice does not accurately predict visceral and subcutaneous adipose tissue changes during weight loss. *Obesity.*(Silver.Spring). 20:2458-2463.
58. Bonora, E., G. Targher, M. Alberiche, R. C. Bonadonna, F. Saggiani, M. B. Zenere, T. Monauni, and M. Muggeo. 2000. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care.* 23:57-63.
59. Khalili, B., P. B. Boggs, and S. L. Bahna. 2007. Reliability of a new hand-held device for the measurement of exhaled nitric oxide. *Allergy* 62:1171-1174.
60. Felgentreff, K., C. Beisswenger, M. Griese, T. Gulder, G. Bringmann, and R. Bals. 2006. The antimicrobial peptide cathelicidin interacts with airway mucus. *Peptides* 27:3100-3106.
61. van Huisstede A., M. C. Cabezas, E. Birnie, G. J. van de Geijn, A. Rudolphus, G. Mannaerts, T. L. Njo, P. S. Hiemstra, and G. J. Braunstahl. 2013. Systemic inflammation and lung function impairment in morbidly obese subjects with the metabolic syndrome. *J.Obes.* 2013:131349.
62. van Huisstede A., A. Rudolphus, C. M. Castro, L. U. Biter, G. J. van de Geijn, C. Taube, P. S. Hiemstra, and G. J. Braunstahl. 2015. Effect of bariatric surgery on asthma control, lung function and bronchial and systemic inflammation in morbidly obese subjects with asthma. *Thorax* 70:659-667.
63. Horvath, I., J. Hunt, P. J. Barnes, K. Alving, A. Antczak, E. Baraldi, G. Becher, W. J. van Beurden, M. Corradi, R. Dekhuijzen, et al. 2005. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur.Respir.J.* 26:523-548.
64. Efthimiadis, A., A. Spanevello, Q. Hamid, M. M. Kelly, M. Linden, R. Louis, M. M. Pizzichini, E. Pizzichini, C. Ronchi, O. F. Van, et al. 2002. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. *Eur.Respir.J.Suppl* 37:19s-23s.

Nederlandse samenvatting

INLEIDING

Obesitas kan longfunctie op meerdere manieren beïnvloeden. In dit proefschrift zijn effecten van obesitas die niet van mechanische aard zijn onderzocht. In het eerste deel van dit proefschrift is de invloed van obesitas en in het bijzonder de invloed van visceraal vet en de daarmee gepaard gaande insulineresistentie en systemische ontsteking op longfunctie onderzocht. Obesitas gaat vaak samen met vitamine D deficiëntie[1;2]. In het tweede deel van dit proefschrift is daarom het verband tussen serum vitamine D concentraties, longfunctie en luchtweginfecties bekeken. Om meer inzicht te verkrijgen in het mogelijk onderliggend mechanisme is in dit deel ook de invloed van behandeling met vitamine D op antimicrobiële peptiden getest.

Astma is een heterogene ziekte die gekarakteriseerd wordt door chronische luchtwegontsteking. Astma wordt gedefinieerd door klachten als een piepende ademhaling, kortademigheid, benauwdheid en hoest. De klachten zijn over het algemeen niet altijd aanwezig en de intensiteit van de klachten varieert. Wanneer patiënten met astma klachten hebben, wordt de longfunctie gekenmerkt door een beperking van de luchtstroom bij het uitademen door vernauwing van de luchtwegen[3]. COPD wordt meestal veroorzaakt door roken en geeft kortademigheid en hoesten. Bij COPD wordt de longfunctie gekenmerkt door een onomkeerbare beperking van de luchtstroom bij het uitademen[4]. Omdat beide ziektes gepaard gaan met een beperking bij het uitademen worden astma en COPD aangeduid met obstructieve longziekten.

Mensen met obesitas hebben vaker astma dan mensen zonder obesitas[5-7]. Daarnaast hebben mensen met astma en obesitas vaak samen een ernstiger vorm van astma[8;9], en heeft afvallen een gunstig effect op astma symptomen [10;11]. Het is bekend dat obesitas de longfunctie beïnvloedt: de totale longcapaciteit is lager en het reservevolume neemt af [12]. Dit wordt voornamelijk veroorzaakt door druk van de buik op het middenrif. Maar er zijn ook andere manieren waarop obesitas longfunctie kan beïnvloeden en in dit proefschrift worden de effecten van obesitas die niet van mechanische aard zijn onderzocht.

Het metabool syndoom is een cluster van symptomen die vaak samen voorkomen en het risico op aan obesitas gerelateerde aandoeningen verhoogt. Volgens de International Diabetes Federation is het metabool syndroom aanwezig als er naast een verhoogde middelomtrek ook twee van de volgende parameters aanwezig zijn: hyperglycaemie, hypertriglyceridemie, een lage high-density lipoprotein cholesterol concentratie en hypertensie of behandeling hiervoor [13]. Er wordt gesteld dat kenmerken van dit syndroom invloed kunnen hebben op longfunctie. In dit proefschrift hebben we het effect van insulineresistentie op longfunctie onderzocht. De middelomtrek geeft een indicatie van de hoeveelheid visceraal vet en is verwerkt in de definitie van het metabool syndroom. Vetweefsel geeft hormonen en cytokines af die een laaggradige ontstekingsreactie (inflammatie) veroorzaken, die kan bijdragen aan obesitas gerelateerde ziekten [14;15]. Vooral visceraal vet zou samengaan met cardiometabole risicofactoren [14;15]. In dit proefschrift hebben we dan ook het effect van de hoeveelheid visceraal vet op longfunctie en op ontsteking in de longen bestudeerd.

Vitamine D, longfunctie en luchtweg infecties

Obesitas gaat samen met lagere bloedconcentraties van vitamine D [1;2]. Dit wordt mogelijk veroorzaakt doordat vitamine D wordt opgeslagen in subcutaan vetweefsel [16;17].
In observationeel onderzoek is een lagere concentratie van vitamine D geassocieerd met een verminderde longfunctie [18-21]. Vitamine D speelt een rol in de regulatie van de productie van antimicrobiële peptiden en eiwitten [22-24]. Door de rol van vitamine D in het immuunsysteem kan behandeling met vitamine D mogelijk luchtweginfecties en longfunctie beïnvloeden [25-30]. Vaak wordt astma veroorzaakt door allergieën en daarnaast hebben astmapatiënten ook meer kans op infecties [31;32]. Deze allergische inflammatie en vooral de Th2 cytokineproductie die gepaard gaat met allergische inflammatie zou het immuunsysteem kunnen beïnvloeden door een verminderde expressie van antimicrobiële peptiden en eiwitten [33;34]. Doordat vitamine D een rol speelt in de regulatie van de productie van antimicrobiële peptiden en eiwitten [22-24] zou het negatieve effecten van allergische inflammatie op antimicrobiële peptiden en eiwitten in astma patiënten tegen kunnen werken.

RESULTATEN PROEFSCHRIFT

In **hoofdstuk 2** hebben we mannen met het metabool syndoom onderzocht. Er was geen verband tussen de kenmerken van het metabool syndroom met de longfunctie (gemeten met FEV_1, Forced Expiratory Volume in 1 seconde, en FVC, Forced Vital Capacity) of stikstofoxide in de uitademing. Visceraal vet was in tegenstelling tot subcutaan vet wel geassocieerd met FEV_1 en FVC. Dit kan een indicatie zijn dat visceraal vet een rol speelt in de achteruitgang van longfunctie. Dit zou mogelijk kunnen worden verklaard door de uitscheiding van pro-inflammatoire cytokines, leptine en adiponectine door visceraal vet [35-38]. In **hoofdstuk 3** is het verband tussen insulineresistentie en longfunctie onderzocht met de homeostasis model assessment-estimated insulin resistance index (HOMA-IR) als maat voor insulineresistentie. Het geobserveerde verband tussen insulineresistentie en longfunctie bleek voornamelijk verklaard door lichaamsvet. Ofwel, lichaamsvet lijkt eerder een oorzaak te zijn van zowel insulineresistentie als longfunctie, dan dat er een direct verband is tussen insulineresistentie en maten van longfunctie. In **hoofdstuk 4** hebben we de reproduceerbaarheid van stikstofoxide in de uitgeademde lucht van mensen met obesitas middels de NIOX MINO gemeten en aangetoond dat een enkelvoudige meting afdoende is in epidemioloisch onderzoek.
Dit maakte het mogelijk om in **hoofdstuk 5** deze meting te gebruiken om het verband tussen visceraal vet en stikstofoxide in de uitademinglucht in de algemene bevolking te onderzoeken. Dit verband bleek niet klinisch relevant te zijn. Dit zou kunnen betekenen dat visceraal vet geen ontsteking in de longen veroorzaakt in de algemene bevolking, of dat de ontsteking die in de longen wordt veroorzaakt niet leidt tot stijging van stikstofoxide in de luchtwegen.
In **hoofdstuk 6** laten we zien dat bij mensen met obesitas een lager vitamine D gehalte samenhangt met een lager FEV_1 en FVC. Dit komt overeen met resultaten uit eerdere observationele studies [18-21]. Dit verband zagen we niet in bij mensen zonder obesitas. Bij mensen met obesitas hing een hoger vitamine D gehalte samen met een lager stikstofoxide gehalte in de uitgeademde lucht, in tegenstelling tot bij de mensen zonder obesitas. Dit kan een indicatie zijn dat vitamine D een rol speelt in de luchtwegontsteking bij mensen met obesitas. Eerdere studies hebben dit verband niet gevonden maar onderzochten mensen met obesitas niet apart [39;40]. In beide groepen is er geen verschil in het voorkomen van verkoudheden. Dit in tegenstelling tot studies waar specifiek werd gekeken naar het effect van vitamine D in longpatiënten. In astmapatiënten kan vitamine D exacerbaties voorkomen [41]. In onderzoek

waar COPD patiënten worden behandeld met vitamine D om exacerbaties van COPD te voorkómen valt het op dat die behandeling alleen een meetbaar effect heeft bij vitamine D deficiënte personen [42;43]. In **hoofdstuk 7** beschrijven we dat de spiegels van antimicrobiële peptide (HNP1-3 and LCN2) in neussecreet lager zijn in astmapatiënten dan in de gezonde controlegroep. Behandeling met actief vitamine D ($1,25(OH)_2D3$) liet deze waarden niet stijgen.

CONCLUSIE EN DISCUSSIE

Het onderzoek beschreven in dit proefschrift draagt bij aan de ontrafeling van de relatie tussen obesitas en longfunctie. In het eerste deel van het proefschrift stellen we vast dat in mannen met het metabool syndroom meer visceraal vet samengaat met een verminderde longfunctie. We laten zien dat in de algemene populatie insulineresistentie niet direct gerelateerd is aan longfunctie. De geobserveerde verbanden in het eerste deel van het proefschrift tonen geen causaliteit aan; hiervoor is longitudinaal onderzoek nodig. Mocht uit longitudinaal onderzoek blijken dat er geen relatie is tussen insulineresistentie en longfunctie, maar wel tussen visceraal vet en longfunctie dan is verder onderzoek nodig naar de biologische mechanismen die hieraan ten grondslag liggen. Mogelijk veroorzaakt de systemische ontsteking die gepaard gaat met visceraal vet ontsteking in de longen en wordt op deze manier de longfunctie beïnvloed. In een onderzoek komt naar voren dat mensen met het metabool syndroom een hoger percentage eosinofielen in het bloed hebben dan mensen met obesitas maar zonder metabool syndroom [44]. Dit zou een eosinofiele ontsteking in de longen kunnen veroorzaken en dat geeft een verhoogd stikstofoxide in de uitademingslucht. Omdat er in ons onderzoek geen verband was tussen visceraal vet en stikstofoxide in de uitademingslucht in de algemene bevolking is het bij het ontwerpen van toekomstige onderzoek belangrijk om ook niet-eosinofiele ontsteking in de longen te meten.

In het tweede deel van het proefschrift tonen we aan dat bij mensen met obesitas het vitamine D gehalte samenhangt met longfunctie. We vonden geen verband met het optreden van verkoudheden; dit komt overeen met eerder onderzoek waar vitamine D wordt gesuppleerd wordt [45-47]. Omdat vitamine D mogelijk infecties zou kunnen voorkómen in astma- en COPD-.patiënten [41-43] is het van belang het mechanisme hierachter te ontrafelen. Daarom hebben we de invloed van vitamine D op antimicrobiële peptiden onderzocht in astmapatiënten en gezonde controlepersonen. Omdat onze groepsgrootte beperkt was konden we niet met voldoende zekerheid concluderen of vitamine D deze peptiden beïnvloedt. In de toekomst is groter opgezet onderzoek nodig om dit verband te onderzoeken.

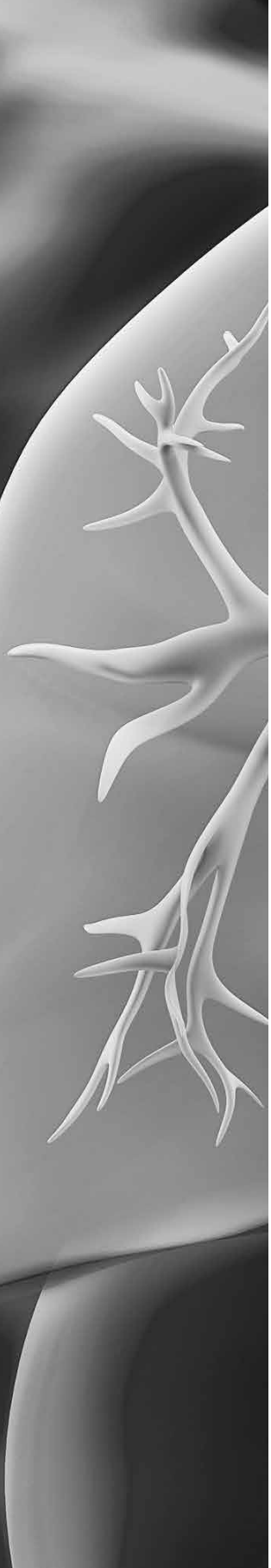
Dit proefschrift geeft inzicht in de wijze waarop obesitas de gezondheid van de longen kan beïnvloeden. In onze cross-sectionele analyses lijkt visceraal vet longfunctie te kunnen beinvloeden, maar dit lijkt niet via mechanismen als insulineresistentie of eosinofiele ontsteking te zijn. Deze informatie kan helpen bij de opzet van toekomstig onderzoek dat zich kan richten op de niet-eosinofiele systemische inflammatie die gepaard gaat met visceraal vet en of deze ontsteking terug te vinden is in de longen. In longitudinaal onderzoek kan het effect van vitamine D behandeling op longfunctie en verkoudheden bestudeerd worden. Een groter onderzoek naar het effect van vitamine D op antimicrobiële peptiden kan meer inzicht geven in de preventieve werking van vitamine D. Dit inzicht zou in de toekomst kunnen leiden tot nieuwe therapieën om luchtweg infecties te voorkomen.

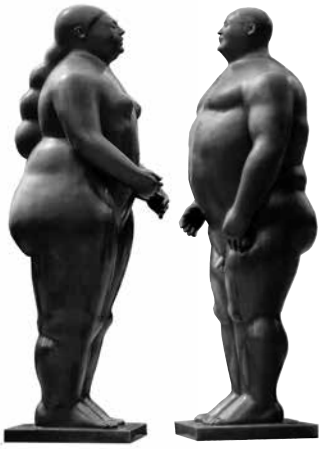
REFERENCE LIST

1. Parikh, S. J., M. Edelman, G. I. Uwaifo, R. J. Freedman, M. Semega-Janneh, J. Reynolds, and J. A. Yanovski. 2004. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J.Clin.Endocrinol.Metab* 89:1196-1199.
2. Lagunova, Z., A. C. Porojnicu, F. Lindberg, S. Hexeberg, and J. Moan. 2009. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res.* 29:3713-3720.
3. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. Revised 2014 Vancouver, *GINA*, 2014. 2014.
4. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for Diagnosis, *Management and Prevention of COPD*. 2015.
5. Brumpton, B., A. Langhammer, P. Romundstad, Y. Chen, and X. M. Mai. 2013. General and abdominal obesity and incident asthma in adults: the HUNT study. *Eur.Respir.J.* 41:323-329.
6. Beuther, D. A. and E. R. Sutherland. 2007. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. *Am.J.Respir.Crit Care Med.* 175:661-666.
7. Assad, N., C. Qualls, L. J. Smith, A. Arynchyn, B. Thyagarajan, M. Schuyler, D. R. Jacobs, Jr., and A. Sood. 2013. Body mass index is a stronger predictor than the metabolic syndrome for future asthma in women. The longitudinal CARDIA study. *Am.J.Respir.Crit Care Med.* 188:319-326.
8. Saint-Pierre, P., A. Bourdin, P. Chanez, J. P. Daures, and P. Godard. 2006. Are overweight asthmatics more difficult to control? *Allergy* 61:79-84.
9. Clerisme-Beaty, E. M., S. Karam, C. Rand, C. M. Patino, A. Bilderback, K. A. Riekert, S. O. Okelo, and G. B. Diette. 2009. Does higher body mass index contribute to worse asthma control in an urban population? *J.Allergy Clin.Immunol.* 124: 207-212.
10. A van Huisstede, A. Rudolphus, C. M. Castro, L.U. Biter, G. J.van de Geijn, C.Taube, P. S. Hiemstra, and G. J. Braunstahl. 2015. Effect of bariatric surgery on asthma control, lung function and bronchial and systemic inflammation in morbidly obese subjects with asthma. *Thorax* 70:659-667.
11. Boulet, L. P., H. Turcotte, J. Martin, and P. Poirier. 2012. Effect of bariatric surgery on airway response and lung function in obese subjects with asthma. *Respir.Med.* 106:651-660.
12. Jones, R. L. and M. M. Nzekwu. 2006. The effects of body mass index on lung volumes. *Chest* 130: 827-833.
13. Alberti, K. G., P. Zimmet, and J. Shaw. 2006. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the Internati onal Diabetes Federation. *Diabet.Med.* 23:469-480.
14. Ferrante, A. W., Jr. 2007. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. *J.Intern.Med.* 262:408-414.
15. Bastard, J. P., M. Maachi, C. Lagathu, M. J. Kim, M. Caron, H. Vidal, J. Capeau, and B. Feve. 2006. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur.Cytokine Netw.* 17:4-12.
16. Earthman, C. P., L. M. Beckman, K. Masodkar, and S. D. Sibley. 2012. The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. *Int.J.Obes.*(Lond) 36:387-396.
17. Piccolo, B. D., G. Dolnikowski, E. Seyoum, A. P. Thomas, E. R. Gertz, E. C. Souza, L. R. Woodhouse, J. W. Newman, N. L. Keim, S. H. Adams, et al. 2013. Association between subcutaneous white adipose tissue and serum 25-hydroxyvitamin D in overweight and obese adults. *Nutrients.* 5:3352-3366.
18. Niruban, S. J., K. Alagiakrishnan, J. Beach, and A. Senthilselvan. 2015. Association between vitamin D and respiratory outcomes in Canadian adolescents and adults. *J.Asthma*1-33.
19. Black, P. N. and R. Scragg. 2005. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. *Chest* 128:3792-3798.
20. Berry, D. J., K. Hesketh, C. Power, and E. Hypponen. 2011. Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br.J.Nutr.* 106:1433-1440.
21. Choi, C. J., M. Seo, W. S. Choi, K. S. Kim, S. A. Youn, T. Lindsey, Y. J. Choi, and C. M. Kim. 2013. Relationship between serum 25-hydroxyvitamin D and lung function among Korean adults in Korea National Health and Nutrition Examination Survey (KNHANES), 2008-2010. J.Clin.Endocrinol. *Metab* 98:1703-1710.
22. Wang, T. T., F. P. Nestel, V. Bourdeau, Y. Nagai, Q. Wang, J. Liao, L. Tavera-Mendoza, R. Lin, J. W. Hanrahan, S. Mader, et al. 2004. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J.Immunol.* 173:2909-2912.
23. Gombart, A. F., N. Borregaard, and H. P. Koeffler. 2005. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB J*. 19:1067-1077.

24. Schrumpf, J. A., M. A. van Sterkenburg, R. M. Verhoosel, S. Zuyderduyn, and P. S. Hiemstra. 2012. Interleukin 13 Exposure Enhances Vitamin D-Mediated Expression of the Human Cathelicidin Antimicrobial Peptide 18/LL-37 in Bronchial Epithelial Cells. *Infect.Immun.* 80:4485-4494.
25. Prietl, B., G. Treiber, T. R. Pieber, and K. Amrein. 2013. Vitamin D and immune function. *Nutrients.* 5:2502-2521.
26. Gunville, C. F., P. M. Mourani, and A. A. Ginde. 2013. The role of vitamin D in prevention and treatment of infection. *Inflamm.Allergy Drug Targets*. 12:239-245.
27. Laaksi, I., J. P. Ruohola, V. Mattila, A. Auvinen, T. Ylikomi, and H. Pihlajamaki. 2010. Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. *J.Infect.Dis.* 202:809-814.
28. Ginde, A. A., J. M. Mansbach, and C. A. Camargo, Jr. 2009. Association between serum 25-hydroxy-vitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch.Intern.Med.* 169:384-390.
29. Monlezun, D. J., E. A. Bittner, K. B. Christopher, C.A. Camargo, and S. A. Quraishi. 2015. Vitamin d status and acute respiratory infection: cross sectional results from the United States national health and nutrition examination survey, 2001-2006. *Nutrients.* 7:1933-1944.
30. Berry, D. J., K. Hesketh, C. Power, and E. Hypponen. 2011. Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br.J.Nutr.* 106:1433-1440.
31. Talbot, T. R., T. V. Hartert, E. Mitchel, N. B. Halasa, P. G. Arbogast, K. A. Poehling, W. Schaffner, A. S. Craig, and M. R. Griffin. 2005. Asthma as a risk factor for invasive pneumococcal disease. *N.Engl.J.Med.* 352:2082-2090.
32. Zambrano, J. C., H. T. Carper, G. P. Rakes, J. Patrie, D. D. Murphy, T. A. Platts-Mills, F. G. Hayden, J. M. Gwaltney, Jr., T. K. Hatley, A. M. Owens, et al. 2003. Experimental rhinovirus challenges in adults with mild asthma: response to infection in relation to IgE. *J.Allergy Clin.Immunol.* 111:1008-1016.
33. Beisswenger, C., K. Kandler, C. Hess, H. Garn, K. Felgentreff, M. Wegmann, H. Renz, C. Vogelmeier, and R. Bals. 2006. Allergic airway inflammation inhibits pulmonary antibacterial host defense. *J.Immunol.* 177:1833-1837.
34. Ong, P. Y., T. Ohtake, C. Brandt, I. Strickland, M. Boguniewicz, T. Ganz, R. L. Gallo, and D. Y. Leung. 2002. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N.Engl.J.Med.* 347:1151-1160.
35. Lemieux, I., A. Pascot, D. Prud'homme, N. Almeras, P. Bogaty, A. Nadeau, J. Bergeron, and J. P. Despres. 2001. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. Arterioscler. *Thromb.Vasc.Biol.* 21:961-967.
36. Fried, S. K., D. A. Bunkin, and A. S. Greenberg. 1998. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J.Clin.Endocrinol.Metab* 83:847-850.
37. He, G., S. B. Pedersen, J. M. Bruun, A. S. Lihn, P. F. Jensen, and B. Richelsen. 2003. Differences in plasminogen activator inhibitor 1 in subcutaneous versus omental adipose tissue in non-obese and obese subjects. *Horm.Metab Res.* 35:178-182.
38. Good, M., F. M. Newell, L. M. Haupt, J. P. Whitehead, L. J. Hutley, and J. B. Prins. 2006. TNF and TNF receptor expression and insulin sensitivity in human omental and subcutaneous adipose tissue--influence of BMI and adipose distribution. Diab.Vasc.Dis.Res. *3:26-33.*
39. Yao, T. C., Y. L. Tu, S. W. Chang, H. J. Tsai, P. W. Gu, H. C. Ning, M. C. Hua, S. L. Liao, M. H. Tsai, C. Y. Chiu, et al. 2014. Serum 25-hydroxyvitamin D levels in relation to lung function and exhaled nitric oxide in children. *J.Pediatr.* 165:1098-1103.
40. Dabbah, H., Y. R. Bar, G. Livnat, F. Hakim, and L. Bentur. 2015. Bronchial Reactivity, Inflammatory and Allergic Parameters, and Vitamin D Levels in Children With Asthma. *Respir.Care* 60:1157-1163.
41. Martineau, A. R., C. J. Cates, M. Urashima, M. Jensen, A. P. Griffiths, U. Nurmatov, A. Sheikh, and C. J. Griffiths. 2016. Vitamin D for the management of asthma. *Cochrane.*Database.Syst.Rev. 9:CD011511.
42. Lehouck, A., C. Mathieu, C. Carremans, F. Baeke, J. Verhaegen, E. J. Van, B. Decallonne, R. Bouillon, M. Decramer, and W. Janssens. 2012. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. *Ann.Intern.Med.* 156:105-114.
43. Martineau, A. R., W. Y. James, R. L. Hooper, N. C. Barnes, D. A. Jolliffe, C. L. Greiller, K. Islam, D. McLaughlin, A. Bhowmik, P. M. Timms, et al. 2015. Vitamin D3 supplementation in patients with chronic obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial. *Lancet Respir.Med.* 3:120-130.
44. A. van Huisstede, M. C. Cabezas, E. Birnie, G. J. van de Geijn, A. Rudolphus, G. Mannaerts, T. L. Njo, P. S. Hiemstra, and G. J. Braunstahl. 2013. Systemic inflammation and lung function impairment in morbidly obese subjects with the metabolic syndrome. *J.Obes.* 2013:131349.

45. Li-Ng, M., J. F. Aloia, S. Pollack, B. A. Cunha, M. Mikhail, J. Yeh, and N. Berbari. 2009. A randomized controlled trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. *Epidemiol.Infect.* 137:1396-1404.
46. Murdoch, D. R., S. Slow, S. T. Chambers, L. C. Jennings, A. W. Stewart, P. C. Priest, C. M. Florkowski, J. H. Livesey, C. A. Camargo, and R. Scragg. 2012. Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. *JAMA* 308:1333-1339.
47. Rees, J. R., K. Hendricks, E. L. Barry, J. L. Peacock, L. A. Mott, R. S. Sandler, R. S. Bresalier, M. Goodman, R. M. Bostick, and J. A. Baron. 2013. Vitamin D3 supplementation and upper respiratory tract infections in a randomized, controlled trial. *Clin.Infect.Dis.* 57:1384-1392.





Appendix

CURRICULUM VITAE

Willemien Thijs was born on October 23rd, 1977 in Loenen aan de Vecht. In 1996 she graduated from the Athenaeum Broklede in Breukelen. She studied medicine at the Free University in Amsterdam and became a medical doctor in 2004. After graduation she worked as an internal resident at the Spaarne Hospital in 2005. In 2006 she started her PhD project at the Department of Pulmonology of the Leiden University Medical Center. In 2010 she commenced her pulmonology training at the Spaarne Hospital (dr. C.F. Melissant, dr F.H. Krouwels en dr I. van der Lee); for the academic part of her training she worked at the Academic Medical Center (Amsterdam, dr. R.E. Jonkers), and at Southampton General Hospital (United Kingdom, prof. dr. L. Richeldi). She finished her training in April 2016 after which she started as a respiratory consultant at the Haaglanden Medical Center in The Hague.

DANKWOORD

Via deze weg wil ik graag iedereen bedanken die op welke wijze dan ook heeft bijgedragen aan dit proefschrift. De volgende mensen wil ik in het bijzonder noemen:

- alle proefpersonen voor het belangeloos meewerken aan de metingen
- Prof. dr. Pieter Hiemstra voor zijn wetenschappelijke kennis, toegankelijkheid, betrokkenheid en natuurlijk zijn geduld
- Prof. dr. Saskia Middeldorp voor haar enthousiasme, daadkracht en mooie formuleringen
- Dr. ir. Renée de Mutsert voor haar epidemiologisch inzicht, haar begeleiding en alle verbeteringen
- Prof. dr. Klaus Rabe voor de geboden kansen en vernieuwende inzichten
- Prof. dr. Frits Rosendaal voor het onderwijs en zijn bijdrage aan de methodes van dit vitamine D onderzoek en het longgedeelte van het NEO-onderzoek
- de leden van de promotiecommissie voor hun bereidheid dit proefschrift inhoudelijk te beoordelen
- alle longfunctielaboranten, studenten en analisten van het laboratorium longziekten voor de inzet en alle metingen
- alle medewerkers van de NEO-studie voor hun inspanningen en het verzamelen van de data
- mijn opleiders in het Spaarne voor de ruimte die ze me voor dit onderzoek hebben gegeven
- de collega's uit het Spaarne, LUMC en HMC voor alle vrolijkheid en collegialiteit
- Bob voor de goede gesprekken en lol tijdens congressen en op de kamer
- Edske, Jasper, Joris en Henk voor alle fijne afleiding
- Richard en Marieke voor het aanhoren van de strubbelingen van het onderzoek en voor de genoeglijke avonden
- Marije voor de steun, de lekkere wijntjes en hulp bij het staartje van het boek
- Dorien voor alle interesse en hulp bij het begin van het project
- Claartje voor haar statistische kennis en de heerlijke en vaak hilarische zus (regressie) momenten en Kevin voor zijn betrokkenheid
- Annemarie voor alle warme zussensteun en plezier, en Arnoud voor de gezellige avonden en overnachtingen die daar mee gepaard gaan
- Stijn, Giel, Melle, Isaac, Leander en Ella voor alle mooie neefjes- en nichtjesmomenten

Babs, ik geniet altijd enorm van je energie, ideeën en vriendschap. Bedank voor alle mooie dingen die we hebben beleefd, ik ben blij dat je mijn paranimf wilt zijn.
Pap, jouw grondige medische kennis, werkethos en compassie hebben mij altijd geïnspireerd, ik ben trots dat je mijn paranimf bent. Mam, je levendigheid, humor en kritische discussies zijn voor mij onmisbaar en daarom heb ik dit boekje aan je opgedragen. Ik wil jullie beiden bedanken voor de oneindige steun, liefde en gezelligheid.

Jules, thank you for sorting out the mucus in the LUMC freezer (I kept my promise).
Clearly I couldn't have done this without your humour, sarcasm and support. I'm looking forward to our freezer-free future together.

BIBLIOGRAPHY

1. **Predominance of left-sided deep vein thrombosis and body weight.**
   W.Thijs, K.F. Rabe, F.R. Rosendaal and S. Middeldorp
   J Thromb Haemost. 2010 Sep;8(9):2083-4.

2. **Association of lung function measurements and visceral fat in men with the metabolic syndrome.**
   W. Thijs, R. Alizadeh Dehnavi, P.S. Hiemstra, A. de Roos, C.F. Melissant, K. Janssen, J.T. Tamsma and K.F. Rabe
   Respir Med. 2014 Feb;108(2):351-7.

3. **Reproducibility of the NIOX MINO measurements in overweight and obese participants.**
   W. Thijs, S. Middeldorp, S. le Cessie, P.S. Hiemstra, C.F. Melissant, F.R. Rosendaal and K.F. Rabe
   BMC Res Notes. 2014 Nov 3;7:775.

4. **Nasal Levels of Antimicrobial Peptides in Allergic Asthma Patients and Healthy Controls: Differences and Effect of a Short 1,25(OH)$_2$ Vitamin D3 Treatment.**
   W. Thijs, K. Janssen, A.M. van Schadewijk, S.E. Papapoulos, S. le Cessie, S. Middeldorp, C.F. Melissant, K.F. Rabe and P.S.Hiemstra
   PLoS One. 2015 Nov 6;10(11):0140986.

5. **Een vrouw door het oog van de naald; diagnose in beeld.**
   B. van Tienhoven, L. Becude and W.Thijs
   Ned Tijdschr Geneeskd. 2017;161(0):D1041.

6. **Pulmonary function, exhaled nitric oxide in asthma patients with obesity- a cross sectional study.**
   M.J. Kasteleyn, T.N. Bonten, R. de Mutsert, W. Thijs, P.S. Hiemstra, S. le Cessie, F.R. Rosendaal, N.H. Chavannes and C. Taube
   Respir Res. 2017 Dec 7; 18(1):205.

7. **Associations of serum 25(OH)D concentrations with lung function, airway inflammation and common cold in the general population**
   W. Thijs, R. Rafiq, R. Prein, R.T. de Jongh, F.R. Rosendaal, C. Taube, P.S. Hiemstra, R. de Mutsert and M. den Heijer
   Nutrients. 2018 Jan 3; 10(1): 35