Obesity-related risk factors for impaired lung function

Thijs, W.

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.

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