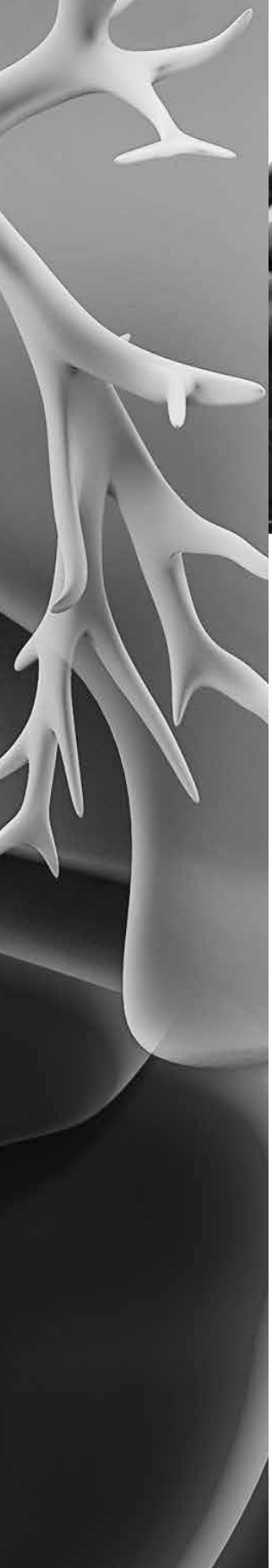
Obesity-related risk factors for impaired lung function

Thijs, W.



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

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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.

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