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# Obesity in women with asthma: Baseline disadvantage plus greater small-airway responsiveness

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## Abstract

**Background:** Obesity is known to diminish lung volumes and worsen asthma. However, mechanistic understanding is lacking, especially as concerns small-airway responsiveness. The objective of this study was therefore to compare small-airway responsiveness, as represented by the change in expiratory:inspiratory mean lung density ratios ( $MLD_{e/i}$ , as determined by computed tomography [CT]) throughout methacholine testing in obese versus non-obese women with asthma.

**Methods:** Thoracic CT was performed during methacholine bronchoconstriction challenges to produce standardized response curves (SRC: response parameter versus  $\ln[1 + \% PD20]$ , where PD20 is the cumulative methacholine dose) for 31 asthma patients ( $n = 18$  non-obese and  $n = 13$  obese patients). Mixed models evaluated obesity effects and interactions on SRCs while adjusting for age and bronchial morphology. Small airway responsiveness as represented by SRC slope was calculated for each third of the  $MLD_{e/i}$  response and compared between groups.

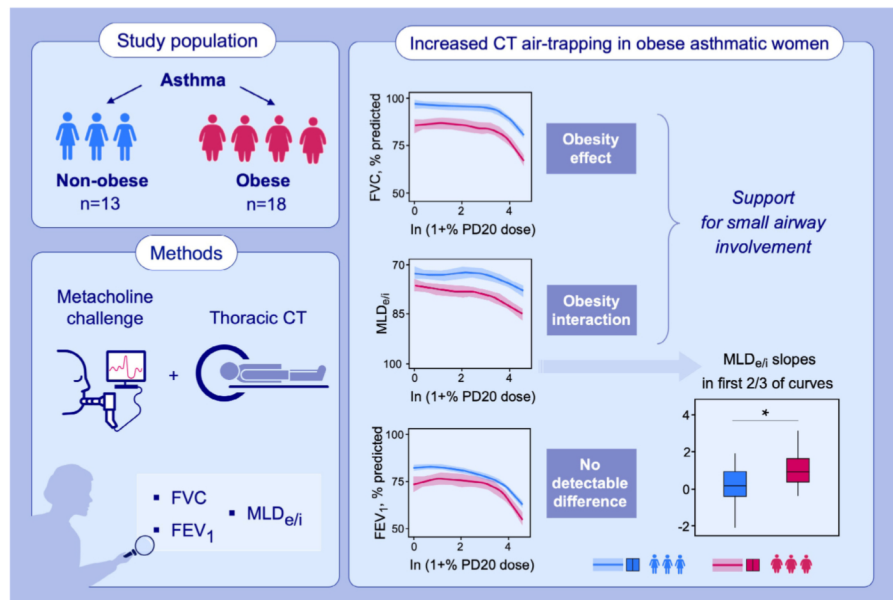
**Results:** Obesity-associated effects observed during experimental bronchoconstriction included: (i) a significant baseline effect for forced expiratory volume in 1 second with lower values for the obese ( $73.11 \pm 13.44$ ) versus non-obese ( $82.19 \pm 8.78$ ;  $p = 0.002$ ) groups prior to methacholine testing and (ii) significantly higher responsiveness in small airways as estimated via differences in  $MLD_{e/i}$  slopes (group  $\times \ln(1 + \% PD20)$  interaction;  $p = 0.023$ ). The latter were pinpointed to higher slopes in the obese group at the beginning 2/3 of SRCs ( $p = 0.004$  and  $p = 0.021$ ). Significant obesity effects ( $p = 0.035$  and  $p = 0.008$ ) indicating lower forced vital capacity and greater % change in  $MLD_{e/i}$  (respectively) throughout methacholine testing, were also observed.

**Conclusion:** In addition to baseline differences, small-airway responsiveness (as represented by the change in  $MLD_{e/i}$ ) during methacholine challenge is greater in obese women with asthma as compared to the non-obese.

**Abbreviations:** ACQ-6, Asthma control questionnaire 6; B1, the right apical bronchus; B8, the anterior basal bronchus; BMI, body mass index; CalvNO, alveolar nitric oxide; CT, computed tomography; FENO, fractionated exhaled nitric oxide; FEV1<sub>%p</sub>, percent-predicted forced expiratory volume in 1 second; FRC, functional residual capacity; FVC%p, percent-predicted forced vital capacity;  $MLD_{e/i}$ , the expiratory to the inspiratory ratio of mean lung density; NO, nitric oxide; RV, residual volume; SRC, standardized response curve.

## KEYWORDS

air-trapping, body mass index, computed tomography, mean lung density, methacholine



## GRAPHICAL ABSTRACT

Non-obese versus obese women with asthma underwent methacholine provocation coupled with CT scanning to determine MLD<sub>e/i</sub> per step. Mixed models on standardized response curves determined an obesity x dose interaction for MLD<sub>e/i</sub>. Greater MLD<sub>e/i</sub> slopes for obese patients were pinpointed in the first two-thirds of curves, corresponding to higher bronchial responsiveness during methacholine provocation. CT, computed tomography; FEV<sub>1</sub>, forced expiratory volume in 1 second (% predicted); FVC, forced vital capacity (% predicted); MLD<sub>e/i</sub>, expiratory: inspiratory ratios of mean lung density; PD20, the end of methacholine provocation, when a drop of at least 20% in FEV<sub>1</sub> is observed

## 1 | INTRODUCTION

Epidemiological links between obesity and asthma<sup>1-4</sup> point towards a potential interaction or accumulation of additive effects between the two. During methacholine challenges, similar response curves for obese and normal-weight groups in terms of percent-predicted forced expiratory volume in 1 s (FEV<sub>1%<sub>p</sub></sub>), forced vital capacity (FVC<sub>%<sub>p</sub></sub>) and FEV<sub>1</sub>/FVC ratios have been demonstrated, suggesting at first no effect of body mass index (BMI) on airway-response dynamics.<sup>5-7</sup> Cross-sectional spirometry studies indicate preserved or even increased FEV<sub>1</sub>/FVC ratios in adult obese asthmatics,<sup>8,9</sup> while FEV<sub>1</sub><sup>8,9</sup> and FVC<sup>9</sup> are reduced.

The fact that obesity (and even simulated obesity<sup>10</sup>) reduces lung volumes has been repeatedly documented in both asthmatic and non-asthmatic adult populations. Lung volume reductions in association with higher BMI have been observed for total lung capacity,<sup>8,9</sup> residual volume (RV),<sup>9</sup> most extensively for expiratory reserve volume (ERV)<sup>5,11</sup> and so subsequently/logically for functional residual capacity (FRC).<sup>5,8,9,12,13</sup> Curiously, the results of certain airway hyperresponsiveness studies suggest that FRC and RV may increase at faster rates in subjects with asthma and obesity as compared to normal-weight asthmatic patients.<sup>5,14,15</sup> The latter hints at increased small-airway involvement in asthmatic hyper-responsiveness as BMI

increases and is supported by evidence of increased small-airway resistance among the non-asthmatic obese.<sup>11</sup> The relative contributions of small-airway closure and large-airway narrowing to changes in FEV<sub>1</sub> during methacholine testing may furthermore be quite distinct for obese versus asthmatic populations.<sup>16</sup> In short, how obesity affects the small airway compartment involved in asthmatic hyper-responsiveness remains un-deciphered, though published observations suggest that subtle BMI effects do nevertheless exist, complicating asthma. The suspicion of small-airway responsiveness undetected by spirometry has been termed the “silent zone”,<sup>17</sup> and is thought to contribute to obesity-associated variation in airway responsiveness.

In this context, recent advances in computed tomography (CT) technology are particularly relevant. Specifically, the expiratory to the inspiratory ratio of mean lung density (MLD<sub>e/i</sub>)<sup>18,19</sup> has proven to be a particularly suitable measure for estimating the intensity, the site and the regional heterogeneity of small airway obstruction.<sup>20,21</sup> For example, MLD<sub>e/i</sub> was the best-performing parameter for the early identification of small airway disease on lung cancer screening CTs.<sup>22</sup> MLD<sub>e/i</sub> also outperforms mosaic attenuation for the assessment of small airway involvement in asthma.<sup>23</sup> MLD<sub>e/i</sub> was also the best-performing parameter for the assessment of small airway obstruction in mild asthmatics as determined by a

single breath nitrogen test.<sup>20</sup> We thus hypothesized that differences would exist between obese and non-obese asthmatic individuals in terms of air-trapping progression visualized via high-resolution CT scans. The primary objective of this study was therefore to compare measures of distal airway involvement (air trapping estimated via  $MLD_{e/f}$  on CT scans) throughout methacholine testing between obese (BMI  $\geq 30$ ) and non-obese (BMI  $< 30$ ) patients with asthma. To reduce heterogeneity due to body shape differences between sexes, this first mechanistic study further focussed on women.

## 2 | METHODS

### 2.1 | Study design and population

In this perspective, a mechanistic cohort study was approved by an independent ethics committee (*Comité de Protection des Personnes Sud Méditerranée III*; reference number: 2011.12.05 bis) on 16 January 2012. Adult female patients ( $\leq 85$  years of age) were recruited between June 2012 and March 2014 at the Montpellier University Hospital, France. Focussing this first study on women helped limit variation in body shape. All patients had asthma (known positive response to a methacholine provocation test with a PD20  $< 2460$  mcg and asthma symptoms) with baseline FEV<sub>1</sub> values  $\geq 80\%$  on maintenance treatment. Patients with other known respiratory diseases (e.g. emphysema or non-obesity-related restriction; see the online repository for detailed criteria) were excluded. Recruitment was stratified according to BMI and subdivided into two groups: nonobese (BMI  $< 30$ ) and obese (BMI  $\geq 30$ ) subjects. All patients gave their free and informed consent before the study commencement.

### 2.2 | Overview of exams performed

The ACQ-6 questionnaire<sup>24</sup> was used to summarize symptomology and each patient participated in exhaled nitric oxide (NO) and alveolar NO testing as a marker of airway inflammation (see the [online supplement](#) for details). Afterwards, a methacholine provocation test coupled with CT scanning was performed. Briefly, baseline chest imaging and spirometry were performed. Then, following each step in the methacholine test, imaging and spirometry were repeated ([Figure 1](#)). Image processing determined mean lung densities from CT scans in both the upper and lower lungs, as well as bronchial morphology at baseline.

### 2.3 | Spirometry and methacholine testing coupled with CT

Baseline spirometry was first performed in a seated position (see [Figure 1](#)). Immediately afterwards, spirometry was re-performed

in a supine position on the CT scan table and a practice sham procedure was explained and simulated. If the patient's FVC in the supine position was at least 90% of that when performed while seated, a whole chest volume was scanned at total lung capacity to establish baseline inspiratory mean lung density and for bronchial morphology measurements. Next, isotonic saline was given via dosimeter, followed by spirometry and a CT scan. If FEV<sub>1</sub> and FVC values were above 80% of baseline values, then the methacholine challenge began. Expiratory CT scan slices (restricted to small subsets in the upper and lower lungs for safety reasons) were acquired just after each step of the methacholine challenge until the PD20 (i.e. the cumulative methacholine dose resulting in an FEV<sub>1</sub> decline  $> 20\%$ ) was reached. Methacholine doses were at a maximum of 5 min apart. Then, four puffs of salbutamol (50  $\mu\text{g}$  each) were given and, 15 min later, a last expiratory CT acquisition was performed.

All spirometry procedures were performed using a Winspiro unit. Methacholine was reconstituted and appropriately diluted to conduct the challenge according to the protocol provided in the online supplement. The escalating cumulative methacholine dose schedule was: 0 (placebo saline), 20, 40, 100, 220, 540, 940, 1500, 2060 and a maximum of 2460  $\mu\text{g}$ . FEV<sub>1</sub> and FVC were reported for each spirometry test. Values obtained at the placebo step were the reference for determining response. The cumulative methacholine dose corresponding to a 20% fall in FEV<sub>1</sub> (PD20) in reference to the saline step was interpolated using the last two FEV<sub>1</sub> measures and log transformed cumulative doses.

### 2.4 | Computed tomography

Imaging was performed immediately after spirometric measurements (see [Figure 1](#)) and without contrast material using a 64-detector row CT unit (LightSpeed VCT, GE Healthcare). Further details are provided in the [online supplement](#), along with representative CT images ([Figure S1](#)).

At baseline, one whole chest volume was scanned at total lung capacity (spirometrically gated at 90%–100% of FVC) for estimating segmental bronchi measurements and inspiratory mean lung density. To minimize patient X-ray exposure according to ethics committee instructions, CT scans for estimating expiratory mean lung density (spirometrically gated at  $20\% \pm 5\%$  of FVC) at each step during methacholine challenges were limited to two small subsets of 10 consecutive slices in the upper (between the apices and the carina) and lower (between the carina and the diaphragm) areas of the lung.

Image data processing was performed (blinded) by a senior radiologist with over 8 years of experience in chest CT (SB).

Bronchial morphology measurements were performed on the baseline inspiratory whole chest scan using commercially available software (Thoracic VCAR, GE Healthcare) that automatically tracks airways and provides cross-sectional data. For each patient, the radiologist selected anatomically fixed sites at mid-distance between

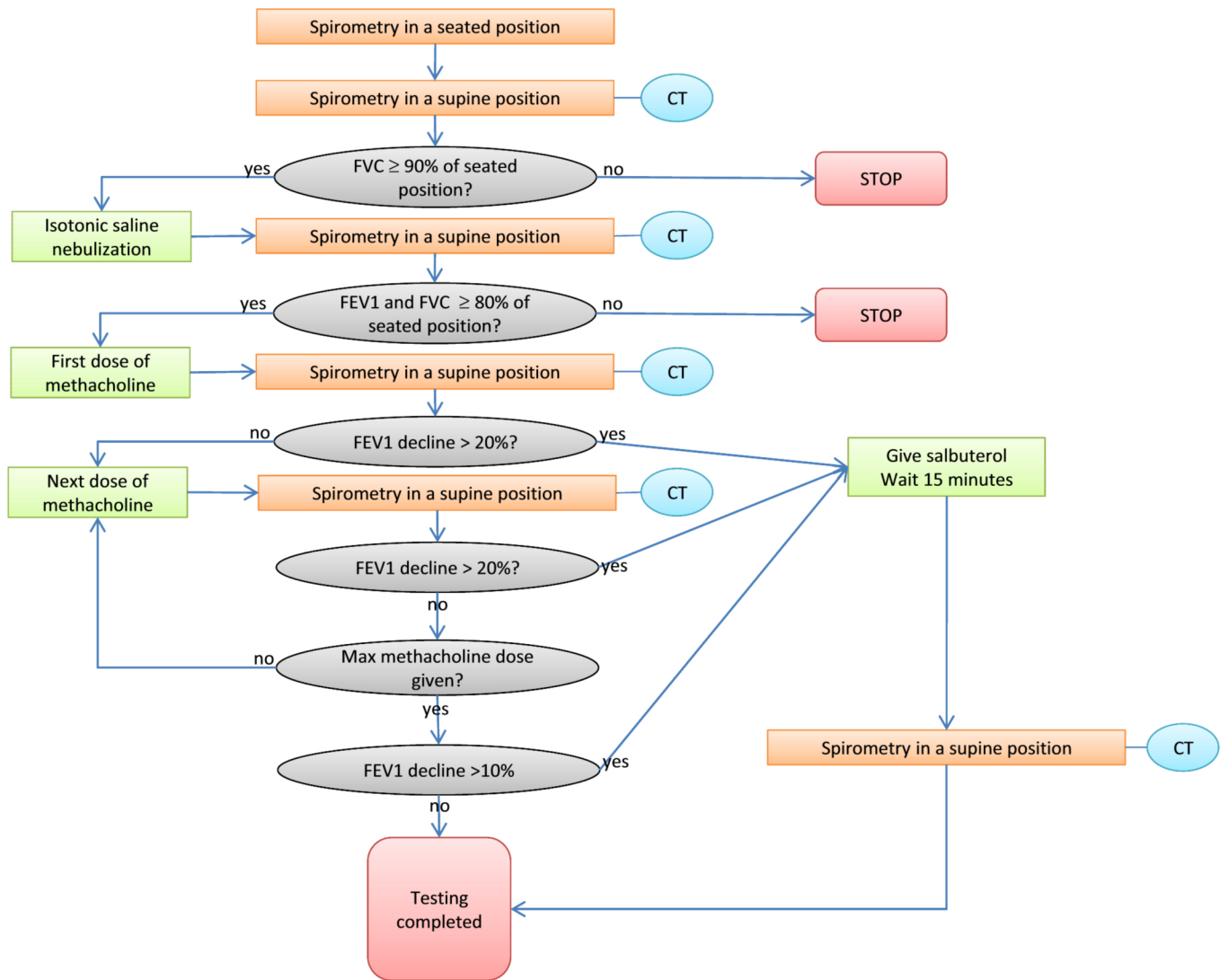


FIGURE 1 Overview of methacholine challenge with spirometry and computed tomography scans. CT, computed tomography; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity

the origin of each chosen segmental bronchus (the right apical bronchus (B1) and the anterior basal bronchus (B8) according to the Boyden classification) and its first division. The B1 and B8 locations were chosen as the least susceptible to artefacts. The percentage of the airway cross-sectional area occupied by the wall area (%WA) and the % wall thickness to airway diameter ratio (%WT) were measured at each site and averaged.

Mean lung densities were quantified (after delineating parenchymal borders) on the whole chest baseline CT scan performed at total lung capacity and on each of the expiratory scans performed at each step of the methacholine provocation test process (Figure 1). This required separate software (Myrian, Intrasure, Montpellier, France) based on thresholding techniques. Expiratory and inspiratory images were matched according to anatomical landmarks (spine, cardiac cavity and bronchial divisions). Finally, average expiratory-to-inspiratory ratios of mean lung density (MLDe/i)<sup>20</sup> were calculated for each step of the methacholine provocation test.

## 2.5 | Sample size and missing data

As previously published imaging data within bronchial provocation tests do not exist, sample sizes could not be based on calculations. Given the logistic complexity of combining sequential series of scanning and spirometry, this first study therefore targeted feasibility and aimed to compare groups of at least 12 patients. No imputation of missing values was foreseen or used.

## 2.6 | Statistics

Statistical analyses were performed in the R programming environment.<sup>25</sup> Descriptive statistics are presented as geometric means [95% confidence interval] for PD20. For the remaining parameters, means  $\pm$  standard deviation are presented for normally distributed continuous variables or medians (interquartile range) for other continuous variables. Qualitative data are summarised via percentages.

Linear regressions (adjusted on variables that differed between groups) were used to test for obesity-group effects at five levels: (i) baseline values, (ii) sensitivity as measured by the PD20 for each patient,<sup>26-28</sup> (iii) percent responsiveness values

at the end of methacholine testing (PD20 percent change values, also known as airway responsiveness), (iv) the closing index (% change in FVC/% change in FEV1)<sup>16</sup> and (v) percent reversibility.

TABLE 1 Baseline characteristics with comparisons between non-obese and obese female asthmatic patients

	Total n = 31	Non-obese: BMI < 30 n = 18	Obese: BMI ≥ 30 n = 13	Group comparisons		
				p <sup>a</sup>	p <sub>adjusted</sub> <sup>b</sup>	Coef ± SE for obesity
Age (years)	35.0(28.5–57.5)	30.5(28.0–42.5)	57(31–65)	0.029 <sup>b</sup>	--	--
BMI (kg/m <sup>2</sup> )	28.11 ± 8.41	22.21 ± 3.62	36.27 ± 5.81	<0.001	--	--
Smoking: never/ former/current	74.19%/6.45%/19.35%	61.11%/11.11%/27.78%	92.31%/0%/7.69%	0.149	--	--
NCS	12.90%	16.67%	7.69%	0.621	--	--
ICS+LABA	45.16%	33.33%	61.54%	0.157	--	--
OCS	3.23%	0%	7.69%	0.419	--	--
LRA	12.90%	11.11%	15.38%	0.999	--	--
SABA	16.13%	5.56%	30.77%	0.134	--	--
Bronchus morphology, an average of B1 and B8 (Boyden classification)						
%WT	23.72 ± 1.98	23.04 ± 1.74	24.93 ± 1.87	0.017	0.011 <sup>d</sup>	2.1 ± 0.76
%WA	71.33(70.00–74.04)	70.65(69.26–72.41)	73.21(72.62–75.07)	0.009 <sup>c</sup>	0.016 <sup>d</sup>	3.99 ± 1.55
Exhaled nitric oxide						
FENO	17.00(15.00–44.00)	24.50(15.75–45.25)	16.00(14.00–35.00)	0.281 <sup>c</sup>	0.835	-0.10 ± 0.49
CalvNO	12.60 ± 6.71	12.57 ± 6.48	12.64 ± 7.34	0.982	0.921	0.05 ± 0.52
Symptoms						
ACQ-6 score	1.41 ± 1.01	1.27 ± 1.02	1.56 ± 1.01	0.455	0.745	-0.15 ± 0.44
Mean lung density on computed tomography scans						
Mean MLD <sub>i</sub> (HU)	-799.53 ± 33.92	-815.25 ± 24.41	-775.94 ± 33.19	0.002	0.014	0.99 ± 0.37
Mean MLD <sub>e</sub> (HU)	-592.87 ± 76.30	-588.12 ± 70.89	-600.00 ± 86.54	0.697	0.174	0.42 ± 0.30 <sup>f</sup>
Mean MLD <sub>e/i</sub> (%)	71.96(66.80–80.85)	70.59(66.80–76.10)	80.72(72.83–84.19)	0.146 <sup>c</sup>	0.653	0.11 ± 0.26
Pulmonary function (spirometry)						
FEV1, % predicted	78.38 ± 11.69	82.19 ± 8.78	73.11 ± 13.44	0.046	0.002 <sup>e</sup>	-1.21 ± 0.34
FVC, % predicted	91.77 ± 11.77	97.28 ± 9.22	84.57 ± 11.07	0.003	0.002 <sup>e</sup>	-1.17 ± 0.34
FEV1/FVC (% L/L)	72.41 ± 7.34	72.81 ± 7.12	71.89 ± 7.89	0.866	0.571 <sup>e</sup>	-0.24 ± 0.42

Note: Descriptive statistics are presented as means ± standard deviation for those variables with a normal distribution according to a Shapiro-Wilks test, and medians (interquartile range) otherwise.

Abbreviations: ACQ-6, asthma control questionnaire 6; BMI, body mass index; CalvNO, alveolar nitric oxide; Coef, standardized coefficient of the obesity term; FENO, fractionated exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; MLD<sub>e/i</sub>, the expiratory to the inspiratory ratio of mean lung density; SE, standard error; %WA, (wall area (mm<sup>2</sup>)/(wall area (mm<sup>2</sup>) + lumen area (mm<sup>2</sup>)) × 100; %WT, (wall thickness (mm)/airway diameter (mm)) × 100.

<sup>a</sup>p values for group comparisons were performed using t-tests or Fisher's exact tests unless otherwise indicated.

<sup>b</sup>p-values determined via linear models adjusting for age and bronchial morphology (Both %WT and %WA adjustments were attempted and provided similar models with negligible changes in parameters and goodness of fit; the best model was retained.) unless otherwise indicated.

<sup>c</sup>Mann-Whitney test.

<sup>d</sup>Adjusted for age only.

<sup>e</sup>Adjusted for bronchial remodeling only.

<sup>f</sup>Note that the direction of adjusted coefficients is counter-intuitive relative to means/medians due to adjustments. For example, there is a tendency for obesity to associate with an increase in baseline MLD<sub>e</sub> once age is taken into account.

As in Niimi et al,<sup>29</sup> CT air-trapping and pulmonary function curves were standardized by plotting response variables against the log of the percentage of the maximum cumulative methacholine dose (ln[1 + %PD20 dose]) used. The resulting standardized response curves (SRCs) visually display the relative amplitude of experimental bronchoconstriction, while controlling for sensitivity.

Mixed models explaining air-trapping/spirometric variables as a function of obesity and obesity-by-ln(1 + %PD20 dose) interactions were performed while adjusting for variables that differed between groups [i.e. baseline-values (as in Deemsomshok et al<sup>5</sup>), bronchial morphometry (as WA% and WT% were highly correlated and differential effects on models were trivial, only WA% was included in mixed models), age (for explaining MLD<sub>e/i</sub> only because spirometry variables are already adjusted for age) and baseline FEV1 (to account for differences in airway obstruction that might affect small airway reactivity as represented by FVC or MLD<sub>e/i</sub>)]. Patients were defined as a random effect. Models including baseline FEV1 as a fixed factor and that minimized the Akaike information criterion were considered appropriate. Following evidence of obesity-by-ln(1 + %PD20

dose) interactions, similarly adjusted linear regressions were used to detect significant obesity-group effects on slopes within tertiles of interpolated individual SRCs. Mixed models were also repeated using BMI as a continuous variable.

### 3 | RESULTS

#### 3.1 | Baseline population description

Descriptive statistics for baseline population characteristics are presented for the total population ( $n = 31$ ), the non-obese group ( $n = 18$ ) and the obese group ( $n = 13$ ) in Table 1. The populations were similar in terms of smoking history and anti-asthma treatments. The obese group was significantly older than the non-obese group, and statistical adjustments for age were thus performed as required throughout the study.

Baseline differences between BMI groups included a greater attenuation (mean lung density) on inspiratory CT scans for the obese,

**TABLE 2** Descriptive statistics and group comparisons for traditional measures of methacholine sensitivity, overall airway responsiveness, a small airway closure index and reversibility

	Total $n = 31$	Non-obese BMI < 30 $n = 18$	Obese BMI $\geq 30$ $n = 13$	Non-obese versus obese		
				$p^a$	$p_{\text{adjusted}}^b$	Coef $\pm$ SE for obesity
Sensitivity						
PD20: geometric mean [95% CI]	390.74 [247.26–617.47]	428.66 [237.06–775.12]	348.64 [154.75–785.45]	0.740 <sup>d</sup>	0.768 <sup>d</sup>	0.15 $\pm$ 0.49
%Reactivity at maximum methacholine dose (Airway responsiveness)						
MLD <sub>e/i</sub> mean–% increase	6.45 (3.76–13.13)	6.41 (4.43–11.92)	9.18 (3.71–17.40)	0.580 <sup>c</sup>	0.054	0.77 $\pm$ 0.38
FEV1 (L)–% fall	24.70 (23.06–28.26)	24.69 (23.05–28.10)	24.71 (23.11–28.31)	0.621 <sup>c</sup>	0.422 <sup>e</sup>	0.29 $\pm$ 0.35
FVC (L)–% fall	20.64 $\pm$ 6.94	18.39 $\pm$ 6.68	23.05 $\pm$ 6.60	0.080	0.170 <sup>e</sup>	0.65 $\pm$ 0.45
FEV1/FVC (% L/L)–% fall	7.70 $\pm$ 6.71	9.67 $\pm$ 5.67	5.59 $\pm$ 7.30	0.120	0.167 <sup>e</sup>	–0.61 $\pm$ 0.43
Airway closure at PD20						
Closing index	0.76 $\pm$ 0.22	0.68 $\pm$ 0.22	0.84 $\pm$ 0.19	0.043	0.166	0.71 $\pm$ 0.49
% Reversibility						
MLD <sub>e/i</sub> mean	4.57 (5.96–2.21)	3.81 $\pm$ 3.20	6.74 $\pm$ 5.59	0.135	0.045	0.93 $\pm$ 0.44
FEV1 (L)	24.80 (20.23–37.23)	21.66 (19.57–29.20)	28.37 (22.88–37.61)	0.145 <sup>c</sup>	0.344 <sup>e</sup>	0.45 $\pm$ 0.47
FVC (L)	17.31 $\pm$ 11.20	13.66 $\pm$ 11.44	22.28 $\pm$ 9.12	0.043	0.078 <sup>e</sup>	0.99 $\pm$ 0.53
FEV1/FVC (% L/L)	9.87 $\pm$ 7.21	11.10 $\pm$ 6.93	8.20 $\pm$ 7.57	0.330	0.479 <sup>e</sup>	–0.40 $\pm$ 0.56

Note: PD20 data are presented as geometric means and their 95% confidence intervals. The remaining descriptive statistics are presented as means  $\pm$  standard deviation for those variables with a normal distribution according to a Shapiro–Wilks test, and medians (interquartile range) otherwise.

Abbreviations: Coef, standardized coefficient of the obesity term; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; MLD<sub>e/i</sub>, the expiratory to the inspiratory ratio of mean lung density; PD20, the extrapolated cumulative dose of methacholine associated with a decrease in FEV1 of 20%; SE, standard error.

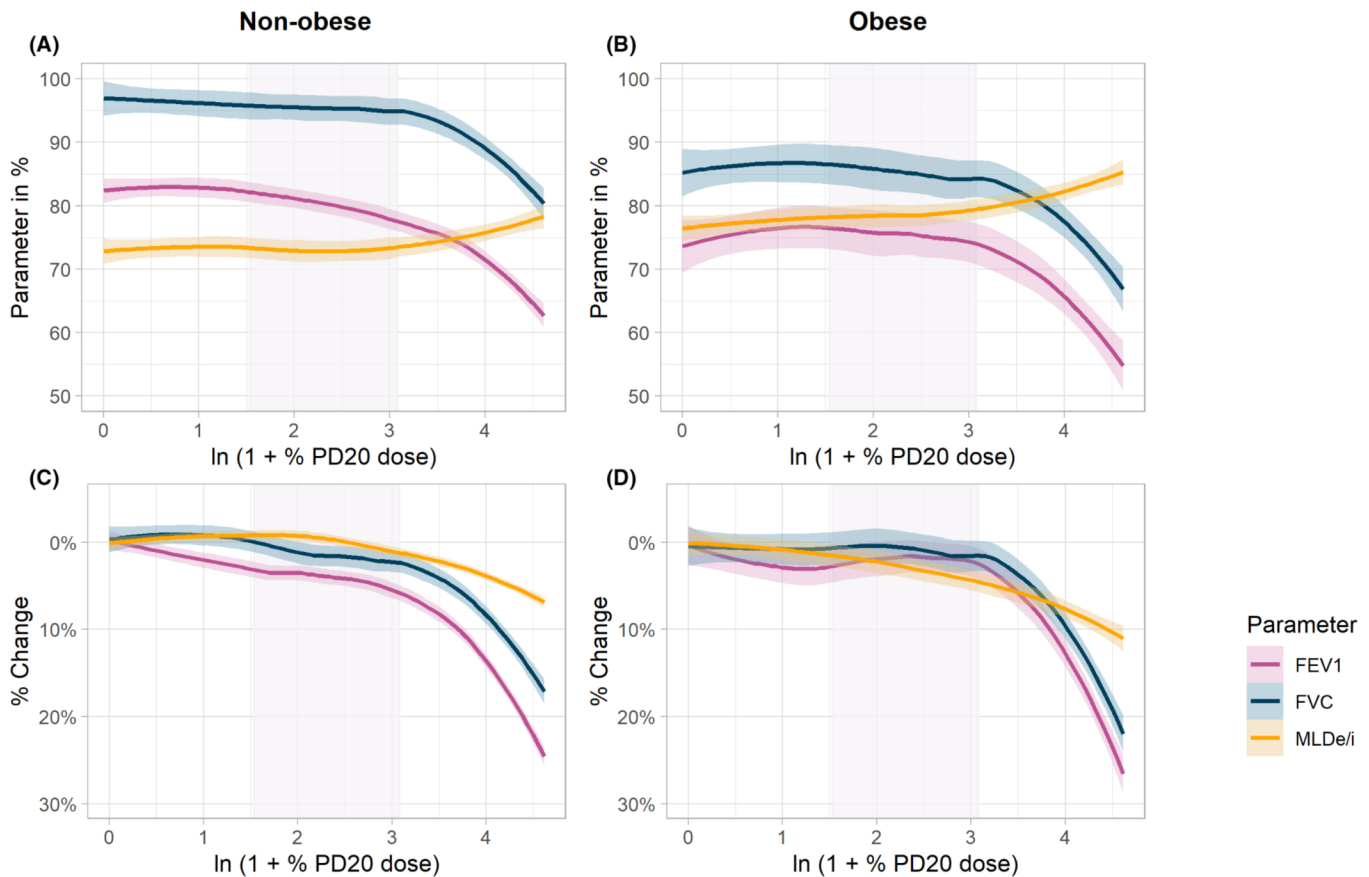
<sup>a</sup> $p$  values for group comparisons were performed using  $t$ -tests unless otherwise indicated.

<sup>b</sup> $p$ -values determined via linear models adjusting for age and bronchial morphology (Both %WT and %WA adjustments were attempted and provided similar models with negligible changes in parameters and goodness of fit; the best model was retained.) unless otherwise indicated.

<sup>c</sup>Mann–Whitney test.

<sup>d</sup>Data were log-transformed.

<sup>e</sup>Adjusted for bronchial morphology only as % predicted spirometric measures already take age into account.



**FIGURE 2** Standardized response curves to methacholine testing (experimental bronchoconstriction). Abscissa represent the log-transformed methacholine dose as a percentage of the maximum dose performed ( $\ln(1 + \% \text{PD}_{20} \text{ dose})$ ), with the shaded background area indicating the middle tertile. Lines indicate a local loess estimate of the mean and pastel zones the accompanying standard error. Left panels (A,C) present data for non-obese patients and right panels (B,D) that for obese patients. Upper panels (A,B) present raw data for FEV1, FVC and  $\text{MLD}_{e/i}$  in %, while the lower panels (C,D) present the relative percentage change for the same parameters during methacholine testing. FEV1, forced expiratory volume in 1 s (% predicted); FVC, forced vital capacity (% predicted);  $\text{MLD}_{e/i}$ , the expiratory to the inspiratory ratio of mean lung density from computed tomography;  $\text{PD}_{20} \text{ dose}$ , the maximum methacholine dose administered (after which a 20% decrease in FEV1 was observed)

indicating that the inflated lung of an obese individual is denser (or less-inflated) than that of a non-obese counterpart ( $-775.94 \pm 33.19$  HU vs.  $-815.25 \pm 24.41$  HU;  $p = 0.014$ ). The baseline expiratory and the ratio of expiratory: inspiratory mean lung densities ( $\text{MLD}_{e/i}$ ) did not differ between BMI groups (Table 1). Spirometric differences previously known to associate with obesity<sup>8,9</sup> were found: after withdrawal of maintenance medication, baseline FEV1 (obese:  $73.11 \pm 13.44$ ; non-obese:  $82.19 \pm 8.78$ ;  $p = 0.002$ ) and FVC (obese:  $84.57 \pm 11.07$ ; non-obese:  $97.28 \pm 9.22$ ;  $p = 0.002$ ) % predicted values were significantly lower for obese patients, while the FEV1/FVC ratio was not.

Bronchial morphology indices were significantly lower among non-obese patients compared to their obese counterparts (%WT:  $23.04 \pm 1.74$  vs.  $24.93 \pm 1.87$ ;  $p = 0.011$ ; %WA:  $70.65$  (69.26–72.41) vs.  $73.21$  (72.62–75.07),  $p = 0.016$ ). No significant baseline differences between groups were found in terms of exhaled nitric oxide measures or symptoms as measured by the ACQ-6 score (Table 1).

### 3.2 | Overall response parameters: airway sensitivity, responsiveness, a closing index and reversibility

Only one patient had a %fall in FEV1 at  $\text{PD}_{20}$  which was under 20%. This patient has a %fall in FEV1 of 18.9% and this was omitted from  $\text{PD}_{20}$  estimates. Sensitivity as measured by  $\text{PD}_{20}$ <sup>26–28</sup> did not differ between obese and non-obese groups (the geometric mean [95% confidence interval] of  $348.64$  [ $154.75$ – $785.45$ ]  $\mu\text{g}$  methacholine for obese individuals vs  $428.66$  [ $237.06$ – $775.12$ ]  $\mu\text{g}$  for the nonobese;  $p = 0.768$ ; Table 2). Airway responsiveness tended to be higher for  $\text{MLD}_{e/i}$  in the obese ( $11.31 \pm 9.51$ ) versus the non-obese ( $7.51 \pm 5.04$ ;  $p = 0.054$ ), while no such differences were found for FEV1, FVC, FEV1/FVC or the Chapman closing index. Finally, there was at least a tendency for increased reversibility for  $\text{MLD}_{e/i}$  and FVC among participants with obesity ( $6.74 \pm 5.59$  and  $22.28 \pm 9.12$ , respectively) as compared to their non-obese counterparts ( $3.81 \pm 3.20$  and  $13.66 \pm 11.44$ ;  $p = 0.045$  and  $p = 0.078$ ; see Table 2).



### 3.3 | SRC during bronchial provocation

Spirometric and  $MLDe/i$  SRCs during methacholine testing are presented in Figure 2. For raw data values of FEV1, FVC and  $MLDe/i$  (Figure 2, panels A and B), the baseline differences between the non-obese and obese (Table 1) in spirometry are visually evident at the start of SRCs. Figure 2C,D present the associated % change in parameters throughout bronchial provocation, thus taking into account such baseline offsets. The latter demonstrates that among the non-obese participants, the first parameter to worsen is FEV1 (Figure 2C, while in the obese (Figure 2D), the first parameter to worsen is  $MLDe/i$ , which is then quickly overtaken by FVC and FEV1. For all curves in Figure 2, the highest levels of responsiveness (i.e. the steepest slopes) are evident in the last third of the curves when the abscissa (i.e. the standardized methacholine step) is approximately  $>3$ .

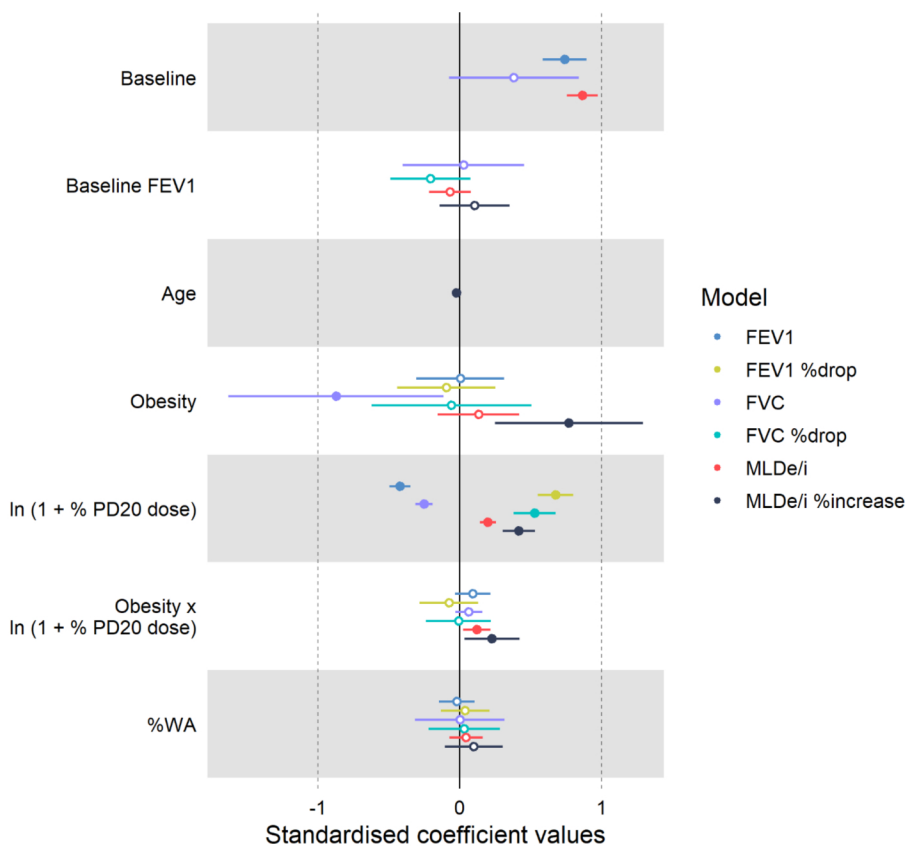
All mixed-effect models for SRCs detected significant ( $p < 0.001$ )  $\ln(1 + \%PD20 \text{ dose})$  effects, indicating the expected significant changes in  $MLDe/i$  and spirometric measures with the increasing relative methacholine dose (Figure 3). Significant ( $p < 0.001$ ) baseline effects were also found for raw values of FEV1 and  $MLDe/i$ , indicating that baseline values influence the course of methacholine response. Age was retained as an adjustor within the best models only for the % increase in  $MLDe/i$ , whereas adjustments for bronchial morphology improved fit but were otherwise insignificant (Figure 3). A fixed effect for obesity was found only for FVC raw values and the % increase in  $MLDe/i$  (Figure 3), indicating that FVC values were lower and the % increase in  $MLDe/i$

was greater in association with obesity in a homogenous fashion throughout the SRCs (Figure 2). However, a significant obesity-by- $\ln(1 + \%PD20 \text{ dose})$  interaction was found for  $MLDe/i$  values (both raw ( $p = 0.016$ ) and % change ( $p = 0.023$ ); Figure 3), indicating differences in slope (and therefore responsiveness) within the  $MLDe/i$  SRCs between non-obese and obese groups. The distribution of slopes within SRCs are therefore presented in Figure 4. Mixed models replacing group effects with BMI as a continuous variable differed in that they lacked obesity effects, but nevertheless indicated significant differences in slopes for  $MLDe/i$  variables (see Figure S2).

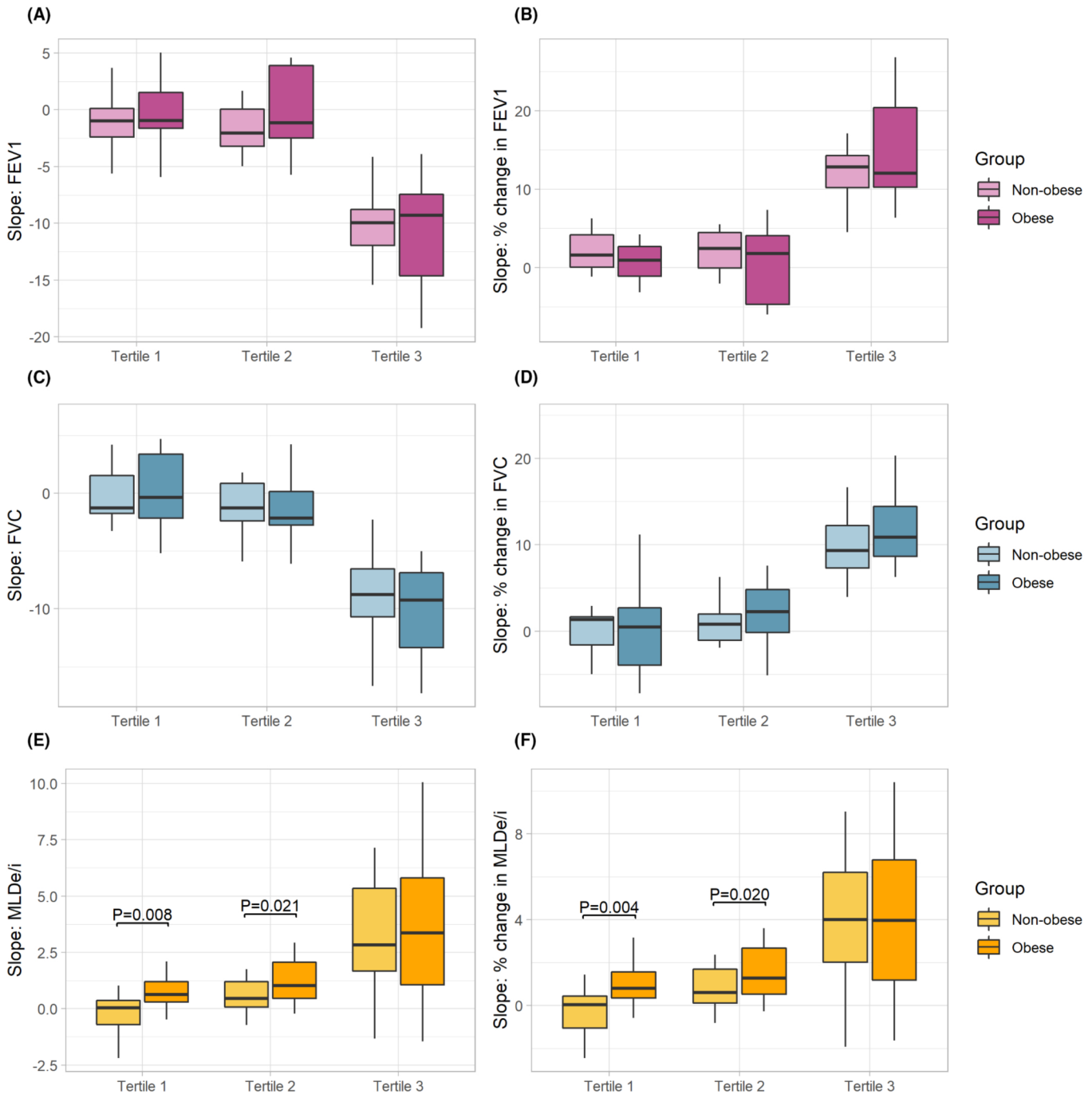
As concerns  $MLDe/i$  slopes, responses were significantly greater for obese patients in the first 2/3 of both the raw value and % change SRCs (Figure 4, panels E and F).

## 4 | DISCUSSION

This study demonstrates that obesity is associated with greater small airway responsiveness during bronchial provocation, as objectively measured by  $MLDe/i$  on CT scans, among patients with asthma as compared to their normal-weight asthmatic counterparts. As shown by significantly higher slopes for  $MLDe/i$ , the air-trapping response to methacholine was significantly greater among the obese (Figures 3,4). This greater responsiveness was detected in the first two-thirds of experimental broncho-constriction and represents a potential mechanism that can explain previous descriptions of a spirometric “silent zone”.<sup>17</sup> For non-obese patients, bronchial



**FIGURE 3** A forest plot presenting the standardized coefficients (dots) and their 95% confidence intervals (horizontal bars) for mixed model parameters (each model explains a given lung function parameter indicated by the legend). Coefficients considered insignificant within their models have white centers (and their 95% confidence intervals cross zero). %WA, percentage of the airway cross-sectional area occupied by the wall area; %WT, the % wall thickness to airway diameter ratio; FEV1, percentage predicted value for forced expiratory volume in 1 second; FVC, percentage predicted value for forced vital capacity;  $MLDe/i$ , average expiratory: inspiratory ratios of mean lung density; PD20, the maximum dose of methacholine achieved



**FIGURE 4** Slopes at the beginning, middle and end thirds (tertiles 1, 2 and 3) of standardized methacholine response curves for forced expiratory volume in 1 s (FEV1, panels A and B), forced vital capacity (FVC, panels C and D) and the average expiratory to inspiratory ratios for mean lung density ( $MLD_{e/i}$ , panels E and F). The panels on the left side (A,C,E) present slope distributions for raw parameters, and those on the right side (B,D,F) slopes for percent changes in the same parameters. Data are expressed as boxes depicting the first, second (median) and third quartiles, non-outlier ranges as whiskers and outliers as dots. *p*-values are significance levels for obesity group effects adjusted for age, baseline FEV1 and bronchial morphology (when not-indicated, results were  $p > 0.1$ )

provocation appears to be more dominated by large-airway constriction as represented by FEV1.

Importantly, these obesity effects that increased the intensity of air-trapping in tandem with decreased FVC during a methacholine response occurred in addition to and independently of baseline effects. Significant baseline effects for the CT air-trapping and FEV1 variables addressed in the present study suggest that the state at

which a patient enters a potential asthma exacerbation influences the course of the subsequent response. Baseline differences associated with obesity, such as well-known reductions in lung volumes (as previously reported<sup>11,30</sup> and demonstrated by decreased FEV1 and FVC values in Table 1), are thus likely to persist throughout an episode of bronchoconstriction, worsening subsequent outcomes. However, our data demonstrate that baseline states, including differences in

baseline obstruction as represented by FEV1, do not represent the full extent of obesity's effects on airway responsiveness. Intensified air-trapping in small airways, as estimated by changes in  $MLD_{e/i}$ , is additionally present in obese patients (Figures 2,4).

Previous studies have implied that overweight and obese people, irrespective of the presence of asthma, suffer from mechanical constraints that limit their ability to achieve physiological distal airspace inflation at full inspiration.<sup>8,10,11</sup> We demonstrated significantly denser MLD among obese patients (as compared to the non-obese, Table 1) during inspiratory CT, providing further support for this mechanism. This decreased preload may subsequently preclude the ability to achieve complete expiration, increasing the potential for breathing cycles within the closing volume, a quite well-known mechanism of obesity-related functional hypoxemia. We previously observed this phenomenon in non-asthmatic women.<sup>31</sup> Alterations of airway compliance and distensibility likely explain at least part of the obesity effects described here.

A limitation of the present study is that it only included women. While this constraint avoids sex-related heterogeneity in the distribution of adipose tissue, it would be interesting to further explore obesity effects in men. Chinn et al have previously demonstrated that the relationship between BMI and bronchial responsiveness is stronger in men than in women,<sup>32</sup> suggesting that our results might be conservative from this point of view. A second limitation of our study is the small sample size, which was necessary due to the logistic complexity of serial CT scanning during methacholine testing. The latter was also restricted to small windows of acquisition in the upper and lower lungs for patient safety, which might not represent the extent of heterogeneity in a full lung acquisition. Finally, and for obvious safety reasons, bronchoconstriction is halted at a drop in FEV1 of 20%. The latter puts an evident limit on responsiveness at the end of bronchial provocation testing, resulting in similar ending slopes. Our populations also differed in terms of baseline FEV1, age and bronchial morphology, thus requiring statistical adjustment. Despite these limitations, we were able to demonstrate  $MLD_{e/i}$  differences during methacholine testing in association with obesity, with surrounding results concordant with the literature. Notably, we reproduced previously demonstrated obesity-associated reductions in FEV1 and FVC<sup>30</sup> and baseline effects.<sup>5</sup>

Burgess et al<sup>33</sup> suggested that airway closure, as represented by relatively large decreases in FVC versus FEV1, mediates a large part of the relationship between BMI and airway hyperresponsiveness. Our data further underline that such airway closure specifically involves peripheral small airways, the dynamics of which cannot be demonstrated by spirometry alone (a phenomenon known as the "silent zone"<sup>34,35</sup>).

Accelerated, more-intense air-trapping following baseline disadvantage in association with obesity has important clinical implications. Among non-obese patients, the progression of  $MLD_{e/i}$  air-trapping is delayed at first (with slopes at or near zero, Figure 4) and then rapidly rises in the last third of SRCs. In contrast, obese patients experience more intense air-trapping early on during the bronchoconstriction process. Given the difference in dynamics, a mild exacerbating insult

is more likely to result in clinically-relevant air-trapping for an obese person than for a normal-weight individual. In this context, lifestyle and weight-loss measures, already demonstrated to improve lung function in some patients,<sup>36,37</sup> take on increased importance.

In conclusion, we demonstrate that obese asthmatic women present with increased CT air-trapping (as measured by the expiratory to inspiratory ratios of mean lung density) during methacholine testing in addition to strong baseline effects. To the best of our knowledge, this is the first objective demonstration that BMI negatively affects the extent and speed of small airway responsiveness in an asthmatic population.

#### AUTHOR CONTRIBUTIONS

AB, GM, SB, IV and NM designed the study. AB, ASG and EA acquired clinical data. SB acquired imaging data. CMS, NM and GM performed statistics. CMS wrote the first draft of the manuscript. All authors participated in manuscript corrections/additions and gave final approval of this version.

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#### CONFLICT OF INTEREST

AB reports grants, personal fees, non-financial support and other from Astra Zeneca, grants, personal fees and other from GSK, grants, personal fees, non-financial support and other from Boehringer Ingelheim, personal fees, non-financial support and other from Novartis, personal fees and other from Teva, personal fees and other from Regeneron, personal fees, non-financial support and other from Chiesi Pharmaceuticals, personal fees, non-financial support and other from Actelion, other from Gilead, personal fees and non-financial support from Roche, outside the submitted work. SB reports previous grants from Astra Zeneca, outside the submitted work. GM, IV, ASG and EA have no conflicts of interest to report. CMS reports fees and a previous grant from Astra Zeneca, and a grant from GSK outside the submitted work. NM reports personal fees from Astra Zeneca, grants from GSK, outside the submitted work.

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