Kan olika deponeringsmönster användas för en mer exakt deponering av partiklar i de små luftvägarna?

Can different deposition patterns be used for a more accurate deposition of particles in the small airways?

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Abstract

Introduction: Asthma and Chronic obstructive pulmonary disease (COPD) are chronic diseases that causes airflow obstruction. If inflammation is present (predominantly in asthma), this will increase bronchial reactivity, which can be tested by bronchial provocation tests, using for example methacholine. An increased degree of reactivity (i.e., hyper-reactivity) supports the diagnosis of asthma, but even healthy persons react with broncoconstriction when exposed to inhaled methacholine. The aim of this study is to investigate if we could measure any difference in outcome using impulse oscillometry when performing methacholine provocations in two different exposures regarding droplet size and inhalation flow rate, supposedly giving two different deposition patterns within the airways.

Method: Eight healthy participants was examined on three separate days. On the first visit, a reactivity test was performed. On the second- and third-day bronchial provocation was made with two different inhalation patterns: A standard normal inhalation patters (approximately 1 L/s) was compared to extremely slow inhalations (ESI) using large droplets. Airway resistance was measured with a plethysmograph. Resistance and reactance were measured with Impulse oscillometry.

Result: The result showed no significant differences between slow and normal inhalation. The peripheral resistance measured by impulse oscillometry did not increase with slow inhalations compared with normal inhalation.

Conclusion: This study did not find any differences between slow and normal inhalation patterns measured with the IOS. Theoretically it should give a difference in depositions patterns in the airways. But whether this means that there is no difference or that it is simply not possible to measure the eventual difference has not yet been identified.

Sammanfattning

Inledning: Astma och Kronisk obstruktiv lungsjukdom (KOL) är två kroniska sjukdomar som orsakar obstruktion av luftvägarna. Om inflammation av luftvägarna förekommer (främst förekommande i astma), kommer hyperaktiviteten i bronkerna att öka, vilket kan mätas med ett bronkialprovokations test, med inhalation av exempel vis metakolin. En ökad grad av reaktivitet (dvs. hyperreaktivitet) stödjer diagnosen astma, men även friska individer reagerar med bronk-konstriktion när de utsätts för inhalerad metakolin. Syftet med studien är att undersöka om vi kan mäta någon skillnad med hjälp av impulsoscillometri när vi utför metakolinprovokationer med två olika exponeringar avseende droppstorlek och inandningsflödeshastighet, vilket antas ge två olika deponeringsmönster i luftvägarna.

Metod: Åtta friska deltagare undersöktes vid tre separata dagar. Vid det första besöket utfördes ett reaktivitetstest för att utvärdera deltagarnas hyperaktivitet. På andra och tredje dagen utfördes bronkial provokation med två olika inandningsmönster: Ett normalt inhalationsflöde (ca 1 L/s) jämfört med ett extremt långsamt inhalationsflöde med större droppar. Luftvägsresistansen och reaktans uppmättes med impulsoscillometri.

Resultat: Resultatet visade inga signifikanta skillnader mellan långsam och normal inandning. Den perifera resistansen mätt med impulsoscillometri ökade inte vid långsam inandning jämfört med normal inandning.

Slutsats: Denna studie fann inga skillnader mellan långsamma och normala inandningsmönster uppmätta med IOS. Teoretiskt sett bör det finnas en skillnad mellan deponeringsmönstren i luftvägarna. Men om detta innebär att det inte finns någon skillnad eller om det helt enkelt inte är möjligt att mäta den eventuella skillnaden har ännu inte identifierats.

Contents

List of abbreviations

ATS	American Thoracic Society
AX	Total reactance
BHR	Bronchial hyper reactivity
COPD	Chronic obstructive pulmonary disease
DPI	Dry powder inhalers
EB-OCT	Endobronchial optical coherence tomography
ERS	European Respiratory Society
FEV1	Forced expiratory volume after 1 second
FRC	Functional residual capacity
FVC	Forced vital capacity
Hz	Sound waves frequency Hertz
IOS	Impulse oscillometry system
k	Constant
MMD	Mass median diameter
P	Pressure
P_a	Alveolar pressure
P_{ao}	Mouth pressure
pMDi	Pressurized metered dose inhalers
R	Resistance
Raw	Airflow resistance
RV	Residual volume
R5	Resistance at 5 Hz (total resistance)
R5-20	5 Hz – 20 Hz (peripheral resistance)
R20	Resistance at 20 Hz (central resistance)
sRaw	Specific airflow resistance
TLC	Total lung volume
V	Volume
$\dot{V}$	Flow
VC	Vital capacity
X	Reactance
Z	Impedance

1 Introduction

1.1 Airflow obstruction

Asthma and chronic obstructive pulmonary disease (COPD) are two major non-communicable diseases worldwide. Both asthma and COPD cause airflow obstruction and are treated with inhaled aerosols. Asthma is most common among children and is an inflammatory disease characterized by airway hyperresponsiveness. COPD is predominantly caused by cigarette smoking and is characterized by alveolar abnormality. COPD symptoms are not reversible with bronchodilating medicine. Further, COPD does not cause hyperreactivity (1).

A maximal expiratory forced maneuver recorded by a spirometer ("flow volume loop") can be used in both diseases. In some cases, a pre-and post-bronchodilator test can be performed (1, 2). The test is quick and equipments are cheap, which is an advantage of the test, but other methods have a higher sensitivity. If further testing is needed, such as measurements of airway resistance, diffusing capacity, and BHR testing, this is done on a case-by-case basis (2).

1.2 Inhalation Treatment

Today's pharmacological treatment for both COPD and asthma are bronchodilation and anti-inflammatory inhalation drugs. The same treatment used in COPD can also treat severe asthma (3). When administrating these drugs, the inhalers create an aerosol of droplets or solid particles (from here on called particles, even if in liquid form). A therapeutical aerosol consists of small, suspended particles with a size of around 2-5 μm. Deposition of particles will occur in all parts of the respiratory tract, most commonly divided in, and defined as, the mouth and throat, bronchii, bronchioles, and alveolar compartment. The particle deposition pattern depends on the mass of the particles and its velocity (i.e., air velocity). High air stream velocities causes turbulence, which accordingly becomes higher in airway generations where the total cross-sectional area of the airways is small (4).

There are different types of hand-held inhalation devices on the market today. The two largest groups are dry powder inhalers (DPI) and pressurized metered-dose inhalers (pMDI). Nebulizers and mechanically pressurized sprays are available, but not as common. The differences between them are that DPI uses the energy from the resistance and pressure drop formed from the patient's inhalation to disperse the dry powder into an aerosol. A pMDI uses a pressurized aerosol propellant gas to distribute the pharmacological liquid into an aerosol (5, 6). In hospitals and home care nebulizers are more common. The jet nebulizer uses compressed air to distribute the liquid into droplets. One advantage of a nebulizer is that you manually put the liquid in the device and by that can choose almost any substance, combination and concentration you want (7).

1.3 Bronchoprovocation

A bronchoprovocation (or bronchial challenge test) test assesses airway reactivity (bronchial hyper reactivity, BHR) and is performed to confirm or rule out the diagnosis of asthma. The commonly used methacholine acts as the neurotransmitter acetylcholine and interacts with muscarinic receptors on the airway smooth muscles, resulting in narrowing of the airways. During the test, the patient inhale an aerosol of methacholine in increasing doses while continuously measuring lung function. If airway narrowing is detected (i.e., at a low concentration), the patient reacts more to the stimuli and then has hyperreactive lungs (8, 9).

1.4 Lung function testing

1.4.1 Flow volume loop

Airway obstruction in asthma and COPD is mainly a disability to exhale. When performing a "spirometry", or more adequately termed "maximum forced expiratory maneuver", the flowrates are registered and evaluated, as an indirect measure of airway dimensions. The main parameters assessed from a flow-volume loop are Forced expiratory volume after 1 second (FEV1), Forced vital capacity (FVC), and the quota FEV1/FVC, also called "Tiffeneau index". An important mechanism in airway obstruction is the mechanism known as dynamic compression (10, 11). This means that the exhalation force increases the thorax pressure and causes the airways to compress when exhaling. In COPD the decrease in elastic recoil (due to emphysema) this effect is enhanced.

When performing bronchial provocations clinically, the outcome measure is a 20% drop of FEV1, which corresponds to about a doubling of the measured airway resistance. If this is reached at a too low inhaled dose, this is defined as hyper-reactivity. The disadvantage of performing a flow-volume curve is that it is well established that deep inhalations (vital capacity) stretch out the smooth muscles of the lungs and reverse airway obstruction induced in healthy or asthmatic subjects (12-14). For that reason, we chose not to use the flow-volume curve in this explorative study.

1.4.2 Body plethysmography

A plethysmograph is a tightly closed chamber ("body box") with a volume of around 700-1000L, used to measure pressure changes in the box during breathing or pressure changes in the patient's lungs measured at the patient's mouth. This method allows calculation of the patient's lung volumes, functional residual capacity (FRC), and (combined with measurement of the vital capacity, VC), also total lung volume (TLC), and residual volume (RV). The physical principle is Boyle's law, which describes the relationship between pressure and volume. A bit simplified, in a closed system, Pressure (P) multiplied by volume (V) is constant (k) (15).

$$PV = k$$

The plethysmograph can also measure airway resistance (Raw) and specific airway resistance (sRaw). *Resistance* is defined as the ratio between pressure and flow. The more pressure needed for a given flow, the higher the resistance. Theoretically there is a very close relation between airway (tube) diameter and flow resistance. Resistance is measured at tidal breathing and is not affected to any significant degree from dynamic compression as forced manoeuvres. The Raw is measured by relating the pressure difference between the alveolar (P_a) and pressure at the mouth (P_{ao}) to the corresponding flow through the airways.

$$Raw = (P_a - P_{ao})/Flow$$

Although Raw = ΔPressure/Flow, the patient's alveolar pressure must be measured to calculate Raw with a plethysmograph. The alveolar pressure can only be calculated from the occlusion manoeuvre, where the relationship between shift volume and alveolar pressure will establish. Raw is therefore computed from sRaw and FRC, measured with a plethysmograph (FRC_{Pleth}) (15, 16).

$$Raw = sRaw \,/\, FRC_{pleth}$$

1.4.3 Impulse oscillometry system

The impulse oscillometry system (IOS) evaluates airflow limitation during tidal breathing (normal resting breaths). The method requires minimal patient cooperation, and is fast and easy to perform, but theoretically, quite complex. In short, a known air pulse is introduced into the airways. The echo of the sound waves (pressure waves) and the resulting flow changes are then continuously recorded at the mouth. The pulse will not be reflected fully from the lungs (i.e., not a complete "echo"). With an advanced mathematical algorithm, the total resistance to the sound waves in the respiratory system, called impedance (Z), is calculated. The impedance consists of resistance (R) and reactance (X), where R is the energy loss from the sound waves propagated down into the lungs, and X is the energy stored in the airways, i.e., the "echo" of the sound wave. The dampening of the pulse will vary with the frequency. Theoretically, lower frequencies will reach longer into the airways, and the higher frequencies will reflect the central airways. A standard IOS measurement assesses airway resistance at 5 Hz (all airways) and 20 Hz (central airways) during 20 s tidal breathing.

Since sound waves with high-frequency, 20 Hz, do not reach beyond the central airways, and low-frequency sound waves, 5 Hz, can propagate through the whole airway tree, the difference between high- and low-frequency sound waves will theoretically represent the peripheral airways (fig 1) (17, 18).

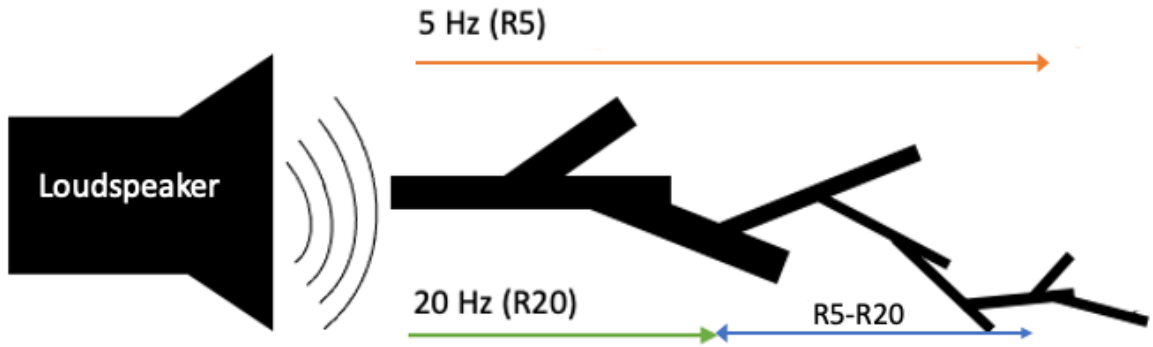

Fig 1. Schematic image of how sound waves with different frequencies propagate in the airways. R5 represent resistance at 5 Hz and the global resistance. R20 represents resistance at 20 Hz in the central airways. R5-R20 represent the peripheral resistance.

This method has advantages for patients who cannot cooperate in standard spirometry (flow/volume-loop), especially in children (19, 20). From the IOS measurements, there are several parameters to evaluate. In this study, the focus is on the parameters of clinical use, the high resistance at 20 Hz (R20), the low resistance at 5 Hz (R5), peripheral resistance (R5-R20), and total reactance (AX) (Fig. 2) (17, 18). Previous studies have shown that X5, R5-R20, and AX will reflect changes in the degree of obstruction in the peripheral airways (21).

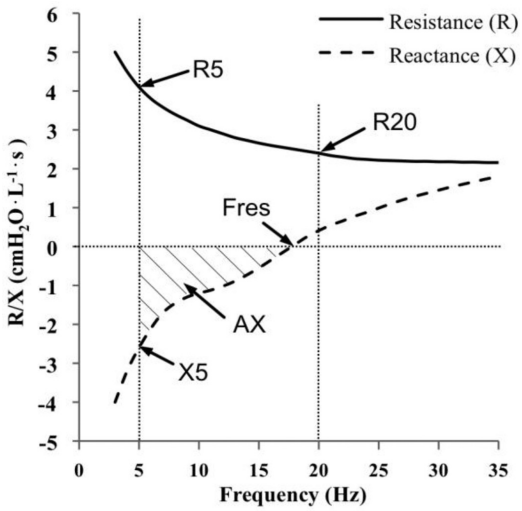

Fig. 2. A schematic illustration of IOS parameters. Includes the parameters: Resistance at 5 Hz (R5), resistance at 20 Hz (R20), reactance at 5 Hz (X5), resonant frequency (Fres) and total reactance (AX). Figure from Yixin Shi et al. 2012 (22).

1.5 Particle deposition

Deposition of a particle means that it makes contact with the mucosa of the airways and stays there (then leaving the aerosol). Where the deposition happens depends on particle factors (size and speed) and other factors like the degree of turbulence and time. When it comes to aerosol-forming drugs, two deposition mechanisms play an essential role: impaction and sedimentation. Impaction means that the particle will deposit due to kinetic energy. The effect of impaction will be highest at locations with much turbulence (i.e. mouth and throat regions and flow-limiting airways, where the cross-sectional area is low). Sedimentation means that the particle settles due to gravity and deposits on the lower border of the airway. The effect of sedimentation will consequently be highest for heavier particles, short distances, and long time frames (7).

1.5.1 Particle size measurements

There are different ways to measure particle size. For monodisperse particles, i.e., they are all the same size, it is simple to do in a microscope. However, all therapeutical aerosols are more or less poly-disperse. In the pharma industry, measurements using impactors are common. They consist of stages that particles have more challenging to get through due to impaction. When working with droplets (as in this study), the effect of drying can decrease the particle size considerably. Measurements using a light scattering instrument are not much affected by drying and are more representative since airways have almost 100% humidity, and no drying occurs (23).

1.5.2 Deposition pattern

Deposition patterns can be assessed in different ways. One strategy is to give the patient pharmacological drugs and then measures the drug's effect with lung function measurements. Another way is to label the particles with a radioactive isotope and evaluate the deposition pattern by lung scintigraphy (24). The challenge with both methods is that neither of them has

a good resolution when it comes to differentiating large and small airways, i.e., to distinguish between bronchii and bronchioles.

1.6 Theoretical calculations of deposition

Since there is no experimental setup to assess whether a particle deposits in, for example, generation 12 or 13, many theoretical models have been developed to enhance precision. Even quite basic models calculating deposition for each generation show good agreement with empirical data (25). The probability of impaction depends mainly on the impaction parameter D^2F. This parameter uses the aerodynamic diameter (D) in the square, multiplied by the inhalation flow (F), thus taking mass and velocity into account. D^2Fshows a very close relationship to deposition in both mouth and throat and the tracheobronchial region. In this study we calculate deposition using previous D^2F data (26).

1.7 Aim and Hypothesis

The aim of this study is to investigate if we could measure any difference in outcome using IOS when performing methacholine provocations with two different exposures regarding droplet size and inhalation flow rate, which theoretically should give very different deposition patterns in the airways (bronchii vs. bronchioles).

The hypothesis of this study is that slow inhalations increase the deposition in the bronchiole (by increased sedimentation) and thereby increase the peripheral resistance measured by impulse oscillometry.

H0 = There is no difference between slow and normal inhalation in peripheral resistance measured by impulse oscillometry.

H1 = Slow inhalation increases the peripheral resistance measured by impulse oscillometry.

2 Materials and Methods

2.1 Subjects and study design

The study was performed at the Department of clinical physiology at Karolinska University Hospital Huddinge, Stockholm. The study population was eight healthy subjects, five women and three men, with a mean age of 32 (range 22-56 years) (Tab I). The clinical inclusion criteria for this study were that the subjects had no previous history or signs of allergy or lung diseases.

Examinations of each subject were performed on three separate days (visit 1-3). On the first visit, a BHR test was performed to evaluate the participant's reactivity, and all subjects were informed about the study. On the second and third visit, provocation with normal or slow inhalations of methacholine was made at a dose found to double Raw for each individual. The participants inhaled methacholine aerosol with two different flowrates, each flowrate using a specific particle size. Output calculation from the two nebulizers was made by measuring weight loss. Airway resistance (Raw) was measured using a plethysmograph. Measurements of oscillometry resistance (R5 and R20) and reactance were made with the IOS at all three visits.

Tab I. Overview of the subject's basal data

	Height cm	**Weight** Kg	**Age** Year	**Resistance** Raw	**Resistance at 5 Hz** R5	**%Predicted** R5
Mean ± SD	171 ± 9	69 ± 10	32 ± 13	0,18 ± 0,06	0,29 ± 0,05	93 ± 12

The data shown is from the first visit before provocation. Resistance (Raw) was measured with a plethysmograph and resistance at 5 Hz (R5) and R5 % predicted was measured with the Impulse oscillometry.

2.2 Provocation

2.2.1 Reactivity testing (Visit 1)

The equipment used for the reactivity test was a PARI LL nebulizer connected to an air compressor (Pari Boy) and a dosimeter (Spira Oy, Hämeenlinna, Finland). The participant inhaled methacholine (5 mg/ml) with increasing doses. The participant inhaled methacholine and then waited 10 minutes after inhalation. Both IOS and plethysmography measurements were made. The test was completed when the participant's Raw increased to at least the double compared to before provocation or if the participant reached the maximum dose of 0.8 mg methacholine.

2.2.2 Normal inhalations

The equipment used during normal inhalation was the same as the reactivity test (droplet size around 5 µm). The participant inhaled methacholine in different doses depending on IOS results, meaning that an additional dose was given if needed. During normal inhalation, the participants inhaled with an inhalation flow of approximately 0.5 to 1.0 L/s. After inhalation, the patient waited for 10 minutes before performing three reproducible measurements with the IOS and plethysmograph. A mean value of the three measurements was used.

2.2.3 Slow inhalation

The equipment used for slow inhalations was a PARI TIA nebulizer (droplet size around 8 µm) connected to an air compressor. The nebulizer inlet for inhalation air was blocked, giving in an inhalation flow rate of approximately 0.1 L/s. The participant inhaled methacholine (5 mg/ml) in different doses depending on the IOS results, then waited 10 minutes before measurements with the IOS and plethysmograph. A mean value of the three measurements was used.

2.3 Lung function measurements

All lung function measurements done with the IOS and body plethysmograph were performed in a sitting position, breathing through the mouthpiece with a nose clip.

The equipment used for the IOS measurements was a Vyntus IOS (Vyntus PNEUMO spirometer, Vyaire Medical, USA) with the SentrySuite-software (SentrySuite Software (cyber-) Security, Vyaire Medical, USA). The subject was tidal breathing into the mouthpiece for 20 seconds. The technicians evaluated the test, and three reproducible manoeuvres from each subject were obtained. An average of these three measurements was used (as recommended by the manufacturer's manual).

The plethysmograph measurements were done with a MasterScreen Body plethysmograph with the SentrySuite-software (Vyaire Medical, USA). The subject was tidally breathing into the mouthpiece until a stable respiratory state. Five reproducible sRaw-loops from each subject were maintained, then the shutter manoeuvre was made, and FRC and Raw-loops were stored. An average of the saved measurements was used. The measurements were performed according to the European Respiratory Society/American Thoracic Society (ERS/ATS) guidelines (16, 27).

2.4 Aerosol measurements

The nebulizer droplet size distribution was measured using a light scattering instrument (Malvern Mastersizer II, Malvern Panalytical Ltd, Malvern, UK). The nebulizer outlet (mouthpiece) was placed at a 2.5 cm distance from the laser beam and a 1.5 cm distance from the lens. Each nebulizer was measured for 5 s. Before each measurement, a control measurement was performed to reset the baseline. For the large particles, the nebulizer inlet for inhalation air was blocked, and for the normal inhalation, an additional flow of 1 L/s was introduced into the nebulizer to mimic exposures.

2.4.1 D^2F calculations

D^2F equations described previously were used in the calculation (26). The equation estimates where the particles will deposit due to impaction, divided into the mouth and throat region, the tracheobronchial part, and the alveolar compartment. The calculation was made for each of the 20 size intervals given by the light scattering measurements, and for both inhalation set-ups.

2.5 Ethical aspects

This project has an approved ethical application with Dnr: 2019-02044. The method used in this study is a well-established, clinically used method. Because the patient inhales a drug, some discomfort may occur, but the method does not pose any risk to the participant.

In this study, the participant's data was stored and coded before, and the general data protection regulation (GDPR) guidelines were followed (28). There was no direct medical benefit for these healthy participants in the study who underwent a bronchoprovocation, unlike the more direct benefit for a patient at the clinic. The benefit of this study is more related to advances in medical research and may improve medical diagnostics.

2.6 Statistics

All data from the participants were coded and recorded in Excel. Descriptive statistic such as, mean, standard deviation and the range was used. As the sample size in this experimental study for practical reasons was small, the results were presented mainly as descriptive statistics and in graphical form. Since airway resistance normally is low, any provocation will lead to skewness in the data and for that reason the non-parametric statistical tests Wilcoxon paired signed-rank test was performed to compare Raw, R5, R20, R5-R20, and AX between the two exposures. The significance level was set to $p<0.05$.

3 Results

A total of eight participants underwent methacholine provocation on three different occasions. At visit 1, the IOS and plethysmograph parameters before provocation were all within the normal range, which confirmed that all participants had normal lung function. During the reactivity test, the mean increase in Raw (that is typically very low) was 381% (range 19-973 %), and the mean increase in the percent of R5 was 93% (range 41-157%). One participant reached the maximal dose of 0.8 mg methacholine (Tab II).

Tab II. Reactivity rest to assess hyperreactivity in the airways.

Subject	Raw % Increase	R5 % Increase
1	19	157
2	108	41
3	639	86
4	198	103
5	387	96
6	973	104
7	209	87
8	513	68
Mean	381	93
SD	317	33

Individual increases in percent of Airway resistance (Raw) and Reactance at 5 Hz (R5).

3.1 Inhaled methacholine doses

There was no significant difference ($p>0.05$) in dose between visit 2 when slow inhalation was performed and visit 3 when normal inhalation was performed. Although it was not primarily intended to achieve the same dose on these two occasions, the result showed that the mean dose was similar. Slow inhalation was 0.29 mg (range 0.05-0.73 mg), and the mean dose given at normal inhalation was 0.28 mg (range 0.04-0.83 mg). The output measurements

showed that the PARI TIIA had an output of 0.0049 µl/s, and the PARI LL had an output of 0.0042 µl/s (Tab III).

When monitoring a methacholine inhalation over time with the IOS parameter R5 in two participants (1 and 2), the result showed that the highest effect of methacholine was around 10 minutes after the inhalation (data not shown).

Tab III. Table of the doses given at slow and normal inhalation.

	Slow inhalation (Mean ± SD)	Normal inhalation (Mean ± SD)	Wilcoxon signed-rank test (p)
Nebuliser output (µl/s)	0.0049	0.0042	
Concentration (mg/ml)	5	5	
Concentration (mg/s)	0.024	0.021	
Number of breaths (s)	17 ± 14	16 ± 12	
Dose (mg)	0.29 ± 0.25	0.28 ± 0.26	>0.05

3.2 Lung function measurements

When comparing slow inhalation with normal inhalation of methacholine, the result showed no significant differences ($p > 0.05$) between the two inhalation patterns and R5, R20, R5-R20, nor Raw (Wilcoxon paired signed-rank test). Even though we created quotas and looked at all the different parameters from the IOS and plethysmograph, no significant differences were found. The peripheral resistance measured by impulse oscillometry did not increase with slow inhalations compared with normal inhalation.

Figure 3 shows the normal compared to slow inhalations close to the identity line for R5. The scatter can be expected from double determinations with the same exposure. The results were much the same for the higher frequency R20 (fig. 4). No significant dirrerence could be established between the two inhalation patterns and R5-R20 or Raw (Wilcoxons signed rank test).

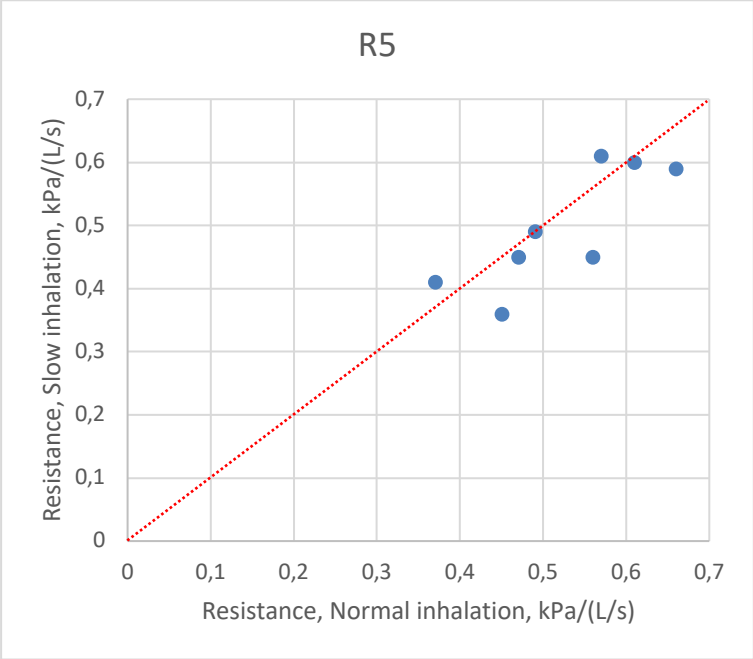

Fig 3. Comparison of resistance at 5 Hz (R5) between slow and normal inhalation.

Measurements was made with the impulse oscillometry system. The red line is the identity line (x=y).

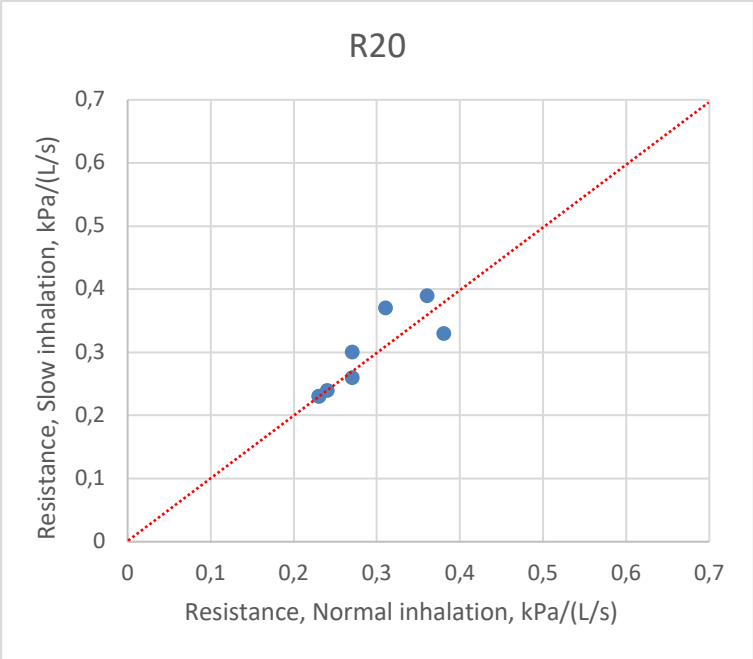

Fig 4. Comparison of resistance at 20Hz (R20) between slow and normal inhalation.

Measurements was made with the impulse oscillometry system. The red line is the identity line (x=y).

The result from visit 1 before methacholine provocation showed a correlation between Raw and R5 and between Raw and R20 (Fig 5). The result also showed no significant difference between Raw and R5, Raw and R20, or Raw and R5-R20 during slow inhalation (Wilcoxons signed rank test).

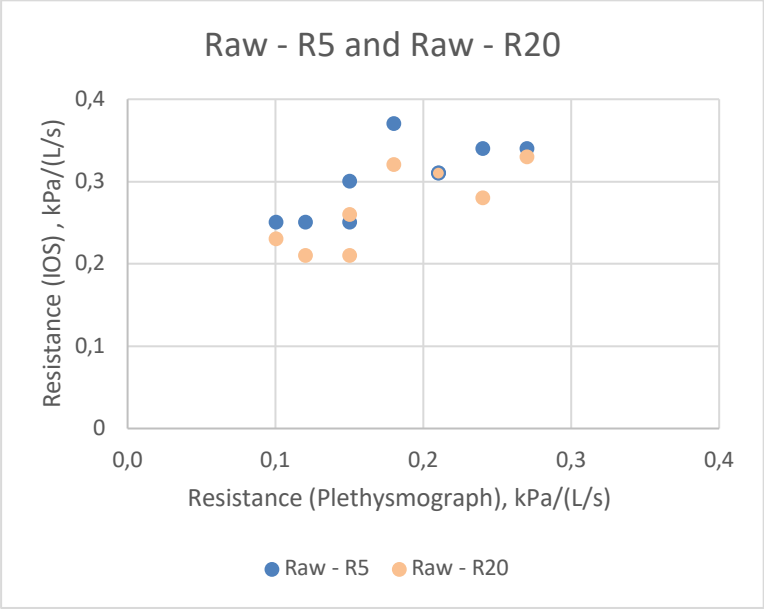

Fig 5. Comparison of air way resistance (Raw), resistance at 5 Hz (R5) and resistance at 20 Hz (R20) before inhalation of methacholine. Raw was measured with a body plethysmograph. R5 and R20 was measured simultaneously in each individual with the impulse oscillometry system (hence two data points for each value of Raw). From the graphics it is clear that R20 (which is a sub-part of the global R5 resistance) accounts for the majority of the resistance at 5 Hz.

3.3 Malvern measurements

The particle sizes from the PARI TIA nebulizer measured with the Malvern instrument had a mass median diameter (MMD) of 7.8 µm. The PARI LL nebulizer had a MMD of 5.2 µm. The distribution of the particle sizes is shown in figures 6 and 7.

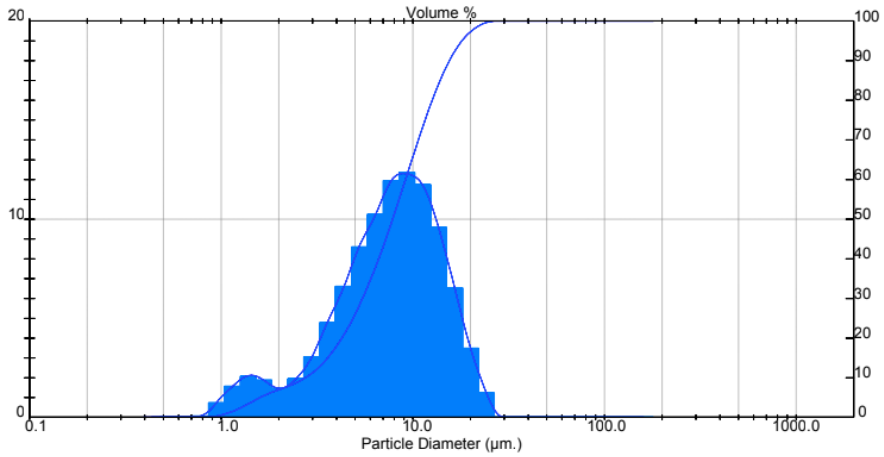

Fig 6. Diagram of the particle size measured with the Malvern instrument. Measurements done on the PARI TIA nebulizer (flow 0.1 L/s). The mass median diameter is calculated as the intercept between the accumulated volyme (S-shaped line) and 50% on the right hand y-axis.

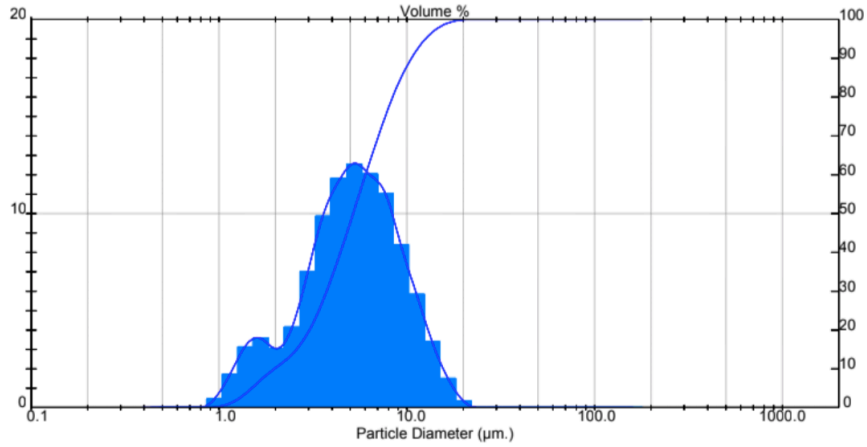

Fig 7. Diagram of the particle size measured with the Malvern instrument. Measurements done on the PARI LL nebulizer (flow 0.5-1,0 L/s). MMD calculated as above (Fig 6)

3.4 D^2F Calculations

The result from the D^2F calculation showed that the PARI TIA nebulizer had a distribution of particle deposition in the mouth and throat of 17%, 18% in the tracheobronchial part, and 65% in the alveolar part of the airways, all using equations from (26) (Tab IV).

The result from the PARI LL nebulizer had a distribution of 48% in the mouth and throat, 25% in the tracheobronchial part, and 27% in the alveolar part of the airways (Tab IV).

Tab IV. Distribution of particles in the airways.

Airway distribution	PARI TIA (Slow inhalation)	PARI LL (Normal inhalation)
Mouth and throat	17 %	48%
Tracheobronchial	18 %	25 %
Alveolar	65 %	27 %

The measurement is based on a mathematical equation that estimates the distribution.

3.5 Side-effects

During methacholine, provocation participants suffered from different side effects, such as irritation in throat, dry cough, and redness of the face, headache, and general discomfort.

4 Discussion

The aim of this study was to investigate if we could measure any clear difference in outcome using IOS when performing methacholine provocations with two different exposures that theoretically should give different deposition patterns in the lower airways (bronchus and bronchioles). The hypothesis stated that slow inhalations using large particles/droplets increase the deposition in the bronchioles, thereby increasing the peripheral resistance. The result showed no significant differences between the two different exposures, measured by impulse oscillometry. The null hypothesis can therefore be accepted.

For R5, the comparison between the two inhalation patterns resulted in data points close to the identity line (i.e., x=y), meaning that the total resistance of the airways was more or less the same during the two different inhalation patterns. The same applies to R20; the resistance in the central airways was the same despite two different inhalation patterns. Furthermore, there was no significant difference between the two inhalation patterns and R5-R20 (Wilcoxon signed rank test).

The lack of difference could have several explanations: 1; We failed to achieve a sufficient difference between the two deposition patterns, 2; we failed to measure the differences with the IOS, or 3; there was a redistribution giving an effect in other location than the deposition.

Previous studies have shown that there should be a significant difference between slow and normal inhalations (29). Slow inhalations results in a lower kinetic energy of the particles (less impaction). Further, it means more time for the particles to settle by sedimentation in a given segment. The effect of impaction is higher in locations with more turbulence, and the effect of sedimentation in the airways is most significant where the distance to the lower surface is the shortest (i.e. the bronchioles) (7).

The calculations using previously obtained data (30) showed that normal inhalation should give higher deposition in mouth and throat (48% of the total distribution), compared to slow inhalation (17%). The calculated alveolar deposition was 65% with slow inhalations compared to 27% with normal inhalations.

The calculations above are based on findings where impaction is the main mechanism of deposition. Theoretically the deposition would increase markedly due to sedimentation using large particles and low flowrates, (29, 30).

The result also showed almost no difference in the deposition in the tracheobronchial part of the airways between inhalation patterns. Due to limited resources, we did not measure or calculate how the particles' distribution looked more specific in the tracheobronchial part, i.e., using a computerized theoretical model. We do not know if there was any difference between the inhalation patterns within the small airways, but there should be, according to previous studies (29). Another deviation from previous studies (29) is that we used a poly-dispense aerosol compared to previous experiments using aerosols with particles of the same size (i.e., monodisperse). The effect of this is that even if the MMD is the same, the global deposition pattern is more “smeared out” for an aerosol with broader distribution than for a monodisperse aersol.

The IOS is conventionally marked as a method that measures small airways, assuming that R5-R20 reflects the peripheral airways (18, 22, 31). The problem with the statement is that there is no "golden standard" method for measuring the small airways, no method with enough good resolution to compare with. For example, there is no equipment such as MRI to verify/compare the measurements with, as in echocardiography. However, there is some research done on reconstructed airways with CT-based imaging. Merryn H Tawhai and colleagues reconstructed airway trees on asthma patients and healthy controls up to generation 6-10 (from segment bronchi to terminal bronchi, Weible's model) and the rest of the airway tree. Generation 11-16 (Start of bronchioles to terminal bronchioles, Weible's model) was computationally reconstructed. Then a simulated measure of R5-R20 was made (32). Brody H Foy and colleagues then compared the simulated measure of R5-R20, from Merryn H Tawhai and colleagues, with actual measurements with the IOS. The study results showed a modest concordance between the stimulated measure and the IOS's actual measurements (31). Although the result from Brody H Foy and colleagues were statistically significant, the validation of the result was based on assumptions, such as computationally reconstructed airways.

Another study presented by Zhu-Quan Su and colleagues used the endobronchial optical coherence tomography (EB-OCT) imaging technique to scan COPD patients, heavy smokers, and non-smokers. EB-OCT is a minimally invasive method with high resolution used clinically to assess the histology of proximal and distal airways up to ninth generation (segment bronchi to almost terminal bronchi, Weible's model) (33, 34). Their study result showed that IOS parameters R5-R20 and resonant frequency correlated with the degree of morphologic abnormalities measured with the EB-OCT. They stated that R5-R20 might be a sensitive parameter that can detect small-airway disorders in the early stage of COPD. Neither the studies by Tawhai, Foy nor Su had enough resolution to provide a "golden standard" to verify IOS findings differentiating bronchi from bronchioles.

On the other hand, R5-R20 has been repeatedly, in several studies, claimed as a sensitive method of detecting early lung changes that spirometry cannot detect (22, 31, 34, 35). Ragia S Sharshar and colleagues showed a significant difference in the IOS parameters R5-R20 and AX between the two groups but not in the spirometry when comparing controlled and uncontrolled asthma patients parameters (35). The study results may show that IOS is a sensitive method for controlling asthma medication. However, the results of this study only confirm that it is different but do not show that the significant differences in R5-R20 between the two groups are in the small airways.

From an ethical point of view, it is better to measure the small airways with the help of lung function measurements which are not invasive methods or do not expose the patient to radiation, which CT-based imaging does. If it is possible to measure the small airways with only lung function measurements, is hard to tell. Further studies need to be done.

A strength of the study was the three separate visits so that the BHR-test could be done from a normal baseline. An additional strength (and deliberate choice) was that this study did not use the flow-volume loop as a measure of obstruction since maximum inhalations extend smooth muscles and affect airway resistance. However, this made it more difficult to compare the results of this study with previous studies. As most previous studies use the flow-volume

curve because it is a well-established method worldwide. A weakness of this study was that the number of participants was too few. Nevertheless, even though the number was small, if there were a clinically meaningful difference, it should be seen in few subjects. The difference between inhalation patterns should create a difference in the deposition of particles at an individual level and not at the group level. Increasing the number of participants would have increased the credibility of the results but probably not affected the conclusion.

One source of error with methacholine dissolved in a fluid is that we do not know how the lung tissue reacts when inhaling the aerosol. Theoretically, by using larger particles and slow inhalation, more deposition of particles will occur via sedimentation instead of impaction and lead to deposition in the small airways, leading to local obstruction. However, possible scenarios that could have occurred are redistribution of the methacholine or simply reflex pathways of the lungs, causing effects in other places than the deposit. The redistribution and the reflex could explain why we could not measure any difference between the inhalation patterns with the IOS. Redistribution and reflexes are factors that cannot be controlled easily, and more research may be needed on this topic.

This study shows that even when using an extreme inhalation with very slow flow and large particles, it is challenging to differentiate large and small airways using methacholine and the IOS technique. We have either not made any difference or failed to get signals from this difference when measuring resistance at various frequencies with the IOS. However, these findings do not rule out possible benefits of using this inhalation technique in the future, for example, with inhaled steroids instead.

Although previous studies show that large particles and slow inhalation flow give a different deposition pattern, we could not show it using conventionally used nebulizers and measuring resistance at various frequencies with the IOS. In conclusion, findings from this study illustrate and once again confirms the difficulties in measuring physiological response in the bronchioles.

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