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Effect of airway opening on production of exhaled particles

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Almstrand A, Bake B, Ljungström E, Larsson P, Bredberg A, Mirgorodskaya E, Olin A. Effect of airway opening on production of exhaled particles. *J Appl Physiol* 108: 584–588, 2010. First published January 7, 2010; doi:10.1152/japplphysiol.00873.2009.—The technique of sampling exhaled air is attractive because it is noninvasive and so allows repeated sampling with ease and no risk for the patient. Knowledge of the biomarkers' origin is important to correctly understand and interpret the data. Endogenous particles, formed in the airways, are exhaled and reflect chemical composition of the respiratory tract lining fluid. However, the formation mechanisms and formation sites of these particles are unknown. We hypothesize that airway opening following airway closure causes production of airborne particles that are exhaled. The objective of this study was to examine production of exhaled particles following varying degrees of airway closure. Ten healthy volunteers performed three different breathing maneuvers in which the initial lung volume preceding an inspiration to total lung capacity was varied between functional residual capacity (FRC) and residual volume (RV). Exhaled particle number concentrations in the size interval 0.30–2.0 μm were recorded. Number concentrations of exhaled particles showed a 2- to 18-fold increase after exhalations to RV compared with exhalations where no airway closure was shown [8,500 (810–28,000) vs. 1,300 (330–13,000) particles/expired liter, $P = 0.012$]. The difference was most noticeable for the smaller size range of particles (<1 μm). There were significant correlations between particle concentrations for the different maneuvers. Our results show that airway reopening following airway closure is an important mechanism for formation of endogenous exhaled particles and that these particles originate from the terminal bronchioles.

airway closure; breath

IN EXHALED AIR, there are particles originating from the airways (15). Chemical analysis of the particles may thus provide information on changes in the composition of respiratory tract lining fluid (RTLF) and be of value for monitoring of pathological processes in the lung. For example, surfactant protein A, an important component of the RTLF in the distal airways, has been shown to be reduced in lung diseases such as acute respiratory distress syndrome (ARDS), pneumonia, and asthma (16). Furthermore, according to recent data, the lipid composition of the RTLF may be altered in severe asthma (9).

In addition to the chemical composition, it is important to know how and where the exhaled particles are produced. Our group has previously developed a method for counting and collecting exhaled particles. With chemical analysis we showed the presence of surfactant lipids and proteins in the particles, confirming that they originate from the RTLF (1). Surfactant phospholipids are produced by alveolar type II cells in the lung and then spread to the conductive airways (3). Thus the presence of these compounds in exhaled particles is not sufficient to establish the location of particle formation. Furthermore, the mechanisms involved in the production of particles have not been delineated and may differ between forceful and calm breathing. The present study deals with slow breathing only.

We hypothesize that the RTLF in the terminal bronchiole forms films that rupture during airway opening, causing particles to form. Closure of peripheral airways, beginning in the lower lung regions and progressing toward the upper lung regions with further decrease of lung volume, was originally demonstrated by Dollfuss et al. in 1967 (6). The lung volume at which airway closure begins during a progressive slow exhalation is termed the closing point (CP), and the volume remaining to residual volume is termed the closing volume (CV). The terminal bronchioles are generally considered the site of airway closure (10). Thus, if exhalations pass the closing point and airways progressively close, the reopening during the following inspiration is thought to produce particles.

The purpose of the present study was to elucidate the effect of airway closure, controlled by the extent of the depth of the preceding slow exhalation, on exhaled particle concentration. If airway opening is an important mechanism for particle production, deep exhalations should lead to higher particle number concentrations and shallow exhalations should result in relatively low number concentrations.

METHODS

Particle counting. The instrument for particle counting has been described in detail previously (1) and was used with small modifications (see Fig. 1). It includes a copper tube/reservoir with a volume of 3.4 liters inside a box with a thermostat set at 36°C to avoid condensation and thus preserve the size distribution of exhaled particles. Outside the box at the mouth end, there is a flowmeter (OEM Flow Sensor Spiroson-AS, ndd Medical Technologies, Zürich, Switzerland) and a valve system that allows inhalation of particle-free air and exhalation either into the room or into the reservoir. The flowmeter is ultrasound based, thus not interfering with the flow and particles. Inside the box and connected to the reservoir at the mouth end, there is an optical particle counter (Grimm Model 1.108, Grimm Aerosol Technik, Ainring, Germany) that draws air at 20 ml/s and counts and sizes particle concentrations in eight size intervals: 0.30–0.40, 0.40–0.50, 0.50–0.65, 0.65–0.80, 0.80–1.0, 1.0–1.6, 1.6–2.0, and >2.0 μm. The instrument sizes particles according to the amount of light scattered by each particle when counted. It measures 1-s mean values with a 90% rise time within 3 s. Size calibration is based on monodisperse latex spheres certified by National Institute of Standards and Technology; an observed particle is assigned the diameter of a latex sphere scattering the same amount of light. The light source is a solid state infrared laser working at 780 nm. Close to the Grimm counter, there is a three-stage inertial impactor (3-stage PM10 Impaktor Dekati, Tampere, Finland, slightly modified) served by a pump

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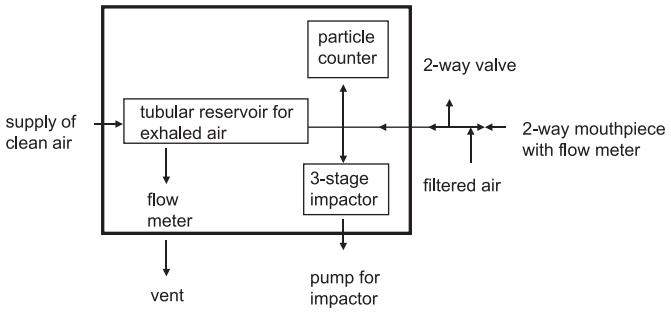

Fig. 1. Schematic picture of the instrument used for particle measurements. The subject inhales particle-free air and exhales into the device. The 2-way valve makes it possible to direct the exhaled air either into the instrument or into the room. Particle concentrations in exhaled air are measured with an optical particle counter. The surplus of exhaled air is drawn through a cascade impactor (not used in this study for collection).

that draws a continuous sample of 230 ml/s. At the opposite end of the reservoir, clean and particle-free air saturated with water vapor at 34°C is added at 280 ml/s to serve as a make-up of the air consumed when no exhalation is taking place. The particle concentrations provided by the optical counter were converted to number of particles per expired liter.

Ten healthy nonsmoking volunteers participated in the study. Spirometry was performed using a flow-based computer-assisted spirometer with the "Spirometry/Flow-Volume" software (MasterScope-PC, VIASYS Healthcare). Spirometry was performed according to international guidelines (13). Closing volume measurements were performed with the single breath nitrogen method with custom-made equipment and procedures according to Oxhoj and Bake (14). In short, flow rate is ~0.2 l/s during inspiration of the initial 0.5 liters from residual volume (RV), i.e., during distribution of the nitrogen in the anatomic and apparatus dead space. Flow rate during expiration from total lung capacity (TLC) back to RV is ~0.3 l/s. Closing point, i.e., the transition between the alveolar plateau (phase III) and phase IV, is defined as the volume point corresponding to the first permanent, convincing, upsloping departure from a straight line through the last part of the alveolar plateau.

The characteristics of the subjects are shown in Table 1. The study was approved by the local research ethics committee of the University of Gothenburg.

Three breathing maneuvers were performed according to the protocol below (illustrated in Fig. 2). In the RV maneuver exhalation proceeds to RV, while in the CP maneuver exhalation proceeds to closing point and in the FRC maneuver there is no exhalation from functional residual capacity (FRC).

Table 1. *Characteristics of the study subjects*

Subject	Sex	Age, yr	Height, cm	FVC, %pred	FEV1, %pred	ERV, liter	CV, liter
1	M	69	173	124	115	0.9	1.2
2	F	48	166	131	117	1.2	0.5
3	M	30	195	126	119	2.7	0.8
4	F	34	162	129	119	1.4	0.2
5	F	38	163	126	114	1.1	0.3
6	M	56	181	128	118	1.6	1.1
7	M	37	170	80	77	1.3	0.3
8	F	56	166	122	103	0.8	0.7
9	M	42	194	129	115	2.6	1.4
10	F	29	156	113	113	1.0	0.1

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; ERV, expiratory reserve volume; CV, closing volume. Predicted (pred) normal values are according to Quanjer et al. (17).

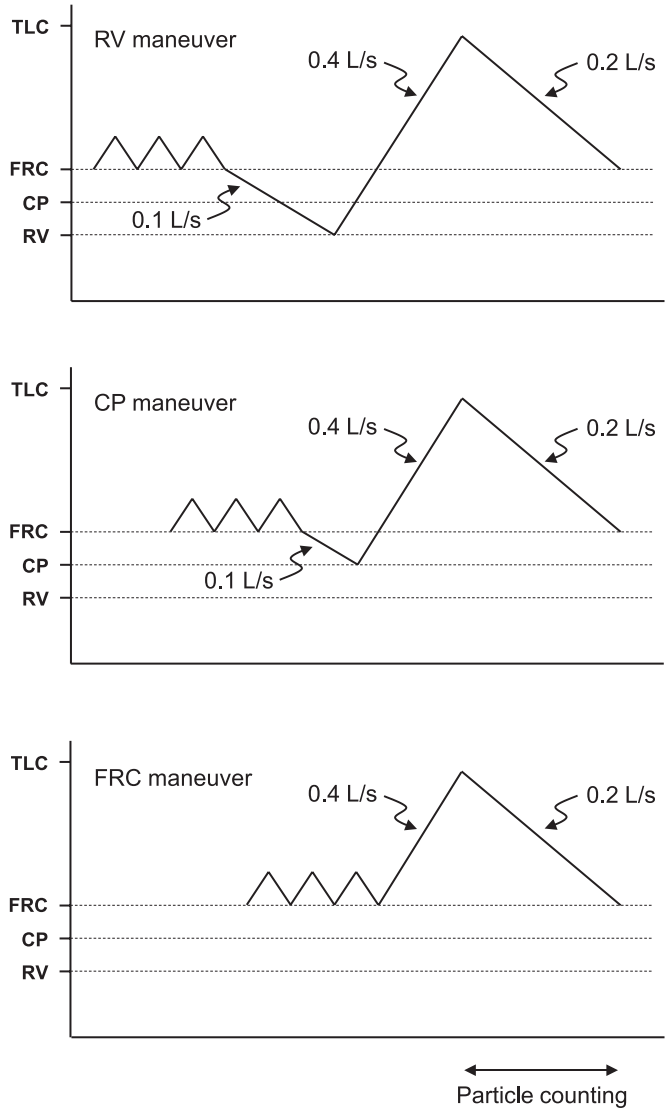

Fig. 2. Schematic measurement protocol for the different breathing maneuvers. During the initial tidal breathing, particle-free air was inspired. Particle counting started after inspiration to total lung capacity and proceeded until expiration to functional residual capacity.

RV Maneuver: Exhalation from FRC to RV at a Flow Rate of 0.1 l/s (±10%)

Inspiration to TLC at a flow rate of 0.4 l/s (±10%).

Exhalation back to FRC at a flow rate of 0.2 l/s (±10%) (particle counting).

CP maneuver: Exhalation from FRC to CP (Closing Point) at a Flow Rate of 0.1 l/s (±10%)

Inspiration to TLC at a flow rate of 0.4 l/s (±10%).

Exhalation back to FRC at a flow rate of 0.2 l/s (±10%) (particle counting).

FRC Maneuver: No Exhalation from FRC

Inspiration to TLC at a flow rate of 0.4 l/s (±10%).

Exhalation back to FRC at a flow-rate of 0.2 l/s (±10%) (particle counting).

The low expiratory flow rates were chosen to be certain to avoid dynamic compression as a possible contributor to particle production.

As dynamic compression occurs at lower flow rate at low lung volume, 0.1 l/s was chosen for exhalations to RV and 0.2 for exhalations to FRC. The inspiratory flow rate at ~0.4 l/s was chosen as it is an ordinary inspiratory flow rate.

Each maneuver was performed 10 times in a randomized order. There was a short break after 10 maneuvers. Before each set of 10 consecutive randomized maneuvers, the subjects breathed particle-free air for 3 min. In between maneuvers and to avoid carry-over effects, subjects continued to breathe particle-free air tidally, exhaling into the room until particle concentrations were back to zero before the next maneuver. Particles were also counted during 10 tidal breaths before each set of maneuvers. The numbers for each set of tidal breaths were summed, and the mean particle number per exhaled liter from the three sets was calculated. In two subjects (*subjects 1* and *8*) CP and FRC were close and so only the FRC maneuver was performed. Nose clips were used throughout.

Exhaled particles are primarily quantified in terms of the exhaled particle number concentration, i.e., number of particles per expired liter (n/l_{exp}). Following the RV maneuver, the exhaled particle number concentration is a mixture of particles produced during inspirations from RV to CP, from CP to FRC, and from FRC to TLC. To separate the production during inspiration of the RV to CP interval, particles from the CP maneuver were subtracted from the RV maneuver. Similarly, particles from the FRC maneuver were subtracted from the CP maneuver to obtain the particle production during the CP to FRC interval. Furthermore, as the volumes of these intervals are very different, the number concentrations were normalized and expressed per unit inspired volume and the dimension is number of particles/(l_{exp}*l_{insp}) where l_{insp} is liter inspired of the volume interval concerned.

To obtain the size distribution, particle number concentrations were normalized with the width of the size interval in question.

Data were analyzed using SPSS 15.0 (SPSS, Chicago, IL). Differences in particle numbers between the RV, CP, and FRC maneuvers were assessed with the Wilcoxon signed rank test. Two-tailed significance below 0.05 was considered statistically significant.

RESULTS

Figure 3 shows the mean particle number concentration for each of the three maneuvers in each subject. The number concentration was significantly higher for the RV maneuver compared to both the FRC maneuver (2–18 times higher) and the CP maneuver (2–8 times higher) in all subjects. The number concentration of particles produced from the CP maneuver was significantly higher than the number concentration produced from the FRC maneuver for all subjects. Overall means and ranges of number concentration of particles for all subjects are presented in Table 2. The mean particle number concentrations produced during inspiration of the three volume intervals, RV to CP, CP to FRC, and FRC to TLC are presented in Fig. 4. For each unit of volume inspired from RV to CP, the produced particle concentration is considerably higher than for each unit of volume in the CP to FRC interval and in the FRC to TLC interval.

Table 2. *Overall mean and range for number concentrations of exhaled particles (0.30–2.0 μm) for the RV, CP, and FRC maneuvers and for tidal breathing*

	Number of Particles (0.30–2.0 μm)/l_{exp}					
	RV maneuver	CP maneuver	FRC maneuver	Tidal breathing	p_1	p_2
Mean	8500	2500	1300	230	0.012	0.012
Range	810–28,000	330–13,000	69–5,300	18–1,000		

Values for tidal breathing are based on the mean of the 3 sets of 10 breaths for each subject. *P* values are presented for the residual volume (RV) and closing point (CP) (p_1) as well as the CP maneuver and FRC maneuver (p_2).

The coefficient of variation (CoV) of the particle number concentration (n/l_{exp}) of the 10 identical maneuvers within individuals, ranged between 15 and 55% for the RV maneuver, between 26 and 50% for the CP maneuver (except for one subject who had 96%), and between 17 and 50% for the FRC maneuver.

There were significant correlations between the number concentrations of the three maneuvers ($r > 0.90$).

The particle size distribution given in Table 3 shows mean values corrected for the differences in size intervals. Figure 5 illustrates the particle size distribution of the three maneuvers.

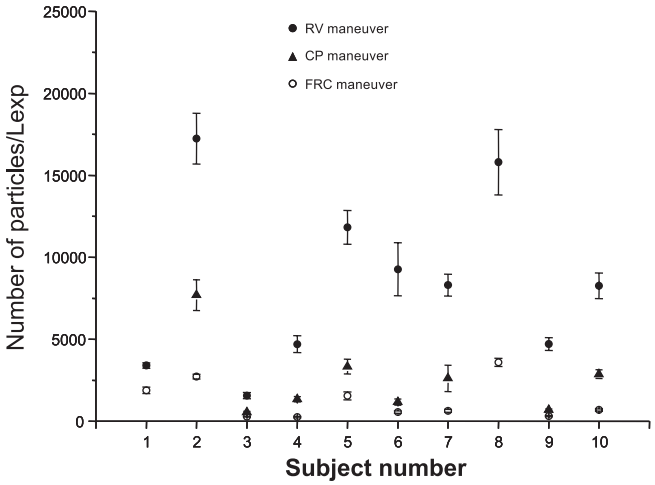

Fig. 3. Mean and standard error of particle number concentrations (size 0.30–2.0 μm) for the RV, CP, and FRC maneuvers for each subject ($n = 10$). The RV maneuver includes a preceding expiration to RV, and the CP maneuver a preceding expiration to closing point, while the FRC does not include any preceding expiration from FRC. In *subjects 1* and *8*, the closing point was close to FRC, and so the CP maneuver was omitted.

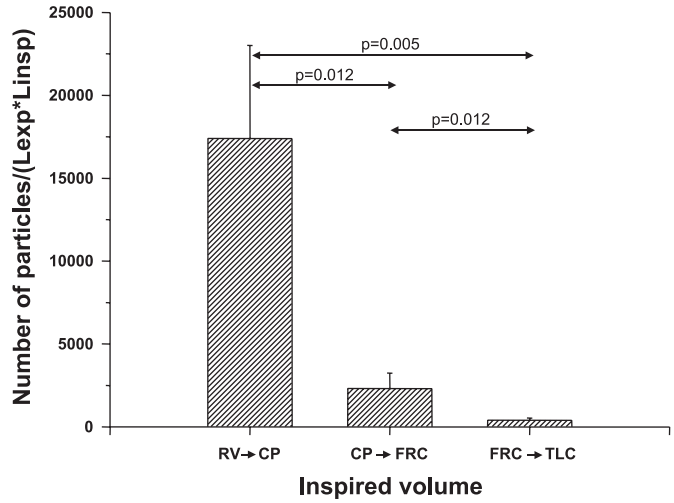

Fig. 4. The 3 maneuvers in Fig. 1 allow for separation of particle production during 3 different inspired volume intervals: RV to CP (i.e., during CV), CP to FRC, and FRC to TLC. The figure presents the particle production during inspiration of these 3 volume intervals and the particle number concentrations are expressed per unit inspired volume of the interval concerned.

Table 3. *Mean number concentrations of exhaled particles normalized by internal width, for the RV, CP, and FRC maneuvers and for tidal breathing for each particle diameter interval*

	Particle Diameter Interval, μm						
	0.30–0.40	0.40–0.50	0.50–0.65	0.65–0.80	0.80–1.0	1.0–1.6	1.6–2.0
RV maneuver Δn/Δd, 1×μm	44,000	21,000	8,800	3,000	870	53	23
CP manoeuvre Δn/Δd, 1×μm	13,000	6,100	2,600	1,000	320	21	9.7
FRC manoeuvre Δn/Δd, 1×μm	6,700	2,700	1,200	530	210	17	9.4
Tidal breathing Δn/Δd, 1×μm	1,300	370	190	120	57	10	4.6
p_1	0.012	0.012	0.012	0.012	0.012	0.012	0.018
p_2	0.012	0.012	0.012	0.012	0.012	0.012	0.063

Δn/Δd is the distribution function where particle concentrations have been normalized by the internal width; n is the particle number concentration per liter and d is the particle diameter (μm). Values for tidal breathing are based on the mean of 10 breaths for each individual. The statistical differences between mean numbers of exhaled particles between the maneuvers are presented as p_1 for the difference between the RV and CP maneuvers and p_2 for the CP and FRC maneuvers.

The distribution was similar in all subjects, in the sense that the concentration was highest in the size interval 0.30–0.40 μm and decreased with increasing size. The RV maneuver produced significantly higher numbers than the CP maneuver in all size intervals. The CP maneuver produced significantly more particles in all size intervals than the FRC maneuver except in the size interval 1.6–2.0 μm. Tidal breathing showed similar distribution of number concentrations and size as the RV, CP, and FRC maneuvers.

DISCUSSION

In the present study, we showed that when a preceding exhalation is deep enough to reach RV, the following exhalation contains a much higher number of particles than when the preceding exhalation is less deep.

The intra-individual variation between identical maneuvers was considerable and approximately similar for the three maneuvers. The mean intra-individual CoV was 37%. Anyhow, it was appropriate to repeat each of the maneuvers 10 times, thereby reducing variation of the mean value. The inter-individual variation was not reduced by expressing particles per exhalation rather than particles per expired liter, and particle concentration was not related to age or size of the subjects. The number of subjects was, however, small.

If a subject produces high number concentration in one maneuver, another maneuver also produced relatively high number concentration as indicated by high correlation between maneuvers. This suggests that there are specific properties of the RTLF governing the general particle formation that may differ between individuals. It should be taken into account that the number concentration produced during the RV maneuver is composed of the production during inspiration from RV to CP, from CP to FRC, and from FRC to TLC. Therefore the number concentrations are bound to be related to some extent. The largest difference between subjects is observed comparing exhalations to residual volume. *Subjects 1, 2*, and *8*, who produced the highest numbers during the FRC maneuver, did not produce the highest particle numbers during tidal breathing, indicating that other production mechanisms may have a role during tidal breathing. Two previous studies have shown that saline delivery to the lungs can diminish the number of exhaled particles (7, 18) during tidal breathing, which suggests that variation in the ionic composition of the RTLF plays a role in the high inter-individual variation. Other properties of the RTLF, such as film thickness and the surfactant composition, may also influence the variation (8).

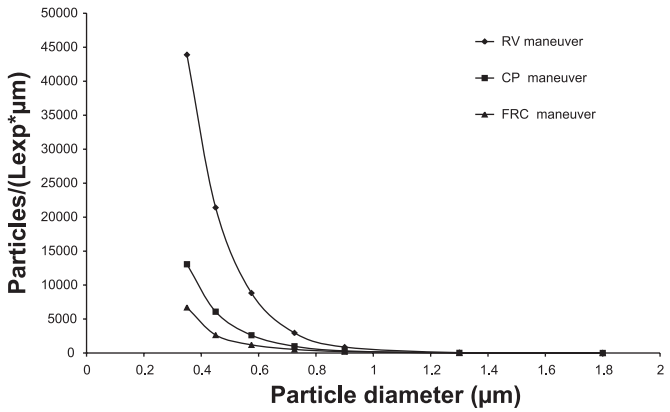

Fig. 5. Particle size distributions based on average number concentrations of exhaled particles.

Expiration to RV clearly produced more particles than less deep expirations (Figs. 3 and 4). Furthermore, when subjects exhaled to CP only, the particle production was substantially less in every subject although the mean difference in the depth of the expiration was only a few deciliters in several subjects (i.e., CV in Table 1). Thus the results are quite compatible with the hypothesis that when expirations result in extensive airway closure, the exhaled particle production becomes substantial. In principle, particles could be produced during the expiratory or inspiratory phase. We consider the most likely mechanism to be the airway opening during inspiration. When airways close, a meniscus of RTLF film is produced (12). On the following inspiration the meniscus ruptures and particles are produced.

The CP and FRC maneuvers and tidal breathing also resulted in particles in exhaled air. Airway closure and opening may still contribute, at least to some extent, because closing point in the single-breath N_2 test only signifies the volume at which a substantial number of airways close simultaneously. Thus if airways close in an uncoordinated manner, this will not be detected, and so this test does not exclude airway closure at higher lung volumes than closing volume. On the other hand, airway closure may not be the only mechanism for particle production under the present circumstances. Hypothetically, mucus meniscus may exist in airways and could rupture during inspiration, thus giving rise to particles. Tidal breathing produced small concentrations of particles compared with any of the maneuvers.

In two subjects, closing point was close to FRC (*subjects 1* and *8*). These subjects had relatively high particle number concentrations for the FRC maneuver. The ratios between the particle number concentration of the FRC and RV maneuvers were 0.55 and 0.23 for *subjects 1* and *8*, respectively, whereas the corresponding ratios for the other subjects ranged between 0.18 and 0.06. Presumably airway closure and opening was a relatively important mechanism also during the FRC maneuver for these subjects

The particle size distributions are difficult to compare because the magnitudes of the number concentrations are quite different. If the size distributions had differed substantially, different production mechanisms had been likely. However, the size distributions as shown in Fig. 5 are rather similar. The difference between preceding exhalations with and without airway closure was most distinct for small particles, suggesting that airway closure is a less important mechanism for large particle formation in the size range studied.

While the present study was in progress, Johnson and Morawska (11) published a somewhat similar study. They showed that deep exhalations result in increased particle concentrations and concluded that this is consistent with particles being produced by film bursting in the respiratory bronchioles during inspiration. This observation is consistent with the present results, although lung volumes and closing volumes were not measured and flow rates were not controlled to support the airway closure hypothesis in the above mentioned study. Low flow rate during the preceding exhalation is important to avoid dynamic compression of the airways, which may be a different cause of particle production. Johnson and Morawska also found a positive correlation between particle formation and age ($R^2 = 0.33$); this is expected, since airway closure increases with age (2, 4). In the present study, the correlation between number of particles per exhalation in the RV maneuver and closing volume expressed as a percentage of the vital capacity was ~0.35 (i.e., $R^2 = 0.12$). However, the small number of subjects does not allow for firm conclusions. We also measured exhaled particles online (no dilution expected to occur) in an environment with a controlled relative humidity and temperature; the size distribution of particles did not change.

The present protocol does not exclude mechanisms for particle production other than airway opening. During forceful exhalations, for example during coughing and sneezing, particles are obviously exhaled. Under these circumstances there is dynamic compression of the airways, with high linear velocities and vibrations of airway walls, presumably tearing off particles from the RTLF (5, 12). In previous studies, we have seen that forceful exhalations (~90% of FEV1) result in an increase in particle concentrations compared with tidal breathing. However, Johnson and Morawska (11) found no increase in aerosol production when increasing the exhalation flow rate.

Future studies that sample particles for analysis of nonvolatiles should consider a strictly standardized breathing pattern to improve reproducibility, both in terms of quantification as well as production mechanisms. In addition, to the extent that small and large particles may have different origin, collection of particles for subsequent chemical analysis should discriminate between small and large particles. This is possible by using, for example, multi-stage impactors (1).

In conclusion, the present results support the hypothesis that airway reopening following airway closure is an important mechanism for formation of endogenous particles in the airways and, thus, that exhaled particles originate from the terminal bronchioles. However, for larger particles other mechanisms may be important.

GRANTS

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DISCLOSURES

No conflicts of interest were disclosed by the authors.

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