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Effects on heart rate variability by artificially generated indoor nano-sized particles in a chamber study

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HIGHLIGHTS

- Exposure to studied particles results in different patterns of HRV.
- HRV may be used for information on physiological responses of particle exposures.
- Particle characteristics may identify their properties for potential health effects.

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ABSTRACT

Background: Airborne particles are associated with increased morbidity and mortality due to respiratory and cardiovascular diseases in polluted areas. There is a growing interest in nano-sized particles with diameter <100 nm and their potential health effects. Heart rate variability (HRV) is a noninvasive method for cardiovascular risk prediction in high prevalent groups.
Aim of study: The aim was to evaluate the impact of nano-sized indoor air particles on HRV for healthy and adult females.
Methods: All exposures were performed as controlled chamber experiments with particle exposure from burning candles, terpene + ozone reactions or filtered air in a double-blind cross over design. Twenty-two healthy females were investigated during 10 min periods at different exposures and the reactivity in high frequency (HF) spectral band of HRV were computed.
Results: Heart rate was unchanged from baseline values in all groups during all experimental settings. HF power of HRV tended to increase during exposure to particles from burning candle while particles from terpene + ozone reactions tended to decrease HF power.
Conclusions: Exposure to nano-sized particles of burning candles or terpene + ozone reactions results in different patterns of heart rate variability, with signs of altered autonomic cardiovascular control.
Practical implications: This study indicates that the HRV method may be used for information on physiological responses of exposure to different nano-sized particles and contribute to the understanding of mechanisms behind health effects of particle exposures.

1. Introduction

An increasing number of studies show correlation between chronic exposure to airborne particles and health problems, for instance from the cardiovascular system, by promoting inflammation and atherosclerosis (Ezzati et al., 2002; Brook et al., 2010; Nawrot et al., 2011; Weichenthal, 2012). Since humans, at least in the industrialized parts of the world, tend to spend more than 85% of their life indoors (Klepeis et al., 2001) particles in these environments are of special interest from a health perspective. As the development of new measurement instruments is progressing, the understanding for which particle properties affect our health is

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increasing, and more extensive studies of aerosols and their health influence are motivated and needed.

Particularly the health impact of airborne nano-sized particles with a dimension <100 nm is of increasing concern, not least as a consequence of the growing industry of engineered nano materials. However, humans are already today frequently exposed to high concentrations of nano-sized particles, in general indoor environments as well as in workplaces. Several indoor activities generate particles in the nano-size range. Recent studies indicate that about 60% of integrated daily residential exposure to nano-size particles by number can be attributed to indoor sources (Bekö et al., 2013; Bhangar et al., 2011; Mullen et al., 2011; Wallace and Ott, 2011). Burning candles have previously been identified as a significant source of nano-sized particles (Stabile et al., 2012; Hussein et al., 2006; Pagels et al., 2009) and other examples of nano-size particle generating sources are heat related activities such as cooking, frying, toasting and laser printing but also cleaning products and furniture polish containing terpenes, which in presence of ozone form particles (Brook et al., 2010).

The mechanisms behind the health impact of fine and nano-sized particles are not fully understood, although some studies in recent years provide a basis for better theoretical understanding of exposure, uptake and kinetics (Brown et al., 2000; Koch and Stöber, 2001; Kreyling et al., 2002; Oberdorster et al., 2004). Most of today's knowledge about airborne particle's health impact is the result of epidemiological studies based on measurements of outdoor particles. Sun et al. (2010) pointed out the need to acquire knowledge about the specific combination of airborne particles which can be blamed for health concerns. A need for toxicological studies exists, as well as means to assess health effects of exposure to indoor particles in humans (Morawska et al., 2013).

Heart rate variability (HRV) is, since long a well-recognized, noninvasive, independent method, for cardiovascular risk prediction in high prevalent groups (Malik, 1996). Influence of the sympathetic and parasympathetic branches of autonomic nervous system regulates heart rate and its variability. The major part of this variability is constituted by alterations in respiratory mediated influence on the parasympathetic load. Further, a link between inflammatory mediators and autonomic cardiac control has been shown (Czura and Traycey, 2005; Sloan et al., 2007; Luttman-Gibson et al., 2010) and an association between low grade inflammation and cardiovascular disease has been reported (Ridker and Morrow, 2003; Celik et al., 2011). In this work, a methodology has been developed with the purpose of exploring the effects on heart rate variability in healthy humans exposed to indoor generated candle particles and particles from terpene–ozone reactions.

2. Aim

The aim of this study was to design a feasible chamber study with laboratory generated common indoor air particles and to evaluate the impact of these nano-sized particles on noninvasive markers of cardiovascular reactivity such as heart rate and its variability. The hypothesis (H0) was defined as no observed changes in the high frequency band (HF) of HRV during exposure and if changes proven, resulting in rejection of H0.

3. Methods

3.1. The experimental chamber

The exposure chamber is a 22 m^3 room where all interior surfaces (except for a window 0.8 m^2) are covered with a layer of stainless steel, see Fig. 1. No air can enter or leave the chamber except through a well-controlled ventilation system. The chamber is supplied with air through a separate conditioning system by which air flow, temperature and relative humidity can be controlled and adjusted. The air passes through an activated carbon filter and an Ultra-Low Penetration Air (ULPA) filter before entering the chamber at roof level. An over-pressure in the chamber is typically maintained at 10 Pa or just below, to avoid undesired penetration of particles from the surrounding laboratory. The chamber can be used for human exposure as well as for source characterization and aerosol transformation studies and is previously described in detail (Isaxon et al., 2013).

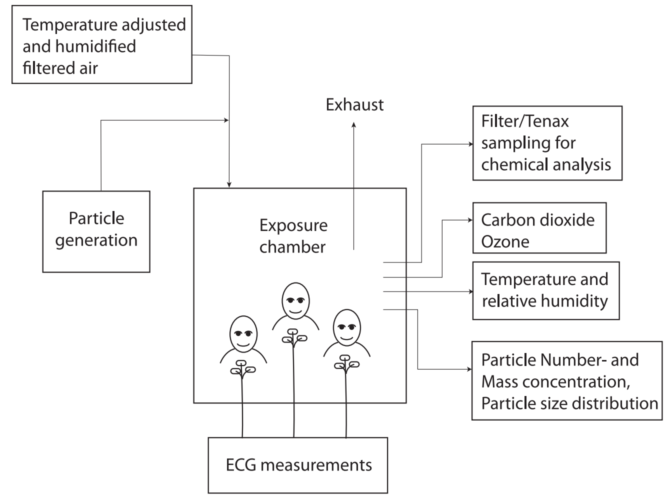

Fig. 1. The experimental set-up for the chamber exposure studies.

4. Particle generation system

The generation system for candle smoke consisted of a glass and stainless steel chamber with a total volume of 1.3 m^3. An inlet for filtered and pressurized air was placed in the bottom of the chamber to provide a steady controlled flow through the generation volume. Ten blue paraffin/stearine candles of a common commercial brand were lit inside the generation volume. A mobile fan was used to make the flames flicker and hence produce soot particles in a way similar to the air streams which are created when there is movement in the vicinity of a candle in a normal indoor setting. The generation chamber was protected from contamination from the surrounding air by a hatch in front of the chamber opening. This hatch was kept closed at all times except for when the generation was initiated (Pagels et al., 2009).

Terpene vapor was generated continuously by passing pure nitrogen through a glass bottle of commercial essential oil (lemon oil, oleum citri, Interlam AB), consisting of 60–95% *d*-limonene. Ozone was generated by a spark discharge generator (Ozone Technology AB, model AM 3000-2) using filtered dry air, and was added to the ventilation air flow before this flow entered the exposure chamber, just downstream the inlet for addition of terpene vapors. The terpene vapor reacts with ozone in the gas phase before entering the exposure chamber which initiates a cascade of chemical reactions generating reaction products distributed between the gas and particle phase.

During the exposures, particle mass concentration was monitored with a Tapered Element Oscillating Microbalance (TEOM, Rupprecht & Patashnic Co Inc.) and particle number concentration and size distribution by a Scanning Mobility Particle Sizer system (consisting of a CPC 3010, TSI Inc and a long column Hauke DMA), all on-line techniques with a time-resolution of minutes. The candle smoke particle chemical composition was characterized by PIXE (Particle Induced X-ray Emission Analysis) and OC/EC (Organic Carbon/Elemental Carbon) analysis. In the terpene–ozone case, Gas Chromatography–Mass Spectrometry (GC–MS) was used to determine the terpene concentration, aerosol mass spectrometry (AMS) to assess chemical composition of particles, while the ozone concentration was measured with a spectrophotometric ozone monitor (Thermo model 92). The temperature, relative humidity, carbon dioxide levels and (in the case of terpene particles) ozone levels were monitored during the exposures to secure steady state conditions. The methodology and technical details of the particle generation and chamber air monitoring is given by Isaxon et al. (2013).

5. Subjects and study design

The study group consisted of 22 healthy adult female test subjects (mean age 32, 19–62) who on different occasions were exposed to: candle particles; particles generated by terpene–ozone reactions or filtered air. At each session a group of 3 test subjects spent 4 h together in the chamber. Twelve test subjects (out of 22) were exposed to all three exposure conditions, eight test subjects to two conditions, i.e. to particle laden air (either candle particles or particles generated by terpene–ozone reactions) and to filtered air, while the remaining two test subject did not undergo zero exposure and were not used in the statistical analysis.

The exposures were conducted according to a double blind protocol, hence the particle levels were blinded both to the volunteers and to the medical investigators. Prior to the commencement of the study, test subjects underwent a physical examination including heart and lung status and skin prick test to assess the atopic status. Medical and work history was registered, from personal communication between medical staff and test persons, according to a structured protocol. They were all found to be healthy without medication.

The study design is illustrated in Fig. 2. Venous blood samples and nasal lavage during the experiments were secured for a separate study. Registration of heart rate variability started around 8:00 in the morning and ended around 12:00. Three HRV recordings were made inside the chamber. The 1st recording was made as a baseline, before exposure commencement i.e. without particle exposure at filtered air condition, where the following two recordings were taken in equal time intervals during the exposure. In the study a 4th recording was also made although later it was excluded from the analysis since the conditions in the chamber were significantly changed i.e. it was made after particle evacuation from the chamber and additionally the noise from the express fan (evacuating the particles) was likely to cause disturbance in these measurements. In the chamber the volunteers were placed in a comfortable reclining chair with feet support which allowed a half-lying position with feet elevated. After a steady state period of 10 min, time series of heart beats were collected during 10 min (sampling frequency 200 Hz) at 3 occasions evenly distributed in time. During the steady state period and the measurement period the lights were shut down and no talking or moving was allowed, as all stimuli that increases sympathetic nervous outflow may affect HRV. Based on previous experience (Hagerman et al., 1996) a steady state period of 10 min were considered sufficient to exclude influence of external stimuli. All recordings were manually edited by an experienced and blinded member of the staff, excluding artifacts (±25% of median heartbeat interval in ms) and ectopic beats (±10% of median heartbeat interval in ms). HRV was analyzed for various spectral components using the Power Lab system (AD Instruments Pty, Ltd. U-PL/QS-05XB, 2006). Times series of minimum 600 heart beats allowed analysis of total variability (TP), power in high frequency band (HF) (0.15–0.4 Hz) and low frequency (LF) band (0.04–0.15 Hz). The autonomic balance expressed by LF/HF was computed.

6. Statistical analysis

As the major part of HRV consists of alterations in respiratory dependent HF band, this parameter was used as primary endpoint for analysis, but low frequency (LF) was also studied as well as the LF/HF ratio. Percentage changes from baseline were calculated for each individual and each exposure scenario to reduce the influence of inter-individual variation for the data from HF and LF/HF. The changes in the selected outcome measures at candle particles or particles generated by terpene–ozone reactions vs. changes at zero exposure were analyzed with repeated-measures analysis of variance using a linear model type of the generalized estimating equation in SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Subject identification, the three exposure scenarios, and the time point of measurements were used to indicate the repeated measurements. All the results were adjusted by age. The 4th recordings (after exposure values) were excluded from analysis since they were performed when the chamber had been evacuated and filled with clean air, which otherwise might have diluted the results from the provocations. Two subjects were excluded from the HF analysis since they were absent from particle free air exposure. Another one subject was excluded from LF/HF analysis since she showed an extreme low LF at baseline. In the analysis comparing only candle and particles generated by terpene–ozone reactions, nine subjects were excluded since they were absent from either of these two

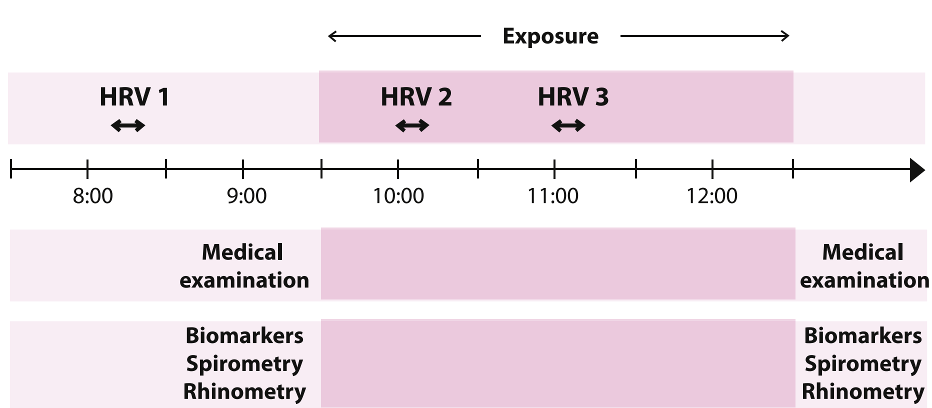

Fig. 2. The experimental study design.

exposure scenarios. Statistical significance refers to $p < 0.05$ (two-tailed).

7. Ethics

The study was approved by the Ethical Committee, Lund University, Dnr 160/207.

8. Results

The typical particle size distributions during exposure are shown in Fig. 3. Mean particle concentrations in number and mass as well as exposure characteristics as air temperature, relative humidity, carbon dioxide and ozone concentrations are given in Table 1. The concentrations were similar of what can be observed in normal indoor settings when for instance having a dinner (candle smoke particles) (Wierzbicka, 2008; Stabile et al., 2012; Bekö et al., 2013) or cleaning (particles generated from terpene–ozone reactions) (Wierzbicka, 2008). The candle combustion resulted in bimodal size distribution (Fig. 3) consisting of: an ultrafine mode of particles (count mean diameter of 23 ± 2 nm and mass mean diameter of 59 ± 4 nm) and a soot mode of particles (count mean diameter of 270 ± 14 nm, mass mean diameter of 477 ± 23 nm). Bimodal size distribution agrees well with previously reported characteristics of particles due to candle burning (Stabile et al., 2012; Pagels et al., 2009; Wallace, 2006) The particles generated by terpene–ozone reactions displayed single mode size distribution (Fig. 3) with a count mean diameter of 98 ± 7 nm and a mass mean diameter of 258 ± 17 nm. Both aerosol sources hence result in particles of the fine and ultrafine type, which have the potential to be inhaled and deposited in the alveolar tract of the respiratory system. As previously reported (Isaxon et al., 2013) the candle particles consisted of 68% elemental carbon (soot), 8% nitrates, 7% of organic carbon, 7% potassium, 6% sodium and minor traces of metals (Cu, Sn) (mass percentages). The particles from the terpene–ozone reactions consisted of 64% carbon, 28% oxygen and 8% hydrogen (mass percentages). The ozone levels in the exposure chamber were low (10 ± 4) ppb compared to average indoor ozone concentrations, which are 28–60 ppb (Rohr et al., 2003), and which can exceed 120 ppb during summertime in urban areas (Singer et al., 2006).

The experimental study was designed in order to obtain double-blind comparisons between three exposure types. Significant differences in HRV were found (Tables 4 and 5). Comparisons of changes from baseline showed more scattered data, with greater confidence intervals. This can be explained by influences on test persons other than exposure (such as differences in initial status of test persons at base-line measurements and differences in influences of the environment) which are likely to have occurred in a random manner. By comparing similar visits in the chamber with different particle exposures to each other significant differences in response could be observed.

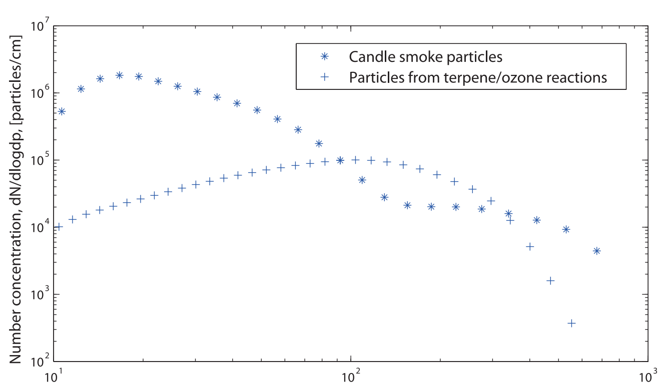

Fig. 3. Typical size distributions from candle smoke exposure and from exposure for particles generated by terpene–ozone reactions.

Table 1
Exposure conditions of candle and terpene/ozone experiments, respectively.

		Mean exposure ± std	Median exposure	Min exposure	Max exposure
Number concentration (#/cm^3)	Candle	$8\cdot10^5 \pm 2\cdot10^5$	$9\cdot10^5$	$4.9\cdot10^5$	$9.1\cdot10^5$
	Terpene	$3\cdot10^4 \pm 4\cdot10^3$	$3\cdot10^4$	$2.3\cdot10^4$	$3.3\cdot10^4$
Mass concentration (µg/m^3)	Candle	200 ± 30	200	170	240
	Terpene	80 ± 10	80	60	90
CO_2 (ppm)	Candle	930 ± 60	920	780	1100
	Terpene	770 ± 50	760	650	970
RH (%)	Candle	33 ± 1.6	33	31	35
	Terpene	33 ± 1.5	32	31	35
Temperature (°C)	Candle	23 ± 0.6	23	22	24
	Terpene	23 ± 0.6	23	22	24
O_3 (ppb)	Terpene	10 ± 4	7.5	7	18

Original data from HF and LF are presented in Tables 2 and 3. We found a mild but significant effect of age, indicating ten years older is related to 8.3% decrement of HF ($p = 0.04$). Exposure to the particles from burning candle increased HF by 22.3% ($p = 0.01$, CI 5.6, 39.1) compared to zero exposure and adjustment for age (Table 4), while exposure to particles generated by terpene–ozone reactions tended to decrease HF by 7.2% ($p = 0.41$, CI −24.5, 10.0) with adjustment of age (Table 4). The LF/HF tended to decrease during exposure for burning of candle and increase during exposure to particles generated by terpene–ozone reactions (Table 4). The changes in HF during candle exposure and particles generated by terpene–ozone reactions exposure were in the different directions, and the difference was −28.9% and was significant ($p = 0.02$, CI −5.4, −52.5) (Table 5). Heart rate (beats/min) was unchanged in all groups during all experimental settings (data not shown).

9. Discussion

Firstly, the aim of this study was to establish a model with chamber exposures together with online registration of time series of ECG in order to explore the effects of common nano-sized indoor particles on HRV in healthy individuals. The two aerosols used contain nano-sized particles and are common in indoor air, thus ethically justified for human exposure and in realistic concentration levels high enough for studies of physiological responses and at the same time avoiding unrealistic and unethical exposures. The experimental chamber design and model system for particle generation used in this study have been evaluated by Isaxon et al. (2013) who demonstrated that aerosols in home environments could be reproduced in this chamber experimental setting with a particle concentration, size distribution and composition comparable to those in field studies. We found that time series of ECG signals could easily be collected with sufficient sampling frequency, from the inside of the chamber, online from 3 individuals at the same time.

This study shows that candle particle exposure increase HF power ($p = 0.01$) and tends to decrease LF/HF indicating a shift in the autonomic balance to a more parasympathetic tone. For particles from terpene–ozone reactions the HF was not changed ($p > 0.4$), but tended to shift LF/HF to a less parasympathetic tone. The hypothesis H0 that the chosen provocations not would affect HF could therefore be rejected. It can also be concluded that the

Table 2
Recorded values from measurements of power in high frequency band (HF) of HRV during different exposures. Index 1 refers to before exposure, 2 refers to 30 min of exposure and 3 refers to 90 min of exposure.

Subject	Zero exposure			Candle particles			Terpene–ozone particles		
	Z_1	Z_2	Z_3	C_1	C_2	C_3	T_1	T_2	T_3
1	611.6	1285.2	605.2	572.1	112.9	694.2	811.4	1509.0	1245.4
2	80.5	120.8	225.1	47.5	60.4	194.2	147.4	230.4	248.3
3	183.9	135.9	89.0	384.1	651.6	315.5			
4	177.1	291.4	180.4	150.5	239.9	106.6	267.7	269.3	305.6
5	631.0	621.5	737.7	420.5	1038.4	702.6			
6	1298.7	915.7	1097.3	1153.2	1288.6	730.1	1300.2	1584.6	2070.6
7	560.0	687.1	404.2	565.5	1277.1	197.9	751.8	267.4	142.4
8	221.9	199.1	175.2	342.1	285.9	485.1			
9	51.3	88.8	98.2	87.5	105.2	184.9	121.4	105.6	61.2
10	477.9	395.3	273.8	482.7	392.8	424.7	216.3	178.0	109.9
11	600.1	410.5	712.8	408.7	723.1	542.8			
12	264.4	154.5	163.2	189.1	368.0	449.9	477.3	451.5	330.8
13	150.7	150.2	179.5	178.0	89.2	221.8	558.6	316.6	446.1
14	1076.4	1236.0	1361.0	737.1	848.3	1226.4	1205.0	759.8	912.8
15	362.5	607.9	208.5	242.3	499.6	357.1	313.3	241.0	321.2
16				212.1	207.9	174.7	328.1	270.1	262.6
17	1384.3	1155.5	877.4	515.6	1068.6	553.4	1023.6	753.0	1159.2
18	1494.4	1511.3	836.6	1291.0	1815.5	1373.9			
19	1070.3	758.0	698.8				798.4	549.4	1326.3
20	2076.9	1793.0	1759.2				1694.5	2360.7	1691.2
21							945.2	625.8	767.8
22	381.1	733.9	764.2				307.9	231.5	209.8

difference of change in HF for candle exposure compared to exposure of particles generated by terpene–ozone reactions was significant ($p = 0.02$). It can also be concluded that with high significance that candle particles did not decrease the variability in HF spectral band.

HF is reported to reflect the influence of respiration and vagal tone in the autonomic modulation of heart rate variability (Malik, 1996; Hagerman et al., 1996; Yasuma and Hayano, 2004; Grossman and Taylor, 2006). The autonomic nervous system has properties to detect inflammatory stimuli. Afferent signals via nervous vagus results in efferent nervous vagus signaling. This cholinergic anti-inflammatory pathway is reported to modulate cytokine production (Czura and Traycey, 2005; Sloan et al., 2007; Huston and Tracey, 2011) and such a mechanism could contribute to changes in HRV during states of inflammation. Various tests for inflammatory parameters were taken during and after the exposure and will be reported in a separate study. Previous field studies of HRV and exposure to fine airborne particles mostly demonstrate reduced HRV and HF. On the other hand, increased HF during low levels of exposure was presented by Riediker et al. (2004), together with increased blood inflammatory and coagulation markers. The results of Wu et al. (2010) were more consistent with previous results with decreased HRV and HF during traffic-related PM exposure although there were a heterogeneity on the individual level. This illustrates the difficulties in HRV measurements and interpretation, with a large inter-individual variability and underlines the need of structured experiments during steady state conditions. Nevertheless, the association between inflammation and effects on vagal tone and possible importance in cardiovascular diseases needs to be studied in different settings.

Table 3
Recorded values from measurements of power in low frequency band (LF) of HRV during different exposures. Index 1 refers to before exposure, 2 refers to 30 min of exposure and 3 refers to 90 min of exposure.

Subject	Zero exposure			Candle particles			Terpene–ozone particles		
	Z_1	Z_2	Z_3	C_1	C_2	C_3	T_1	T_2	T_3
1	604.4	725.6	426.6	609.1	224.5	764.7	507.0	649.8	651.0
2	204.8	229.4	289.5	88.9	102.9	431.7	138.8	395.0	273.9
3	274.5	184.1	82.4	230.5	276.1	191.7			
4	183.8	238.1	230.3	292.0	435.8	87.9	264.0	388.1	315.4
5	268.3	266.9	374.9	280.4	245.1	304.4			
6	586.3	442.0	324.1	652.6	682.3	649.4	506.6	483.5	441.9
7	323.2	463.5	362.3	393.1	474.4	447.0	409.1	390.3	452.3
8	65.7	136.2	71.7	45.3	336.2	394.4			
9	139.1	146.6	314.5	118.4	133.0	198.5	194.7	164.9	127.2
10	267.4	318.2	398.7	554.8	38.9	369.4	276.7	234.1	317.1
11	388.1	210.9	669.5	204.2	525.4	686.1			
12	330.3	276.5	133.5	214.2	440.4	439.8	386.5	468.5	596.0
13	176.5	351.6	353.8	394.9	135.7	212.4	459.6	187.6	268.1
14	269.7	388.3	614.2	322.0	499.1	468.0	310.7	306.1	427.4
15	224.2	331.4	201.9	614.5	357.5	295.7	260.2	233.0	231.2
16				288.1	119.8	96.2	192.3	112.2	178.8
17	461.5	327.6	262.6	822.6	459.5	349.0	164.4	219.4	214.5
18	426.5	522.5	739.9	292.7	486.3	623.1			
19	668.2	408.3	456.3				401.2	221.1	239.2
20	735.4	605.8	551.0				657.6	671.5	781.8
21							919.4	738.1	1025.5
22	155.0	412.9	218.5				174.6	159.9	439.8

In several field studies in homes, candles have been identified as sources of ultra-fine particles (Hussein et al., 2006; Matson, 2004; Wierzbicka, 2008). During normal steady burn, in principle all soot formed in the flame can be oxidized, resulting in very low elementary carbon (EC) emissions. Elemental carbon concentrations emitted from candles can be very high as shown in various experiments by Pagels et al. (2009), and a potential health hazard related to candle burning is the occurrence and release of metal additives from the wick and color pigments.

It is likely that particles from indoor sources can cause health effects (Schneider et al., 2003; Eriksson and Stenberg, 2006). For example, Li et al. (2003) demonstrated that ultrafine particulate pollutants induced oxidative stress on a cellular level with mitochondrial damage. In this study with adult and healthy female volunteers direct health effects are unlikely. Heart rate variability was chosen as being potential pre-cursors of health effects at exposures of particles from different sources which emit particles of different characteristics. In a review on relations between particles and health effects (Schneider et al., 2003) it was pinpointed that in order to draw conclusions about potential health-effects of indoor

Table 4
Percentage changes in the High Frequency (HF)[a] and the Low Frequency/High Frequency (HF/LF)[b] ratio values.

Exposure		% changes from base-line (mean)	% changes from base-line (95% CI)	% changes compared to zero exposure (mean)	% changes compared to zero exposure (95% CI)	P values (compared to zero exposure)
Zero	HF	5.7	(−78.4, 89.6)	–	–	–
	HF/LF	9.8	(−78.2, 97.8)			
Candle	HF	28.0	(−97.2, 153.2)	22.3	(5.6, 39.1)	0.01
	HF/LF	−1.0	(−105.9, 103.9)	−10.8	(−31.2, 9.5)	0.30
Terpene + ozone	HF	−1.5	(−69.7, 66.7)	−7.2	(−24.5, 10.0)	0.41
	HF/LF	32.1	(−147.0, 211.2)	22.2	(−7.8, 52.3)	0.15

[a] Statistic is based on 20 subjects and with adjustment for age.
[b] Statistic is based on 19 subjects and with adjustment for age.

Table 5
Percentage changes in High Frequency (HF) values.[a]

Exposure	Percentage changes from base-line (mean)	Percentage changes from base-line (95% CI)	Difference of percentage changes (mean)	Difference of percentage changes (95% CI)	P value
Candle	25.2	(−109.3, 159.7)	–	–	0.02
Terpene + ozone	−3.8	(−71.4, 63.8)	−28.9	(−52.5, −5.4)	

[a] Statistic is based on 12 subjects and with adjustment for age.

particles, several properties of the particles has to be known. These were particle concentrations (number, surface area and mass), chemical composition, solubility, hygroscopicity and biological constituents. In our study, characteristics of the two types of particles used for exposures i.e. from candle burning and from terpene–ozone reactions, varied considerably. The levels of particles were generated to reflect real concentrations encountered in indoor environments and the differences are seen in: mass concentrations (200 μg/m^3 versus 80 μg/m^3 for candles and terpenes/ozone, respectively), number concentration ($8 \cdot 10^5$ #/cm^3 versus $3 \cdot 10^4$ #/cm^3, for candles and terpenes/ozone, respectively), size distribution (bi-modal versus single mode size distribution for candles and terpenes/ozone, respectively) and chemical composition (elemental carbon dominated candle particles [68% by mass] versus solely soluble organics in terpenes/ozone particles). All these listed characteristics are determinant for the fraction that deposits in respiratory tract and causes given effect. The candle particles consisted of a mixture of elemental carbon (68% by mass) and water soluble salts (such as phosphates and alkali nitrates), while the secondary organic aerosol particles from terpene–ozone reaction products consisted of highly oxidized water soluble organics and no elemental carbon at all. Controlled human exposure to ozone is reported to increase markers of inflammation and markers that affect autonomic control of heart rate (Devlin et al., 2012). In our experiments efforts were made to keep the ozone concentration (see Table 1) as low as possible to avoid potential adverse health effects. As compared with Devlin study of 0.3 ppm ozone concentration our levels were 30 times lower.

A limitation of our study is the lack of controlled respiration but all tests were performed during carefully observed steady state conditions at the same time point of the day and with no intake of coffee, tea or other substances, factors that could have an impact on HRV. Heart rate response to a change in parasympathetic efferent activity is extremely rapid, occurring on a beta-to-beat basis while the change due to sympathetic outflow is a slower process (Hagerman et al., 1996). This, together with other influences on HRV for example hormones, pre- and after-load of the heart, vascular endothelial function results in a signal which is composed of several oscillators with overlapping time scales. The HRV signal is therefore complex and sensitive to both internal and external influences. This could contribute to the large inter-individual variability in heart rate variability measurements and in part explain the heterogeneity and conflicting results between studies of HRV and air pollution (Mills et al., 2011). As the major part of HRV is constituted by respiratory mediated alterations in the HF band and the results of our study, where different kinds of nano-sized particles seem to affect HF in different ways support the very idea that the airway is important for modulation of the autonomic nervous system. The effects on HRV would then depend on to what extent and where particles are deposited together with their biochemical properties mediating inflammatory responses. In this study, it was not possible to determine exactly to which extend the different particle characteristic (mass, number concentration, size or chemical composition) could be linked to the observed effects on HRV. Although it has to be emphasized that the two particle types, having very different characteristics, not only concerning concentration, gave different effects. Therefore further studies on the effects of various particles on HRV, with detailed determination of particle characteristics, are indeed needed. If conducted in a systematic way, studies such as the one we have described can allow mapping of specific particle characteristics to observed effects, which in consequence can enable identification of key particle property (or combination of properties) responsible for observed health effects. Further studies on the effects of various particles on HRV are therefore needed.

10. Conclusions

Exposure to nano-sized particles of burning candles and terpene–ozone reactions seem to have an impact on heart rate variability in healthy individuals, although significant statistical correlations only were demonstrated in part. The highly significant difference in HF between the two aerosols with significantly different characteristics in: particle mass and number concentration, size distribution and chemical composition strengthens these results. Future work will include particles of various characteristics in order to get information on how particle characteristics may influence HRV and studies of different cohorts with increased risk for cardiovascular health problems. The design of the chamber study together with the HRV method may be used to get information on physiological response of exposure to particles of different concentration, sizes and characteristics which may contribute to the understanding of mechanisms behind health effects of particle exposures.

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