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Chapter 8

CONTROLLED HUMAN EXPOSURE STUDIES

Maria Helena G. Andersen[1,*], Steffen Loft[2], Jakob H. Bønløkke[3], Anne T. Saber[1], Ulla Vogel[1] and Peter Møller[2]

[1]The National Research Centre for the Working Environment, Copenhagen, Denmark
[2]Department of Public Health, University of Copenhagen, Copenhagen, Denmark
[3]Department of Occupational and Environmental Medicine,
Danish Ramazzini Centre, Aalborg University Hospital, Aalborg, Denmark

ABSTRACT

Combustion produces complex mixtures of ambient air pollutants, depending on the fuel, oxidant availability, temperature, turbulence, engine, among others. This complexity combined with the complexity of exposure to the pollutant, with variable duration, frequency, spatial position, multiple-sources and co-pollutants, activity level, and inter-individual variation in susceptibility, makes it challenging to assess human health effects from exposure to ultrafine combustion particles. Controlled human exposure studies refer to those where selected study participants are exposed under controlled conditions of exposure and activity for the investigation of selected health outcomes and biomarkers. This is usually done in an exposure chamber although some studies have also attempted to control conditions in real-life settings. This design has been particularly used to investigate acute health effects from short-term exposures to combustion particles, providing mechanistic support of observations from epidemiological and animal studies. This chapter reviews and compares controlled human exposure studies in chambers, on healthy subjects, involving combustion-generated particles, which are dominated by the ultrafine size mode. In total, 64 studies including 12 chamber studies on wood smoke, 33 on diesel exhaust, 15 on concentrated ambient particles and 4 other carbonaceous particles (candles and others) were identified. The studies differ by designs (crossover or sequential), protocol definitions (with or without physical activity), particulate matter source (diesel exhaust, wood smoke or others), exposure metrics (with or without ultrafine size mode description), combustion generation conditions (for the same source), administrated doses and duration of exposure, as well as biomarkers and functional markers under study. The biological effects assessed have been mainly focused on lung function, cardiovascular function, heart rate variability,

* Corresponding Author's Email: mga@nrcwe.dk.

and inflammatory, oxidative stress and genotoxicity markers. The strengths and limitations of the controlled human exposure studies are revisited. The importance of the ultrafine particles is discussed and the results are compared with studies using real-life air concentrations of pollutants to generate a gradient of exposure.

1. CONTROLLED HUMAN EXPOSURE STUDIES

Controlled human exposure studies (CHES) are experiments where volunteer participants agree to be intentionally exposed to pollutants, in a controlled short-term scenario, to provide information on measurable biological changes caused by the exposure. The scientific relevance is to provide evidence of causal or mechanistic relations between exposure and biological effects to support epidemiological observations on hard outcomes such as incidence or mortality of diseases in the human population.

CHES on particulate matter pollution are able to provide unique information to the body of knowledge of air pollution-induced health effects, namely demonstrating specific mechanisms of action for the development of clinical effects, and thus supplement evidence obtained from animal and epidemiological studies (National Academies of Sciences Engineering and Medicine, 2017).

CHES aim to study early, transient and reversible effects without inducing disease, triggered by ambient range or higher equivalent concentrations, ensuring the safety of the participants, using accepted scientific principles and methods and following international ethical standards. The protocols of the studies have to be approved by local ethic authorities, written informed consents have to be collected and the participant's rights ensured.

1.1. Exposure Setting

CHES are generally performed in an exposure chamber with controlled air composition, ventilation, temperature, and humidity conditions with single or multiple subjects exposed simultaneously, as illustrated in Figure 1. The particles are generated by combustion and/or concentration processes or resuspension and forced into the chamber, where dispersion is controlled, also allowing control of mixtures and co-exposures. In these types of chambers, the participants are whole-body exposed. Alternatively, some CHES are performed with exposure administered using a mask that covers the subject's nose and mouth (Figure 2). Intermediate solutions also exist, where subjects are in a confined individual chamber with a particle generation outlet pipe entering the chamber at the chest height of the subject, also exposing the whole-body. The exposure facility design will define protocol requirements, namely concerning flow rates and exposure duration, with larger particle generation capacity and exposure duration being required for bigger chambers, in contrast to facemask delivery.

The level of activity of the participants can also be controlled, with many exposure chambers equipped with treadmills or bicycle ergometers (as illustrated in Figure 1), where participants follow specific protocols of moderate physical activity to increase inhalation rates and deposition.

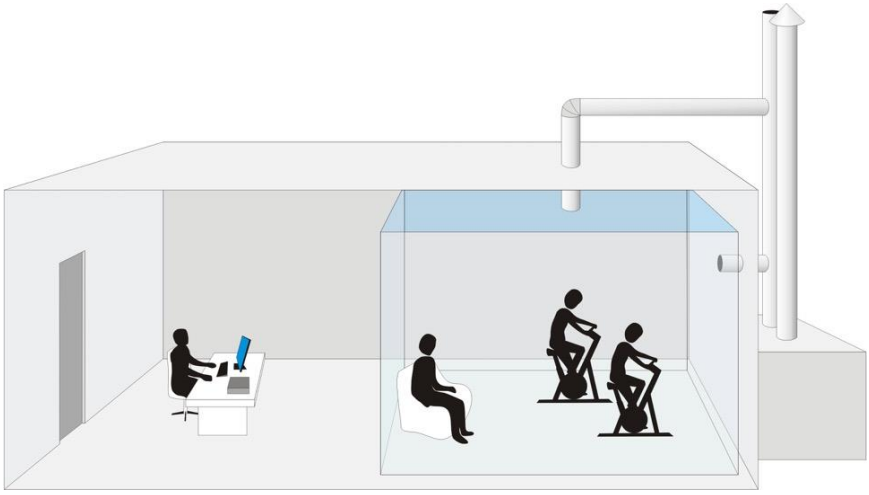

Figure 1. Schematic figure of a typical exposure chamber facility, with subjects under physical exercise or at rest (illustration by Manuel Guerra).

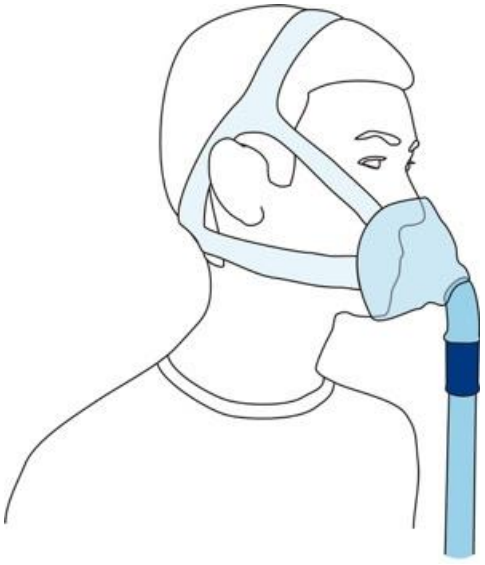

Figure 2. Schematic figure of exposure administrated using a facemask (illustration by Manuel Guerra).

2. Controlled Human Exposure Studies on UFP

Different sources of ambient particle pollution, known to be dominated by ultrafine size mode, have been studied in CHES, namely wood smoke, diesel exhaust and concentrated ambient particles (CAP), but also indoor sources (printers, candles, cooking) and particles from occupational settings (welding fumes and its individual components).

Globally, we have limited information about ambient exposure to ultrafine particles (UFP) to infer health effects from epidemiological observations. The spatial and temporal variability

in concentrations and composition, the lack of monitoring networks, the non-uniformity in exposure metrics, the complexity of mixtures of air pollution, and the uncertainty about mechanisms, makes it difficult to establish associations. However, the likelihood of UFP exposure-effects causality has been accepted (Morawska et al., 2019) and CHES constitute a tool to investigate PM, and particularly UFP, short-term effects and mechanisms.

2.1. Strengths and Limitations

The following table summarizes general CHES strengths and limitations (Ghio et al., 2012a; McDonnell, 1993; National Academies of Sciences Engineering and Medicine, 2017; Utell and Frampton, 2000):

Table 1. Strengths and limitations of controlled human exposure studies

Strengths	**Limitations**
• Control over temporality; • Control over exposure conditions (PM source, size, mixtures, concentrations, duration, activity level); • Control over co-exposures, allowing approaches from individual pollutant component to mixtures and vice versa; • Control for confounders, variability and selection bias by selection of participants, randomization, blinding and crossover design; • Offers possibility to recruit susceptible participants; • Access to gold standard instrumentation, methods and staff for multi-endpoints assessment, not feasible in field studies; • Development of new and emerging markers and outcome measurements, that can be used in other study types; • New portable and reliable equipment available that can be used in *quasi*-controlled or mobile-chamber exposure studies; • Allows a pre-selection of more responsive individuals to be studied further;	• Ethical limitations concerning risk of induction of permanent effects and use of more susceptible subjects; • Inability to assess long term effects and rare events; • Time and logistic limitations concerning large number of subjects; • Statistical limitations from small number of subjects; • Limited control for effects of other recent exposures, diet, life style or susceptibilities; • Expensive and time consuming; • Invasive characteristic of some endpoint assessments limits their use and may interfere with other outcome assessments; • Under-representation of the population in many studies (with studied effects on adult, male, white, young, healthy, never smoking, not on medication subjects) and therefore the limitations to generalize; • For studies on CAPs, daily variations in street level exposures and weather conditions limit the control over target exposures in the chamber, increasing the variability and consequently limiting the power of observations;

CHES have the ability to control the experiment at multiple levels, by:

- Selecting, controlling and quantifying the exposure source of the pollutant of interest (namely UFP and mixture source), using real-life particle sources;
- Selecting participants with known clinical status and characteristics. Some studies select more susceptible subjects, looking for a stronger response (using patients, subjects with asthma or other comorbidities, or elderly), but other variability factors

can then be introduced. Therefore, many of the researchers choose to expose healthy, homogeneous groups of subjects, often recruited in university campus, limiting generalization, but facilitating the inter-comparison of results among studies;

- The use of sequential or cross-over study design where the participants serve as their own control furthermore increases the statistical power to detect biological effects because inter-individual variation is controlled;
- Control exposure dose (in terms of concentration, duration and participants activity level). Control of other recent exposures, diet and susceptibilities, is difficult as CHES control short-term variables on the setting, but not beyond that. Nevertheless, attempts have been made to include adjustment for ambient concentrations from prior days in the analysis (Gong et al., 2004; Pope et al., 2011);
- Select endpoints, samples and time windows of assessment.

CHES have been designed and developed to include methodological aspects that empower a causal inference for the health effects from ambient combustion PM exposure (National Academies of Sciences Engineering and Medicine, 2017). One of the design characteristics of the majority of the CHES is randomization, which was pioneered in clinical trials by Bradford Hill (Armitage, 2003), who in the 1960s defined 9 criteria to evaluate the adequacy of observed associations (Hill, 1965), that remains a useful road map for causality inference. In light of these criteria, CHES can be used to establish causal relationships between exposure and biological effects as follows:

- *Strength of association* – This was the first criteria presented by Bradford Hill when he formalized his arguments of causation while addressing the section of occupational medicine of the Royal Society of Medicine in January of 1965. The strength of the association is no longer interpreted as just its magnitude, but through statistical inference, clinical relevance and comparison with the weight of evidence (Fedak et al., 2015). Literature from CHES generally have these dimensions considered in discussions;
- *Consistency* – CHES have been reproduced in multiple studies on different locations, populations and exposure methods enabling the test of validity of findings across different independent studies;
- *Specificity* – CHES allow to separate the effects of the specific PM component and size fraction of different combustion sources from effects associated with the complex mixtures of ambient air pollution, with studies investigating some of these feature components (Liu et al., 2015; Mills et al., 2011b; Nightingale et al., 2000), and by design (namely through randomization) additionally controls for confounder factors that could interfere with the exposure-to-effects relationship;
- *Temporality* – the majority of the CHES use a crossover design enabling a timing assessment for causality. A true crossover design would use parallel chambers, therefore CHES use a crossover approach adaptation, but still enabling a control over temporality, although with some degree of uncertainty, namely if the source exposure concentration, or even the endpoint assessed would have a longitudinal variance. Some CHES use a sequential design exposing the same participants to clean air first, followed by an exposure session with the PM of interest, also enabling the inference

of temporality, although less optimized for confounders. Even CHES that use separate groups for the exposure and control scenarios (rare), also integrate the temporality with the pre- and post-assessment;

- *Biological gradient* – CHES enable the study of specific exposure concentrations and durations and even synergistic and cumulative exposure's approach, for example by testing both air pollution mixtures, particle filtration and resuspensions of PM, or by designing cumulative exposures;
- *Plausibility* – CHES on PM short-term effects can be used to investigate the biological plausibility (i.e., mechanism of action) of health effects on the respiratory system, as well as cardiovascular and other systemic effects. CHES enable the assessment of multiple biomarkers and physiological responses within the same study, which also allows for test of other alternative mechanisms and further support the endpoints assessed in epidemiological studies;
- *Coherence* – CHES are used to strengthen the evidence of a coherent description of a causal pathway by integrating toxicological and epidemiological study findings and testing hypotheses of specific exposure-to-effects relationships;
- *Experiment* – The CHES are by definition an experimental manipulation of exposure factors, with the majority of the studies comparing the exposure situation with a control, no-exposure scenario, and therefore supporting the causal inference of exposure drawn;
- *Analogy* – CHES are very suitable for the assessment of analogy as the comparable exposure circumstances can be set up (e.g., if diesel exhaust causes effect on a certain biomarker, it would be assumed that a similar response of CAPs exposure is a true positive response).

CHES are a useful and strong study design, nevertheless the outcome under focus is not a hard endpoint of disease onset, but transient mechanistic effects. Moreover, even a well-designed CHES may have limitations because of little analytical validity, clinical validity or clinical utility of the selected types of biomarkers (Gallo et al., 2008).

2.2. Selection of CHES

We performed a systematic literature search on ambient particle CHES on PubMed in January 2020 (n=488), with 78 relevant peer reviewed articles after screening, and added hand-searched articles (n=24), resulting in 102 articles reporting assessment of biological changes following exposure of healthy, non-smoking subjects to fine or ultrafine particles from wood smoke, diesel exhaust, concentrated ambient particles and indoor carbonaceous sources (candle burning, toasting, frying, printing devices and secondary particles generate by terpene-O_3 reactions).

Table 2 summarizes the selected studies by combustion source, and the total number of exposed subjects included. Diesel exhaust was the most commonly used ambient combustion source in the CHES, with a larger number of studies, number of exposed subjects (here only considering healthy subjects), more dedicated exposure facilities and country representation.

Table 2. Selected studies, exposed subjects and exposure facilities description per combustion source

Source	Number of studies (articles)	Number of exposed-subjects	Exposure facilities description (reference)
Wood smoke	12 (20)	243	Borås, Sweden: chamber 44 m^2 (129 m^3)(Sallsten et al., 2006); Umeå, Sweden: chamber 6 m^2 (18 m^3)(Sehlstedt et al., 2010); Århus, Denmark: chamber (79 m^3) (Riddervold et al., 2011); Provo, US-UT: chamber 10.4 m^2 (27 m^3) (Kuprov et al., 2011); Chapel Hill, US-NC: chamber 3.3 m^2 (8.2 m^3)(Ghio et al., 2012b); Missoula, US-MT: breathing facemask (Ferguson et al., 2016); Fort Collins, US-CO: chamber 9.8 m^2 (26.5 m^3)(Fedak et al., 2019);
Diesel exhaust	33 [a)] (51)	580 [a)]	Umeå, Sweden: chamber 9 m^2 (21 m^3)(Rudell et al., 1994); Lund, Sweden: chamber 9 m^2 (21.6 m^3)(Isaxon et al., 2013); London, UK: chamber 2.4 m^2 (5.5 m^3)(Nightingale et al., 2000); Edinburgh, UK: single-person whole body chamber (Mills et al., 2011b); Athens, Greece: chamber 30 m^2 (Tousoulis et al., 2020); Brussels, Belgium: chamber 7 m^2 (18.6 m^3)(Wauters et al., 2013); Vancouver, Canada: chamber 2.2 m^2 (4.8 m^3)(Birger et al., 2011); Chapel Hill, US-NC: chamber 3.3 m^2 (8.2 m^3)(Ghio et al., 2012a); Seattle, US-WA: chamber (116 m^3)(Gould et al., 2008); Piscataway, US-NJ: chamber (25 m^3) (Pettit et al., 2012); S. Paulo, Brazil: breathing facemask (Vieira et al., 2016);
Concentrated ambient particles	15 [a)] (25)	391 [a)]	Toronto, Canada: cabinet with breathing facemask (Petrovic et al., 2000); Ann Arbor, US-MI: mobile facility with breathing facemask (Dvonch et al., 2004); Rochester, US-NY: breathing facemask (Breitner et al., 2019) Chapel Hill, US-NC: chamber 2.8 m^2 (5.5 m^3) (Ghio and Huang, 2004); Los Angeles, US-CA: whole-body chamber (Gong et al., 2000);
Indoor sources	4 [a)] (6)	124 [a)]	Düsseldorf, Germany: laboratory room 16 m^2 (48 m^3)(Soppa et al., 2014); Munich, Germany: chamber 12.6 m^2 (32 m^3)(Karrasch et al., 2017); Lund, Sweden: chamber 9 m^2 (21.6 m^3)(Isaxon et al., 2013)
All exposures	64 (102)	1,338	24 exposure facilities (from 9 countries)

[a)] Considering assumptions of overlapping studies.

3. EXPOSURE PROTOCOLS

The study protocols are firstly challenged by the feasibility of the exposure protocol, as it is complex to control the exposure source and keep it relevant and representative during the entire exposure session. Besides the UFP source choice (wood, diesel, CAPs or others), the combustion conditions (and consequent co-pollutants), the target doses, the duration of exposure, the particle generation and control capacity, along with logistic determinants, are among other circumstances inadvertently incorporated as unique descriptors and contribute to the difficulty of comparison among study results. That is to say, that despite the controlled

design, other factors need to be taken into account in addition to the UFP source descriptor in order to understand the potential effect of variability of all other exposure protocol characteristics on the biological effects. Moreover, different measuring methods and metrics of reporting and integrating the UFP concentrations in the data analysis may also determine the ability to detect possible effects (Baldauf et al., 2016). In the following paragraphs, we shortly introduce the various exposure protocols used among the selected studies, besides the source of pollution.

The *wood smoke* studies have mainly generated emissions from standard woodstoves for home use, while burning specified and different types of wood, in incomplete combustion processes, but also studying different phases, such as start-up and burn-out combustion phases (Stockfelt et al., 2013; Stockfelt et al., 2012), or wood smoke generated from different cook stove technologies (Fedak et al., 2019). All the selected wood smoke studies used mass-based particle concentration descriptors and half of them reported the particle number concentrations. The majority (67%) reported size metrics, describing a bimodal particle size distribution of the aerosol and/or exhausts dominated by UFP and PM_1 size modes. Eleven of twelve wood smoke CHES reported co-pollutants, primarily among by-products of the combustion process such as nitrogen oxides, carbon oxides, polycyclic aromatic hydrocarbons, volatile organic compounds and ozone.

In the majority of the CHES on *diesel exhaust,* the combustion mixture was freshly produced from engines consuming diesel fuels on idling mode, but also some studies with the engine operating on urban transient cycle conditions (Barath et al., 2013; Barath et al., 2010; Lucking et al., 2011), at load (Carlsten et al., 2007; Cosselman et al., 2012; Madden et al., 2014), in transient mode burning biodiesel (Gouveia-Figueira et al., 2018) or using resuspension of diesel exhaust particles (Nightingale et al., 2000). Thus, only one study assessed the effects of exposure to diesel exhaust particles, while the other studies assessed the effects of diesel exhaust emissions. The engine models also differ among the facilities, from cars, trucks or generators, as well as different model years and consequently different compliances with emission standards (namely considering UFP co-exposures). The majority of the diesel exhaust studies used mass-based particle descriptors (31 studies, 94%), with 18% (19 studies) also describing number concentrations and 36% (12 studies) additionally reporting size metrics, also in this source case with reported dominant UFP size mode. All the studies monitored and to some degree reported co-pollutants including nitrogen oxides, carbon oxides, hydrocarbons and formaldehyde, with the exception of the study using re-suspended diesel particles.

The studies on *CAPs* have concentrated street level ambient particles using inertial impaction technology for fine and coarse mode sizes and adaptations based on condensational growth and subsequent thermal restauration of ultrafine size mode, for UFP studies (Gupta et al., 2004). The organic and volatile compounds, part of the particulate phase, are preserved in the concentration process, which allows the study of these real-life air pollution particles. Studies that exclusively focused on coarse particles ($PM_{2.5-10}$) have not been considered here. The selected studies on CAPs are all from North America urban areas (US and Canada). As CAPs are concentrated from the chamber facility outdoors, different geographies with different sources and climate may be expected to influence the CAPs composition. Therefore, generalization from this exposure may be limited, even though they represent real-life ambient particle pollution. All CAP studies used mass-based particle descriptors, with 7 studies (47%) also reporting number concentrations and 6 (40%) reporting size measurements. The target size

in CAP studies was typically $PM_{2.5}$, with 5 studies (33%) targeting UFP. CAPs exposure did not include co-pollutants as such, except in the cases of co-exposure of CAPs and ozone (4 studies) and CAPs and nitrogen dioxide (2 studies), which investigated the effect with and without the co-pollutant. Moreover, some of these studies investigated associations between effects and specific components of CAPs, namely endotoxins and elemental carbon.

From *indoor* ambient UFP sources, we identified CHES on candle burning, particles from terpene-O_3 reactions, toasting and frying processes and printing device emissions. This group of CHES include studies using UFP of different genesis, thus unlike the other source-origin groups these studies were grouped under the common characteristic of exposing people to UFP from indoor environments. The 4 included studies described particle metrics in number concentration, 3 of them also with mass and size descriptors dominated by the UFP size mode. The control over exposure in the case of these indoor sources studies has a different paradigm compared with diesel exhaust or wood smoke studies, as the exposure levels are not controlled by mixing externally generated pollution with ambient air entering the chamber with a target concentration. Instead, the exposure is maintained by the processes generally happening inside the chamber, for example with the amount and conditions of the candles burned, or the number of sausages fried, and protocols for the combustion process repetition. The indoor CHES focused on differential size modes of PM, and only one study (Hagerman et al., 2014) monitored other co-pollutants or particle constituents, namely ozone and organic/elemental carbon characterization.

Table 3 summarizes the exposure designs and protocols per PM source, using the mass metric descriptor for the particle concentration (as it is the most common metric descriptor, used in 94% of the selected studies, while number concentration metric is used in 55%). The large majority of the selected studies has a crossover design (86%), and all except two (with parallel groups of subjects exposed to either air pollution or filtered air), use the exposed subjects as their own control. All studies reported statistical analysis considering the nature of their longitudinal data (except for the studies with parallel groups).

Table 3. Exposure design and protocols from the selected studies (n=62)

Source	Design	PM concentration average (range) ($\mu g/m^3$)	Exposure duration	Physical exercise	Wash out period[a)]
Wood smoke	4 sequential, 1 parallel groups and 7 crossover	368 (50-1100)	1 h to 4 h	7 with and 5 without	1 to 3 weeks
Diesel exhaust	2 sequential and 31 crossover	252 (25-388)	21 min to 3 h	19 with, 12 without and 2 unknown	2 days to 1 month
CAPs	1 sequential, 1 parallel groups and 13 crossover	149 (50-278)	2 h	6 with, 8 without and 1 unknown	2 days to 1 month
Indoor	4 crossover	101 (38-200)	1 h to 4 h	4 without	5 days to 2 weeks

[a)] Not applicable to studies with parallel groups design.

In general, the concentrations used in the wood smoke studies were higher than in the other studies, also due to the inclusion of one chamber study on firefighters that used more than 1 mg/m^3. CAPs and indoor sources CHES used lower mass concentrations, however 7 out of the 15 CAP studies used facemasks, probably with a more efficient delivery of the dose, and the lower range mass concentrations correspond to focus targets on UFP. One of the lowest mass concentrations used in indoor CHES of 48 µg/m^3 (PM_1) corresponds to an exposure session of candle burning with a 2-h average of 1.9x10^6 particles/cm^3 (UFP). Mass metrics may be questionable for describing small PM size modes, but are the ones more generally reported.

4. BIOLOGICAL ENDPOINTS

The outcomes assessed in CHES are early measurable, transient and reversible changes that are believed to be involved in particle toxicity mechanisms. Those mechanisms include airway and systemic inflammation and oxidative stress, triggering events that will affect the vascular function, thrombogenicity, heart rate variability, in addition to genotoxicity and neural pathways, affecting respiratory reflexes and autonomic control (Stone et al., 2017). The controlled nature of CHES in laboratory facilities allows the use of equipment, staff and sampling conditions not possible in field studies. Therefore, CHES are useful for the investigation of multiple endpoints within the same exposure study, also explaining the common overlap of study groups in different published material.

Table 4 summarizes the biological endpoints assessed in the selected studies with the number of studies reporting the different biomarkers presented per type of exposure source. The most common markers assessed in CHES are inflammation and vascular function, with less studies focusing on neurotoxicity, arrhythmia or genotoxicity.

Table 4. Biological endpoints investigated in the selected studies

Marker and matrix/functional method		**Wood smoke (n=12)**	**Diesel Exhaust (n=33)**	**CAPs (n=15)**	**Indoor (n=4)**	All studies (n=64)
Airway inflammation	Nasal secretions, BL, BAL and FeNO	8	11	8	2	29 (45%)
Lung function	Spirometry and plethysmography	5	10	7	3	25 (39%)
Systemic inflammation	Blood	6	19	12	1	36 (56%)
Oxidative stress	Blood, urine and airway samples	6	8	3	2	19 (29%)
Genotoxicity	Blood and urine	2	1	2	1	6 (9%)
Thrombogenicity	Blood	4	10	8	-	22 (34%)
Heart rate variability	ECG or frequency counter	3	5	9	1	18 (28%)
Vascular function	Vascular challenge, BP, pulse wave analysis and blood	5	15	6	1	27 (42%)
Arrhythmia	ECG	2 [a)]	2 [a)]	4	-	8 (13%)
Neurotoxicity	Blood, urine and EEG	-	3	1	-	4 (6%)

BAL, bronchoalveolar lavage; BL, bronchial lavage; BP, blood pressure; CAP, concentrated ambient particles; ECG, electrocardiogram; EEG, electroencephalography; FeNO, fractional exhaled nitric oxide. a) The ECG data recorded in these studies was analysed for arrhythmia in a meta-analysis (Langrish et al., 2014), and included here.

Table 5. Observed effects in the selected studies. Number of studies with assessment (and total number of subjects involved)

Marker	Studies (subjects)	Wood Smoke (n=12)			Diesel exhaust (n=33)			CAP (n=15)			Indoor (n=4)		
		×	÷	?	×	÷	?	×	÷	?	×	÷	?
Airway inflammation	29 (539)	**5 (102)**	2 (39)	1 (14)	**9 (126)**	2 (36)	0 (0)	2 (49)	4 (106)	2 (18)	1 (26)	1 (23)	0 (0)
Lung function	25 (476)	0 (0)	**5 (73)**	0 (0)	5 (81)	5 (73)	0 (0)	1 (17)	**6 (121)**	0 (0)	1 (55)	**2 (49)**	0 (0)
Systemic inflammation	36 (658)	2 (23)	3 (47)	0 (0)	4 (51)	**15 (268)**	0 (0)	4 (112)	7 (134)	0 (0)	0 (0)	1 (23)	0 (0)
Oxidative stress	19 (377)	2 (23)	3 (53)	1 (13)	4 (61)	4 (73)	0 (0)	**2 (80)**	1 (25)	0 (0)	0 (0)	**2 (49)**	0 (0)
Genotoxicity	6 (151)	1 (13)	1 (20)	0 (0)	0 (0)	1 (18)	0 (0)	**2 (75)**	0 (0)	0 (0)	0 (0)	1 (23)	0 (0)
Thrombogenicity	22 (383)	2 (26)	2 (36)	0 (0)	5 (69)	5 (113)	0 (0)	1 (34)	**7 (105)**	0 (0)	0 (0)	0 (0)	0 (0)
Heart rate variability	18 (320)	2 (24)	1 (20)	0 (0)	3 (34)	2 (46)	0 (0)	**8 (157)**	1 (19)	0 (0)	1 (20)	0 (0)	0 (0)
Vascular function	27 (647)	2 (62)	3 (50)	0 (0)	**13 (228)**	2 (33)	0 (0)	**5 (185)**	1 (34)	0 (0)	1 (55)	0 (0)	0 (0)
Arrhythmia	8 (176)	0 (0)	2 (29)	0 (0)	0 (0)	2 (46)	0 (0)	**3 (82)**	1 (19)	0 (0)	0 (0)	0 (0)	0 (0)
Neurotoxicity	4 (103)	0 (0)	0 (0)	0 (0)	2 (20)	1 (28)	0 (0)	0 (0)	1 (55)	0 (0)	0 (0)	0 (0)	0 (0)

×, effect observed; ÷, no effect observed; ?, inconsistent observation; Highlighted if 75% of the studies that investigated the effect marker from the same source were consistent (and with more than 50 study subjects), **red (or dark grey) indicates effect** observed, **blue (or light grey) indicates no-effect.**

Table 5 presents a crude summary of the observed effects, presenting the number of studies that reported one effect finding for the class of endpoint markers, and the correspondent sample of subjects included in that assessment. Study-specific details are summarized in tables organized by endpoint in the end of this chapter (Tables 6-10). The most consistent results observed are highlighted. Effect size is not included in the table due to the diverse endpoints assessed and its reporting for each marker, but considerations are discussed in the following sub-sections.

Other controlled study designs with exposure to ambient ultrafine particles resulting from different ambient sources that are not possible to ascribe are not included in the tables and endpoint analysis. These include airport air pollution (Lammers et al., 2020), exposure inside diesel-powered passenger trains (Andersen et al., 2019), among other *quasi*-controlled real life exposure studies (Avogbe et al., 2005; Brauner et al., 2007; Habre et al., 2018; Jensen et al., 2014; Vinzents et al., 2005) that are mentioned throughout the endpoint synopsis.

4.1. Airway Inflammation

Airway inflammation was assessed in secretions and tissue from the upper and lower airways and also through the fraction of exhaled nitric oxide (FeNO), as nitric oxide is produced by cells involved in the inflammatory response. Levels of neutrophils or inflammatory mediators and FeNO are markers of lung inflammation. Another marker studied is Clara cell protein 16 (CC16), a protein secreted into the lung epithelial fluid that is believed to protect the respiratory tract against inflammation and oxidative stress. One study also investigated the immune response to inoculated attenuated influenza virus after wood smoke exposure (Rebuli et al., 2019). Inflammatory mediators are a battery of different molecules released by cells of the immune system that will stimulate a network of other signals of cell recruitment and healing responses, amplifying local and systemic inflammatory reactions. CHES have assessed a large number of these signalling molecules, namely numerous cytokines, platelet aggregation factors, adhesion molecules, and differential cell counts.

FeNO was assessed in 5 *wood smoke* studies with two of them detecting increases, although for different exhalation flow rates, respectively, an increase in $FeNO_{50}$ after burn-out wood smoke (Stockfelt et al., 2012) and an increase in $FeNO_{270}$ (Barregard et al., 2008). Low flow rates (as 50 mL/s, $FeNO_{50}$) mostly represent conducting airways and high flow rates (as $FeNO_{270}$) reflecting the alveolar compartment (Jorres, 2000), thus results do not clearly agree, with 2 studies finding associations to one and not the other region, and 3 studies with null effect on FeNO. Nevertheless, different concentrations of wood smoke and differences in burning conditions may explain the discrepancy of effects. CC16 was assessed in 4 wood smoke studies, 2 of them reported increased levels in serum 4 to 20 h post-exposure (Barregard et al., 2008; Stockfelt et al., 2012), with no changes in urinary CC16 in any study, and no change in BL or BAL CC16 levels (Muala et al., 2015). Nasal secretions of atopic subjects presented unchanged levels of cytokines after wood smoke exposure, and the authors also noted high variation between participants, suggesting that atopy introduced high degree of variance in the response (Riddervold et al., 2012). A study that sampled fluids from bronchial and bronchoalveolar lavages (BL and BAL) reported unaltered differential cell counts (Sehlstedt et al., 2010), but another study observed a small neutrophilic increase in both BL and BAL (Ghio et al., 2012b). Also Burbank and colleagues (Burbank et al., 2019) observed increased number of neutrophils

in sputum 24 h after exposure to wood smoke. However, a decrease in neutrophils (and lymphocytes) has also been observed in BL (and BAL) after exposure to sooty PAH rich wood smoke from incomplete combustion (Muala et al., 2015). A complementary *in vitro* study demonstrated that the particles from the incomplete combustion were cytotoxic and the authors suggested that the decreased levels of neutrophils could be due to the loss or impairment of airway phagocytic cells (Muala et al., 2015). Wood smoke exposure followed by attenuated influenza virus nasal inoculation suppressed nasal lavage cytokines and with a sex-specific change in inflammation-related gene expression profiles (Rebuli et al., 2019)

Two studies assessed FeNO after *diesel exhaust* exposure with non-conclusive effect as one reported a change for lower flows (reflecting inflammation in the central airways) of 13% for $FeNO_{50}$ and 18% for $FeNO_{10}$ (and no changes for higher flows) 6h post-exposure to 1h of 300 $\mu g/m^3$ $PM_{2.5}$ (Barath et al., 2013) while another study did not detect changes 0h post-exposure to 2h of 300 $\mu g/m^3$ $PM_{2.5}$ (Wauters et al., 2015). Besides different time points after exposure (and although exposure duration was shorter in the first case), activity protocols and equipment methodology were different between the studies. Differential cell counts, namely neutrophils increased after diesel exhaust exposure in BL (Behndig et al., 2006; Stenfors et al., 2004), sputum (Nightingale et al., 2000), biopsies (Salvi et al., 1999) and BAL (Rudell et al., 1990; Wooding et al., 2019). Inflammatory mediators have also been elevated after diesel exhaust exposure, namely in bronchial lavage (Behndig et al., 2006; Stenfors et al., 2004) and biopsies (Salvi et al., 2000), but not in the sputum (Nightingale et al., 2000) or nasal lavage (Xu et al., 2013). Furthermore, bioactive lipids have been reported elevated in BAL after biodiesel exhaust (Gouveia-Figueira et al., 2017). Also, the combined action of allergens was investigated and diesel exhaust augmented the allergen-induced effects, the non-allergic inflammation and suppression of macrophage activity (Carlsten et al., 2016; Hosseini et al., 2016).

The majority of studies with exposure to *CAPs* reported no effects in the airways with no difference in sputum assessed in 5 studies, although two of them observed a decrease in the number of columnar epithelial cells in both young and elderly healthy subjects (Gong et al., 2005; Gong et al., 2003), probably representing an effect on larger central airways with no clear known mechanism, nevertheless not seen after UFP CAPs exposure (Gong et al., 2008). In BL and BAL, one study observed a 3 fold increase in neutrophils in BL and 5.6 fold in BAL (Ghio et al., 2000), while other two studies reported no changes in cell counts in BAL (Huang et al., 2012; Samet et al., 2009). IL6 levels were also unchanged in both induced sputum (Gong et al., 2003; Urch et al., 2010) and BAL (Huang et al., 2012; Samet et al., 2009), while IL8, only assessed in 2 studies, showed a modest elevation in BAL (Samet et al., 2009) and no change in sputum (Gong et al., 2003).

Assessment of airway effects after exposure to *indoor* sources was only performed in 2 studies after exposure to laser and 3D printing emissions, through measurements of FeNO and nasal secretions. FeNO did not change after laser printing exposure (Karrasch et al., 2017) but a small FeNO increase was reported after 3D printing with the use of the high UFP-emitting acrylonitrile butadiene styrene (Gumperlein et al., 2018). No changes in a battery of cytokines from nasal secretions assessed after exposure to both device types, although with a small increase detected in IL6 levels after the low level exposure of laser printer.

Small effects in *airway inflammation* were observed after all types of sources, but more consistently after diesel exhaust exposure, namely in terms of neutrophils increase in fluids from both upper and lower regions in the airway tree.

4.2. Lung Function

Lung function has mainly been assessed through conventional spirometry (assessing forced expiratory volume in 1 second, FEV_1, forced vital capacity, FVC, and peak expiratory flow, PEF), but also with more specialized body plethysmography used in 2 studies (Karrasch et al., 2017; Rudell et al., 1996), allowing the assessment of functional residual capacity besides the ventilated gas changes.

No lung function changes have been observed after 1.5 to 3 h of *wood smoke* exposure of relatively high mass concentrations (from 224 to 500 $\mu g/m^3$) (Ferguson et al., 2017; Ghio et al., 2012b; Muala et al., 2015; Sehlstedt et al., 2010). In addition, more susceptible atopic subject's spirometry was not affected (Riddervold et al., 2012).

After *diesel exhaust* exposure spirometry FEV_1 and FVC endpoints were unchanged, but 3 studies observed decreased pulmonary specific resistance and 2 studies measured FEV_1 decrements after subsequent exercise or ozone exposure (Giles et al., 2012; Madden et al., 2014). A study that assessed PEF during exposure measured a statistically significant decrease of 10 L/min registered at 75 min of exposure time, and although not significant at the end of the 3 h of exposure to 276 $\mu g/m^3$, a decreasing trend was observed (Xu et al., 2013). Furthermore, lung function decrements were observed in a *quasi*-controlled exposure study after 3-cumulative days of exposure inside diesel trains (Andersen et al., 2019) and after 5h exposure to ambient air near an airport (Lammers et al., 2020).

After *CAP* exposure, spirometry was generally unchanged. One study focusing on UFP CAPs reported a 2% decrease in FEV_1 after exposure (Gong et al., 2008). From *indoor* sources, one study reported a small decrease in lung function associated with particle-surface-area concentration from candle burning (Soppa et al., 2014). Interestingly, the researchers also reported an inverse association for both candle burning and frying sausages with particle size-specific-particle-mass, but not for number concentrations or specific surface area, suggesting that size-specific-particle-mass could be a better descriptor for the observed effects (Soppa et al., 2014).

Overall, *lung function* assessed after exposure of healthy subjects in CHES appears to be affected at a mild degree, with small effect sizes and with the majority of the spirometry results with no changes detected.

4.3. Systemic Inflammation

Systemic inflammation was assessed as blood levels of neutrophils, inflammatory mediators, acute phase reactants and cell adhesion markers. Levels of cytokines have been the most commonly assessed endpoint of systemic inflammation (24 studies reported cytokine levels). Some of these inflammatory mediators (as IL6 and TNF) that can be identified within hours after particle exposures, induce production of acute phase reactants in the liver. Among

the acute phase reactants, C-reactive protein (CRP), a biomarker clinically established to determine disease progression (Gabay and Kushner, 1999) detectable within 24 h after exposures, was assessed in 56% of the studies that investigated systemic inflammation. Neutrophils play an important role in inflammation, recruited from the blood to the inflammation site through cell adhesion markers (as ICAM1, VCAM1, P-selectin and E-selectin), which are found in soluble forms in the blood, and usually elevated in serum during inflammatory processes. Neutrophil counts and adhesion markers were assessed in 50% and 39% of the systemic inflammation assessment, respectively.

From *wood smoke* studies, increased level of serum amyloid A (SAA), also an acute phase protein produced as a response to inflammatory stimuli, was reported in a single study at different time-points following exposure (0, 3 and 20h post-exposure) (Barregard et al., 2006). However, the elevated SAA was not confirmed in another study from the same research group, using the same sampling times, but with lower exposure doses (Stockfelt et al., 2013). The mass and number concentrations of the exposures were similar in the studies, but with different exposure durations (4h and 3h, respectively), burning conditions and participants activity level (with moderate exercise and at rest, respectively) which might suggest a dose-dependency in short-time effects (Barregard et al., 2006; Stockfelt et al., 2013). Neither of these two studies reported changes in CRP levels. Another wood smoke study reported levels of IL1β, a pro-inflammatory cytokine, as increased in blood immediately after exposure (Ghio et al., 2012b). No effect on IL6 levels were reported after controlled wood smoke studies (Barregard et al., 2006; Bonlokke et al., 2014; Ghio et al., 2012b; Muala et al., 2015; Stockfelt et al., 2013). Nevertheless, in a real-life exposure condition, forest firefighters had increased blood levels of IL6 and IL8 after 4h of firefighting activity and cytokine levels were associated with lung inflammation (in sputum), but unchanged (for the same subjects) after strenuous physical work without wood smoke exposure (Swiston et al., 2008). The authors suggested that a systemic inflammation was triggered by the wood smoke, although the impact of the heat could not be ruled out. Ghio and colleagues measured a 17% increase in blood neutrophils at 20h post-exposure, an effect also observed at a smaller degree (and the same time point) in the airways (Ghio et al., 2012b). Adhesion molecules (P-selectin, E-selectin, ICAM1 and VCAM1) were unaltered after exposure (Bonlokke et al., 2014; Forchhammer et al., 2012; Muala et al., 2015; Stockfelt et al., 2013).

Peripheral blood levels of neutrophils markedly increased after 1h exposure to 300 $\mu g/m^3$ of *diesel exhaust* particles (Salvi et al., 1999). The same study reported increased levels of airway neutrophils and upregulation of adhesion molecules, suggesting that diesel exhaust triggered a release of neutrophils from the bone marrow and their translocation from blood to the airway tissues (Salvi et al., 1999). An increase of neutrophils was also observed after 2h exposure to 200 $\mu g/m^3$ diesel exhaust particles (Tong et al., 2014) but with no reported effect in systemic levels of neutrophils in 8 other diesel exhaust studies with 200-350 $\mu g/m^3$ particle mass concentrations for 21min-3h exposure (Barath et al., 2010; Krishnan et al., 2013; Lucking et al., 2011; Lucking et al., 2008; Mills et al., 2011a; Mills et al., 2005; Vieira et al., 2016; Xu et al., 2013). CRP was also unaffected after diesel exhaust exposure at all the assessed time points (0, 2, 4, 6, 8, 18, 20 and 24h post-exposure) (Barath et al., 2010; Carlsten et al., 2007; Lucking et al., 2011; Lucking et al., 2008; Mills et al., 2005; Tong et al., 2014; Tousoulis et al., 2020; Vieira et al., 2016; Xu et al., 2013). Törnqvist and colleagues reported increased blood concentrations of IL6, TNF and P-selectin after 24h of diesel exhaust exposure (Tornqvist et al., 2007), but the effect was not observed in an earlier time point of the same study (Mills et

al., 2005). Moreover, other studies that investigated IL6 and TNF at similar time endpoints (20-24h) found no changes in levels of pro-inflammatory mediators (Cliff et al., 2016; Nightingale et al., 2000; Stiegel et al., 2016; Xu et al., 2013), neither at earlier time points (0, 2, 4, 6 or 8h post-exposure) (Barath et al., 2010; Cliff et al., 2016; Lucking et al., 2011; Lucking et al., 2008; Nightingale et al., 2000). ICAM1 levels in blood were also unchanged through different time points (Barath et al., 2010; Krishnan et al., 2013; Lucking et al., 2011; Lucking et al., 2008; Tornqvist et al., 2007).

After *CAP* exposure, two studies reported small changes in blood neutrophils (Behbod et al., 2013; Brook et al., 2009), whereas two other studies reported no changes (Gong et al., 2004; Huang et al., 2012). The findings are not coherent for the time points assessed, with the positive results observed immediately after exposure but not after 24h (Brook et al., 2009) or at 24h post-exposure but not before (Behbod et al., 2013). There is not a consistent explanation by exposure dose (i.e., metric that depends on the concentration, duration and ventilation rate), as the positive findings were observed with 2h exposure at rest of 150 or 235 $\mu g/m^3$ of CAP particles and the non-observed effects after 2h exposure, with moderate exercise and slightly lower concentrations of 98 and 200 $\mu g/m^3$. Nevertheless, locations were different, and different locations might have different particle composition. Levels of cytokines and acute phase proteins were collectively unchanged after CAP exposure at different time points, and for different doses, except in a UFP CAP study from Chapel Hill that showed increased levels of CRP and SAA (at 20h post-exposure but not earlier at 1h) (Devlin et al., 2014). Other two studies on UFP CAPs that did not detect changes on CRP levels used similar exposure doses (1.2 and $1.5x10^5$ versus $1.9x10^5$ particles/cm^3, all three using 2 hours of exposure). However, the sampled population was different in age and condition, with the change in CRP and SAA observed in a sample of 34 middle-aged individuals with metabolic syndrome, a cluster of risk factors for cardiovascular disease and associated low-grade systemic inflammation (Devlin et al., 2014).

From *indoor* sources, only one study assessed markers of systemic inflammation (a battery of cytokines) with no observed changes after exposure to printer laser emissions (Karrasch et al., 2017).

CRP is a widely used and well established clinical inflammation marker that in CHES is not consistently affected. Nevertheless, occupational populations exposed to welding, and epidemiological studies on children's levels showed consistent associations with PM levels (Li et al., 2012) or long-term air pollution with focus on ultrafine particles (Pilz et al., 2018), The CRP response is likely to be affected by susceptibility, dose, PM source and characteristics, and timeframe of effect (Li et al., 2012; Pilz et al., 2018).

Overall, the inflammatory effects in chamber studies seem to be mainly observed for markers of airway inflammation, with eventually later and less consistent results on a *systemic inflammation* level.

4.4. Oxidative Stress

Oxidative stress biomarkers have been assessed in urine, blood and airway samples, namely through lipid peroxidation products (8-isoprostane and malondialdehyde, MDA),

reactive oxygen species producing enzymes (myeloperoxidase, MPO), serum antioxidant capacity and oxidative stress response genes (heme oxygenase 1, *HMOX1*).

After *wood smoke* exposure, 2 studies reported increased levels of lipid peroxidation by assessment of 8-isoprostane in exhaled breath and urine (Barregard et al., 2006; Ferguson et al., 2016) but it was also reported as unchanged in exhaled breath (Murgia et al., 2016; Riddervold et al., 2012) and decreased in urine (Stockfelt et al., 2013). The study from Ferguson et al. observed effects in exhaled breath 1h post-exposure, while Riddervold et al. assessed the effect at later time points (3.5 and 6h post-exposure), which may suggest an earlier onset of response detectable in exhaled breath. In one study with endpoints assessed in different samples and times, the authors reported effects in urine (20h post) but not in exhaled breath across different time points (0, 3 or 20h post) (Barregard et al., 2006; Murgia et al., 2016). A slight increase in exhaled breath levels of 8-isoprostane was observed, but it was not statistically significant after multi-analysis correction and there was no correlation between exhaled breath and urine levels of 8-isoprostane. However MDA, another lipid peroxidation biomarker, was significantly increased in exhaled breath in the same study (Barregard et al., 2006). Moreover, other real-life exposure studies have observed elevated levels of 8-isoprostane following wood smoke exposures, collectively suggesting increased lipid peroxidation by inhalation of smoke from wildland fires among firefighters (Gaughan et al., 2014) and chronic exposures from wood smoke emissions from cook stoves (Commodore et al., 2013). Therefore, the decrease in the urinary excretion of isoprostanes observed by Stockfelt and colleagues is surprising. Nevertheless the authors noted that there was an increase in the levels of isoprostanes assessed after filtered air, which might explain this unexpected observation, affected by the control results (Stockfelt et al., 2013). *HMOX1* gene expression was assessed in 3 studies in blood and biopsies with unchanged levels (Forchhammer et al., 2012; Sehlstedt et al., 2010) and increased levels were observed after exposure in one study (Danielsen et al., 2008). MPO, a cellular source of reactive oxygen species, was also unchanged in BL, BAL (Muala et al., 2015; Sehlstedt et al., 2010), and exhaled breath (Ferguson et al., 2016). Antioxidants examined in BAL after wood smoke exposure showed an increase in glutathione but no increase on levels of the oxidised glutathione (glutathione disulfide produced by antioxidant enzymes during the reduction of peroxides) suggesting the absence of oxidative stress measured by this marker (Muala et al., 2015; Sehlstedt et al., 2010).

Only one study has assessed lipid peroxidation (MDA) following *diesel exhaust* exposure and without detecting changes (Blomberg et al., 1998). Levels of the neutrophil degranulation product MPO were increased in BL but not in BAL (Behndig et al., 2006) and with a borderline increase in blood (Krishnan et al., 2013), whereas another study did not detect any changes in BL and BAL (Stenfors et al., 2004). The 2 studies used the same mass concentrations (100 $\mu g/m^3$) and duration of exposure investigating BL and BAL after diesel exhaust, but sampling times were different (Behndig et al., 2006; Stenfors et al., 2004). With bronchoscopy samples at 18h post-exposure the authors observed an upregulation of endogenous antioxidant defences (glutathione and urate) in the alveolar lavage and MPO in BL without an onset of inflammation triggered in the alveolar region, suggesting a compartmentalised response with a protective effect from the antioxidant defences, apparently effective for the low-dose exposure (Behndig et al., 2006). Another study did not detect any changes in proximal and distal airway antioxidant defences at 6h post-exposure (Blomberg et al., 1998). Following inhalation of diesel exhaust, one study identified increased gene expression of oxidative stress pathways in blood cells (Pettit et al., 2012), although *HMOX1* expression in blood cells was unaltered in one study

(Hemmingsen et al., 2015a). Nevertheless, another study reported that glutathione S-transferases (GST) genotype modulate responses in lower airway inflammation and lung function of atopic subjects (Carlsten et al., 2016; Zhang et al., 2016). The GST family of enzymes have antioxidant and detoxifying roles in the respiratory tract. Among a group of atopic subjects exposed to diesel exhaust with a following co-exposure to an allergen, the authors observed that GSTT1 genotype enhanced the inflammatory response (Carlsten et al., 2016) and that GSTT1 null individuals presented a higher reduction in lung function (FEV1) (Zhang et al., 2016).

Lipid peroxidation (MDA) was assessed in blood and urine after 2 controlled exposure studies of fine and ultrafine *CAPs* (Liu et al., 2015). No changes were detected in blood, and urinary excretion of MDA was detected after fine but not ultrafine CAPs (Liu et al., 2015). One study investigated an emerging possible maker associated with lipid peroxidation, the high-density lipoprotein cholesterol (HDL) antioxidant/anti-inflammatory function (Li et al., 2013), that has been observed to be associated with coronary intima-media thickness in humans (Khera et al., 2011). The exposure to CAPs elicited effects on HDL functionality (Ramanathan et al., 2016).

Two studies on *indoor* printing emission sources assessed oxidative stress through hydrogen peroxide in exhaled breath (Karrasch et al., 2017) and urinary excretion of 8-isoprostaglandin (Gumperlein et al., 2018) without finding associations to the exposure.

There is a lack of assessment of the same *oxidative stress* markers for the different exposure sources and sampling times, but overall the results may indicate a small effect on lipid peroxidation and gene expression of oxidative stress markers.

4.5. Genotoxicity

Genotoxicity has been assessed through direct DNA damage in peripheral blood mononuclear cells (in 3 studies reporting strand breaks and oxidative damage), through markers of DNA repair mechanisms measuring the excretion of oxidized nucleobases (also in 3 studies), gene expression of repair enzyme (*hOGG1*) (assessed in 2 studies) and through DNA hypomethylation, an epigenetic alteration assessed in 1 study (Bellavia et al., 2013).

After wood *smoke exposure*, no effect in the levels of oxidatively damaged DNA in blood cells was observed, investigated in 2 studies using the modified comet assay with a repair enzyme treatment (Danielsen et al., 2008; Forchhammer et al., 2012), although one of the studies reported an increased mRNA expression of a repair enzyme (*hOGG1*) and associated with a larger excretion of the repair product (8-oxoGua) and decrease in strand breaks, suggesting an enhanced repair activity (Danielsen et al., 2008). Nevertheless, a *quasi*-controlled study where 11 young subjects were exposed to wood smoke cumulatively over 1 week did not show changes in levels of DNA strand breaks and oxidative damage to DNA (Jensen et al., 2014).

Only one of the selected studies on *diesel exhaust* investigated oxidative damage to DNA and found unaltered levels of DNA strand breaks, oxidative damage and expression of DNA repair *hOGG1* (Hemmingsen et al., 2015a).

From *CAP* and *indoor* sources exposure, the oxidized nucleobase 8-oxodG levels were studied in urine (Liu et al., 2015) and serum (Karrasch et al., 2017). Although 8-oxodG has not

been proven to arise from repair of DNA, it was found to correlate with the excretion of the direct product of the base excision repair, 8-oxoGua (Loft et al., 2012). However, an additional concern from these studies relates with the assay used in the quantification of the biomarker, ELISA method, which is not optimal for accurate quantification due to unspecific detection of the oxidized nucleobase (Cooke et al., 2008), being a warning for a cautious interpretation of the reported increase of 8-oxodG in urine after UFP CAP exposure (Liu et al., 2015) and unaffected in serum after UFP laser printer emissions (Karrasch et al., 2017).

In contrast to the lack of clear associations of particle exposure and DNA oxidation in the few chamber studies that assessed this biomarker, increased levels of oxidative damage to DNA, measured by Fpg-sensitive sites, have been observed in human air pollution studies (Avogbe et al., 2005; Brauner et al., 2007; Novotna et al., 2007; Vinzents et al., 2005) or unaltered but with significantly increased levels of 8-oxodG (Sorensen et al., 2005; Sorensen et al., 2003). Besides, air pollution was also found not to affect Fpg-sensitive sites in a study of bus drivers using other air pollutant surrogates (Bagryantseva et al., 2010). The differences among study designs and exposure assessment may influence the observations. Positive associations were observed in crossover design studies with 24h exposure duration with study subjects performing exercise in air with or without traffic-related PM dominated by UFP (Brauner et al., 2007; Vinzents et al., 2005) or cross-sectional studies where sample populations were highly exposed to UFP (Avogbe et al., 2005; Novotna et al., 2007). Collectively, contrasting with the findings from CHES, the observed effects in air pollution studies may suggest an effect on oxidative damage to DNA from longer exposure duration, co-pollutants and possibly PM source.

Another biomarker of genotoxicity assessed after CAP exposure is DNA methylation. DNA methylation is a natural epigenetic mechanism where the addition of methyl groups to the DNA suppresses gene expressions, switching them off. PM exposure was suggested, from observational studies, to cause DNA hypomethylation in blood cells (Baccarelli et al., 2009; Madrigano et al., 2011) which can potentially affect expression of inflammatory genes (Madrigano et al., 2011; Tarantini et al., 2009). Moreover, blood hypomethylation was associated to cardiovascular diseases (Baccarelli et al., 2010; Castro et al., 2003). After 2h of CAP exposure Bellavia and colleagues observed DNA hypomethylation.

Very few CHES investigated *genotoxicity*, not allowing a general analysis, although suggesting effects in terms of increased repair activity and hypomethylation.

4.6. Thrombogenicity

Thrombogenicity was measured through blood markers of hypercoagulability, decreased fibrinolysis and changes in coagulation factors or through ex-vivo thrombus formation capacity.

Four studies assessed thrombogenicity markers after *wood smoke* exposure. Increased coagulation factors (Factor VIII, FVII and von Willebrand factor, vWf) were observed in 2 studies (Barregard et al., 2006; Stockfelt et al., 2013) although Stockfelt et al. observed some ambiguous results with time-dependency for FVII (increased at 4 and 47h post-exposure but not in between) and decreased fibrinogen and platelets (Stockfelt et al., 2013). Another study investigating the effects of wood smoke in a sample of atopic subjects reported unchanged

levels of vWf (Bonlokke et al., 2014). Also ex-vivo thrombus formation did not show an effect after wood smoke exposure (Hunter et al., 2014).

After *diesel exhaust* exposure, levels of platelet aggregates showed mixed results, with 6 studies not observing significant changes in different time points (0, 2, 3, 6, 8, 22 and 24 h post-exposure) (Carlsten et al., 2007; Langrish et al., 2013; Lucking et al., 2011; Lucking et al., 2008; Mills et al., 2011b; Mills et al., 2005), whereas 3 studies reported increased levels at 6, 18 and 20h post-exposure (Krishnan et al., 2013; Salvi et al., 1999; Tong et al., 2014). Ex-vivo thrombus formation was increased in samples from 2 studies (from 2 and 6h post-exposure) (Lucking et al., 2011; Lucking et al., 2008). Additionally, Carlsten and colleagues observed unchanged levels of the coagulation factor vWf and a decrease in plasminogen activator inhibitor 1 (PAI1) at 20h post-exposure, although not statistical significant (Carlsten et al., 2007). Pettit et al., following diesel exhaust inhalation identified in blood cells increased gene expression of coagulation pathways (Pettit et al., 2012).

Eight studies assessed thrombogenicity after *CAP* exposure, and all measured unaltered plasma levels of coagulation factors (Devlin et al., 2014; Ghio et al., 2003; Gong et al., 2008; Gong et al., 2003; Gong et al., 2004; Huang et al., 2012; Samet et al., 2009; Tong et al., 2015). Tissue plasminogen activator (tPA) and PAI1 have also been measured as unaltered after CAP exposure (Devlin et al., 2014; Gong et al., 2008; Tong et al., 2015). Likewise, there were no changes in platelet counts (Gong et al., 2003; Gong et al., 2004). However, after exposure of subjects with metabolic syndrome to UFP CAPs, Devlin and colleagues observed decreased levels of 2 proteins involved in the fibrinolytic pathway: plasminogen and thrombomodulin. Interestingly, the association was observed using particle number concentration as metrics and not for mass (Devlin et al., 2014).

CHES on *indoor* sources did not assess thrombogenicity endpoints.

Collectively, CHES provide very limited evidence of *thrombogenicity* effects, with potential differences for different particle sources.

4.7. Vascular Function

Functional markers of vascular vulnerability include blood pressure, endothelial dysfunction, arterial stiffness, arterial pressure and blood levels of a vasoconstrictor substance secreted by vascular cells (endothelin 1). The vascular function was one of the markers more commonly investigated in the selected studies, namely by endothelial dysfunction, an early predictor of cardiovascular disease (Sandoo et al., 2010). The endothelium has an impaired response when it has reduced production of mediators to induce relaxation of the smooth muscle cells (endothelium-dependent response). However, the vascular smooth muscle may also have a reduced response to vasodilation mediators (endothelium independent response). Different techniques to evaluate endothelium function have been used, based on the dilation response to a stimulus, noting that different methods may assess it in different vascular beds (Sandoo et al., 2010). The gold-standard method to assess endothelial function is considered to be the venous occlusion plethysmography with infusion of vasodilators and effect estimation through forearm blood flow (FBF) (Lekakis et al., 2011). It is an invasive technique that measures the changes in the microvasculature, requiring cannulation and allowing a thorough study of the endothelial function. Another technique used in chamber studies is flow-mediated

dilation (FMD), a non-invasive method that assesses the reactive hyperemia response to increased blood flow conduit vessels by released pressure in a blood cuff, with the use of an ultrasound detector. Another technique used is peripheral artery tonometry (PAT) with fingertip sensors to analyse pulse wave changes after the hyperemia response to deflation of a blood cuff on the upper arm. Pulse wave analysis was also used to measure central arterial pressure waveform, arterial stiffness and pulse wave velocity.

Vascular function measured through FBF or EndoPAT (Itamar Medical Ltd., Israel) was unaffected after *wood smoke* exposures (Forchhammer et al., 2012; Hunter et al., 2014; Pope et al., 2011). Hunter and colleagues studied a population of male firefighters and used a high exposure concentration (1h of 1000 $\mu g/m^3$ PM_1), with no changes detected in blood flow at baseline neither after infusions of endothelium dependent and independent vasodilators. Similarly, no changes were detected in reactive hyperemia index measured by means of the non-invasive technique of EndoPAT in atopic subjects (3h, up to 385 $\mu g/m^3$ $PM_{2.5}$) (Forchhammer et al., 2012), or in a sample of young healthy subjects after 3h of 180 $\mu g/m^3$ $PM_{2.5}$ (Pope et al., 2011). Interestingly, the study from Pope et al. showed a decline in vascular function associated with 2-days previous ambient exposures, suggesting an effect from cumulative exposure. Blood pressure was investigated in 3 CHES after wood smoke exposure with no changes reported in 2 studies assessed at 6 and 24h post-exposure by Hunter and colleagues and 1h post-exposure by Unosson et al. (Hunter et al., 2014; Unosson et al., 2013). However, an increased systolic pressure was observed with some consistency at 24h post (and not earlier) exposure of wood smoke generated by different stove types, investigated in a sample of 48 healthy and young subjects (Fedak et al., 2019). Moreover, Unosson and colleagues reported an increased arterial stiffness immediately after exposure assessed through tonometry and measured as augmentation index, arterial pressure and pulse wave velocity (Unosson et al., 2013). Nonetheless, no change in arterial stiffness was observed by Hunter and colleagues in a study from the same lab in Umeå (Sweden), using healthy firefighters as study subjects, and also assessed in the same time frames after the exposure challenge (Hunter et al., 2014). Physical activity has been suggested to be a potential effect modifier of the association of air pollution and arterial stiffness, observed in a Swiss cohort of older adults (Endes et al., 2017) and in a panel of young males from China (Chen et al., 2018). Assuming that the young firefighters are a physically more active population, it might be suggested as an explanation for the inconsistent findings.

A study from Umeå, using pulse wave analysis methodology, measured an arterial stiffness increase immediately after *diesel exhaust* exposure with a transient effect recovered within 30 minutes after the exposure, recovery timing that was also observed by Unosson et al. after wood smoke (Lundback et al., 2009). Endothelial dysfunction, assessed by the gold-standard method of venous occlusion plethysmography with infusion of vasodilators was consistently reported after diesel exhaust exposure (Barath et al., 2010; Langrish et al., 2009a; Lucking et al., 2011; Mills et al., 2011b; Mills et al., 2005; Tornqvist et al., 2007), and particularly observed with infusion of endothelium-dependent vasodilators. However, reactive hyperemia measured by FMD or by the EndoPAT showed collectively no effect of diesel exhaust exposure (Barath et al., 2010; Giles et al., 2018; Langrish et al., 2009a; Lucking et al., 2011; Mills et al., 2011b; Mills et al., 2005; Peretz et al., 2008b; Sack et al., 2016; Tong et al., 2014; Tornqvist et al., 2007; Vieira et al., 2016), although with one study reporting decreased FMD and increased augmentation index up to 24h post-exposure (Tousoulis et al., 2020). The venous occlusion plethysmography with infusion of vasodilators is considered to be a more sensitive method

besides the fact that the assessment is made in the microvasculature, which should be taken in consideration when comparing different techniques, as the endothelial cells have morphological and physiological variations along the vascular tree (arteries, veins and capillaries) (Ghitescu and Robert, 2002). The blood pressure was assessed in 9 studies of diesel exhaust exposure, with 6 studies reporting no effects (Barath et al., 2010; Giles et al., 2018; Langrish et al., 2009a; Lucking et al., 2011; Lundback et al., 2009; Tornqvist et al., 2007), increases reported for systolic pressure in 2 studies (Cosselman et al., 2012; Mills et al., 2011b) and 1 study with increased diastolic pressure (but not systolic)(Tong et al., 2014). The blood pressure assessments were performed with different time endpoints (0, 0.5, 1, 2, 6 and 24h post) without consistent trends in the results. Another biomarker without consistent findings after diesel exhaust exposure was the plasma levels of endothelin-1, increased at 3h post-exposure (Peretz et al., 2008b) and unaltered at 6 and 24h post-exposure (Langrish et al., 2009a).

The vascular function after *CAP* exposure was assessed only by FMD, with unaltered results reported in 3 studies (Brook et al., 2002; Brook et al., 2009; Devlin et al., 2014) and decreased in 2 studies (Brook et al., 2009; Tong et al., 2015). Brook et al., who performed 2 studies in different locations (Toronto and Ann Arbor) with the same protocol, timings, matched doses (2h, 150 $\mu g/m^3$ and the same physical activity level) and even the same mean age of participants, did not find a consistent response in FMD, suggesting the differences to be related with the particle composition from the different sites (Brook et al., 2009). Blood pressure was measured in 5 CAP studies, with changes observed mainly for diastolic blood pressure (Brook et al., 2009; Urch et al., 2005), with one study also reporting an increase in systolic blood pressure (Zhong et al., 2015) and 1 study with no changes in blood pressure (Devlin et al., 2014). Moreover, the increase in blood pressure was associated with endotoxin and beta-glucan constituents of the PM (Zhong et al., 2015). The levels of endothelin-1 were also not consistent, with no change in plasma at 0 and 24h post-exposure to CAPs from Toronto (Brook et al., 2009) and increased levels at 20h post-exposure to CAPs from Chapel Hill (Tong et al., 2015).

From *indoor* sources studies, one assessed vascular functional markers finding increased blood pressure associated with particle mass concentration, particle surface area concentration and particle number concentration from toasting bread, but no consistent changes were observed after exposure to UFP from candle burning or frying sausages (Soppa et al., 2017). The same exposure study also showed increased arterial pressure and augmentation index, indicating increased immediate and mostly transient effect on arterial stiffness (Soppa et al., 2019).

Overall, *vascular function* effects were observed after all sources of exposure in CHES: arterial stiffness seems to increase transiently for all types of exposures. The endothelial function was consistently impaired after diesel exhaust, less consistent after CAP exposure, unchanged after wood smoke, and not assessed after indoor sources. Nevertheless, differences in the assessment method might explain these different results.

4.8. Heart Rate Variability

Heart rate variability (HRV) was generally assessed from electrocardiogram analysis, with 2 studies using other frequency counter monitors. Among more than 70 possible HRV variables

(Bravi et al., 2011), CHES generally reported 6 variables on time (standard deviation of normal-to-normal intervals, SDNN; root mean square of successive differences in normal-to-normal intervals, RMSSD and proportion of successive normal-to-normal intervals differing per more than 50 ms, pNN50) and frequency domains (low frequency, LF; high frequency, HF and the power ratio of both).

After *wood smoke* exposure, HRV had mixed results with unchanged time and frequency domains in 2 studies (Bonlokke et al., 2014; Ghio et al., 2012b) and one study with decreased time-domain variables (SDNN, RMSSD and pNN50) and decreased HF (Unosson et al., 2013), with Ghio et al. also reporting a marginally increased HF immediately after particle exposure (Ghio et al., 2012b). However, the wood smoke sources were different, as well as exposure doses, study designs, and data collection methods. The changes in HRV were observed in studies with collection of raw electrocardiograms, which might have an advantage in data accuracy and additionally those studies used different duration of recording periods (Ghio et al., 2012b; Unosson et al., 2013).

Following *diesel exhaust*, 5 studies assessed HRV, also with mixed results. Mills et al. and Vieira et al. reported unaltered HRV in time and frequency domains while other 3 studies observed changes in time domain variables (Tousoulis et al., 2020) and changed frequency domain components, in terms of increased HF and decreased LF/HF (Peretz et al., 2008a), and decreased very low frequency component and total power (Tong et al., 2014). Mills et al. exposed subjects (mean age 26 years old) to 1h of 300 $\mu g/m^3$ with unaltered time and frequency variables of HRV. Tousoulis et al. observed a decrease in SDNN at 2 and 24h post-exposure to 2h of 25 $\mu g/m^3$ diesel exhaust particles using an older sample population (mean age 41 years old)(Tousoulis et al., 2020). Peretz et al. and Tong et al. had a gradient concentration on different sessions and reported changes in HRV only for the higher concentrations used (more than 200 $\mu g/m^3$, for 2h), and for sample populations with means of 32 and 58 years. Vieira et al. also used a sample population with an older mean age (45 years) but with shorter exposure time (21min of 325 $\mu g/m^3$) and collecting the data in a more basic signal (counter watch) than electrocardiogram (Vieira et al., 2016). Collectively, the diesel exhaust effects on HRV may suggest a threshold dose with observed effects in an older population.

Nine studies assessed HRV after *CAP* exposure, with changes observed in both healthy older (Devlin et al., 2014; Gong et al., 2004; Tong et al., 2012) and younger subjects (Gong et al., 2008; Gong et al., 2003; Samet et al., 2009; Zhong et al., 2017). The exposure doses varied between 2h of 50-100 $\mu g/m^3$ UFP CAPs (10^5 particles/cm^3) (Devlin et al., 2014; Gong et al., 2008; Samet et al., 2009) and 2h of 174-278 $\mu g/m^3$ $PM_{2.5}$ CAP (Gong et al., 2003; Gong et al., 2014; Tong et al., 2012; Zhong et al., 2017). One study only detected changes with ozone co-exposure and a CAP exposure dose of 2h on 127 $\mu g/m^3$ $PM_{2.5}$ (Fakhri et al., 2009) and one study did not find changes in time domain variables after exposure of UFP CAP for 2h with 159 $\mu g/m^3$ (Breitner et al., 2019). Moreover, from an air pollution study that investigated healthy subjects walking in a polluted street with or without wearing a face mask, a significant decrease in time domain HRV was observed (Langrish et al., 2009b). A more susceptible population (overweight and older subjects) had decreased HRV after exposure to street air (Hemmingsen et al., 2015b). Furthermore, a meta-analysis from air pollution epidemiology studies involving 18 667 participants reported an association between particulate air pollution and decreased HRV (Pieters et al., 2012).

From *indoor* sources, only one study assessed HRV with changes in frequency domain by increased HF power and tends to decreased LF/HF after candle burning particles exposure, and

unaltered HF with a no significant shift of LF/HF to a less parasympathetic tone during exposure to terpene-ozone particles (Hagerman et al., 2014).

Heart rate variability was consistently affected after CAP exposure and with mixed responses after diesel exhaust and wood smoke.

4.9. Arrhythmia

Arrhythmia in CHES has been assessed by electrocardiogram analysis in terms of the heart T-wave complexity, namely through QT interval, with some studies reporting the interval between the peak and the end of the T wave (Tp-Te) and Tp-Te/QT ratio, markers of ventricular arrhythmogenesis (de Luna and Baranchuk, 2017). Other markers included ectopic beats, tachycardia and bradycardia (Langrish et al., 2014).

From an analysis of the electrocardiograms from 4 of the selected studies, Langrish et al. did not find effects after *wood smoke* and *diesel exhaust* exposures (Langrish et al., 2014). Moreover, the analysis also included electrocardiogram analysis from other exposure sources, namely engineered carbon nanoparticles, ozone and ambient air pollution, as well as included studies on patients with coronary diseases.

After *CAP* exposure, and also associated with changes observed in HRV, 3 studies reported effects on the prolongation of QT interval after exposure, which may indicate the likelihood of increased arrhythmia vulnerability (Devlin et al., 2014; Samet et al., 2009; Tong et al., 2012) and one study reported no consistent associations between particle metrics and T wave complexity (Breitner et al., 2019). In addition, a study on coronary heart disease patients did not find differences in incidence of arrhythmias or the number of arrhythmias after CAP exposure (Langrish et al., 2014).

Arrhythmia was not assessed after CHES on *indoor sources.*

Arrhythmia was not observed after wood smoke and diesel studies but it was reported consistently in 3 out of 4 studies after CAP exposure.

4.10. Neurotoxicity

Neurotoxicity has been the endpoint with least focus on the selected CHES, assessed only in 4 studies. It was assessed through quantitative electroencephalography during and post-exposure, postural stability, plasma levels of sympathetic markers and markers of central nervous system damage in blood and urine.

Three studies assessed neurotoxicity endpoints after *diesel exhaust* exposure. Crüts et al. observed an increased median power frequency in electroencephalogram during the 1h exposure to 300 μg/m^3 PM, which further increased in the period post-exposure, demonstrating functional changes in brain activity resulting from diesel exhaust exposure, but the authors noted that the effect may have other mediators than UFP (Cruts et al., 2008). In another study, Peretz et al. observed increased plasma levels of sympathetic markers 3h post-exposure to the higher concentration used (2h of 200 μg/m^3) (Peretz et al., 2008b). And another study did not

find changes in circulating markers of neurotoxicity after 2h exposure of 290 µg/m^3 (Cliff et al., 2016). Cliff et al. measured 3 serum markers related to traumatic brain injury or degeneration (S100b, a calcium protein and NSE, neuron-specific enolase, a glycolytic enzyme) and oxidative stress (BDNF, brain-derived neurotrophic factor), without detecting changes after the exposure (0, 3 and 24 h post) (Cliff et al., 2016). From the same study, postural stability was also not associated to the exposure (Curran et al., 2018).

Liu and colleagues reported unaltered effects on neural biomarkers in blood (i.e., S100b, NSE, BDNF), blood and urine levels of cortisol, and urinary metabolites of neural hormones after *CAP* exposure (Liu et al., 2017).

Very few studies assessed *neurotoxicity* endpoints, and with different markers assessed, not allowing an analysis, although suggesting a general response that lacks definition of involved pathways.

CONCLUSION

Overall, CHES have been used to investigate a large number of hypotheses, mechanisms of action and endpoints, using an enormous number of different markers and a diversity of methods making it difficult to compare. In a schematic graph with the plausible mechanisms of UFP toxicity, we have highlighted the pathways where CHES give some degree of consistency in the assessed effects (Figure 3).

Briefly, after inhalation, the PM deposits in the respiratory tract with a size, hygroscopicity and ventilation rate dependency (Londahl et al., 2007). The UFP deposition is known to occur in the nasopharyngeal, tracheobronchial and in the alveolar region by Brownian diffusion (ICRP, 1994). Very small particles (UFP<10 nm) efficiently deposit in the nasal cavity, and may enter the brain by nose-to-brain transportation (Ali et al., 2010). Small amounts may be able to translocate across the alveolar epithelium and reach the circulation (Kermanizadeh et al., 2015), or through clearance through the lymph nodes followed by translocation to the blood stream (Stone et al., 2017). Local and systemic inflammation and oxidative stress are thought to be the mechanisms driving particle toxicity, triggering events that will affect the vascular thrombogenicity, vascular function, HRV, as well as genotoxicity and alternative neural pathways (Knol et al., 2009; Stone et al., 2017). It is also plausible that an activation of alveolar neural receptors affects the respiratory reflexes (Stone et al., 2017). Besides the DNA damage by oxidative stress, UFP adsorbed substances may directly damage the DNA (by covalent binding, by the induction of reactive oxygen species production or by exacerbation of the local inflammation with production of reactive oxygen species).

The vast majority of the CHES included particles outside the ultrafine range as well as gases. Thus, it is not possible with certainty to ascribe the observed effects to UFP.

Diesel exhaust CHES show some consistent effects on airway inflammation and vascular dysfunction. CAP studies support effects in vascular dysfunction, HRV, arrhythmia and oxidative stress. Wood smoke and indoor sources have been less studied and fewer studies and less focus on some of the endpoints limits the assessment. Even though the consistency of the observed effects may differ with the UFP source, generally, there seems to be a trend of

exposure-response across different sources for the consistent results observed individually per source, suggesting that UFP might have a role in triggering short-time effects.

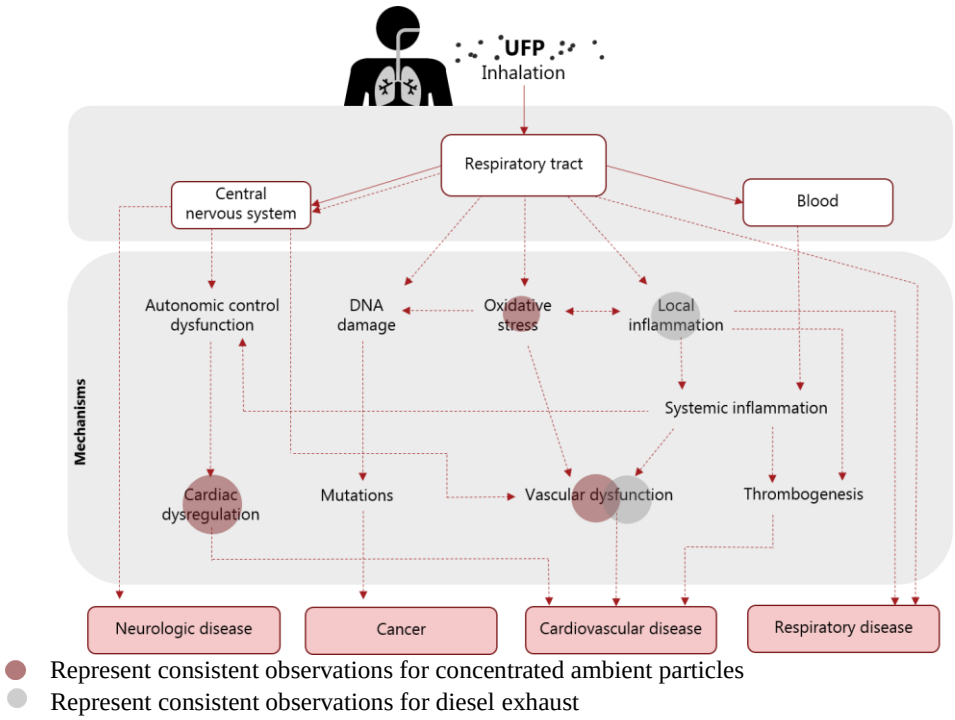

Figure 3. Summary of the more consistent effects observed in controlled human exposure studies over a graphic representation of plausible ultrafine particle (UFP) mechanisms for toxicity and health effects. The solid lines represent the passage of particles and the dashed lines represent biological mediators. The size of the circles represent the degree of number of consistent studies (for more than 75% consistent results and involving more than 50 human subjects in the assessment). Adapted from (Andersen, 2019) and inspired by (Brook et al., 2010; Chen et al., 2016; Stone et al., 2017).

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APPENDIX: STUDY-SPECIFIC DETAILS

The following Tables 6-10 present the selected studies-specific details, organized by biological endpoints assessed, grouped under a general criteria of assessment (endpoints usually assessed in the same studies), in order to simplify the tables reducing repetition of studies entrance.

Table 6. Lung function and airway effects in healthy humans after controlled exposures

Exposure	Effect	Study and subjects	Reference
WS: 4h (with exercise), 240-280 µg/m^3, 9.5x10^4-18x10^4 particles/cm^3	Increased serum CC16 (20 h post); Increased $FeNO_{270}$ (3 h post) but not at 0 or 24h (neither for $FeNO_{50}$)	Sequential. N=13, mean age 34, MF	(Barregard et al., 2008) Borås, Sweden
WS: 3h (with exercise), 224 µg/m^3 ($PM_{2.5}$), 6.7x10^4 particles/cm^3 (UFP)	Unaltered FEV1 and FVC (0, 4 and 24 h post); Unchanged cell counts in BL and BAL (24h post); unaltered $FeNO_{10,\ 50,\ 100\ an\ 270}$ (0, 4 and 24h post)	Crossover. N=19, mean age 24, MF	(Sehlstedt et al., 2010) Umeå, Sweden
WS: 3h, *LOW:* 222 µg/m^3 ($PM_{2.5}$), 13.3x10^3 UFP; *HIGH:* 385 µg/m^3, 33.9x10^3 UFP	Unaltered FEV1, FVC, PEF (0 and 6 h post); unaltered serum CC16 (0, 6 and 24h post) and urine CC16 (24h post); unaltered FeNO; unaltered cytokines in nasal lavage (3h and 6h post)	Crossover. N=20 (atopic), mean age 25, MF	(Riddervold et al., 2012) (Bonlokke et al., 2014) Århus, Denmark
WS: 3h, *START-UP:* 295 µg/m^3 ($PM_{2.5}$), 1.4 x10^5 particles/cm^3; *BURN-OUT*: 146 µg/m^3 ($PM_{2.5}$), 1.0x10^5 particles/cm^3	Increased serum CC16 after start-up fire (4h post); Increased $FeNO_{50}$ after burn-out fire (20 h post); increased $FeNO_{270}$ (for both fire-types, 20h post, and after 3h for start-up and 0h for burnout)	Sequential. N=13, mean age 34, MF	(Stockfelt et al., 2012) Borås, Sweden
WS: 2h (with exercise), 485 µg/m^3 ($PM_{2.5}$), 4.6x10^4 particles/cm^3	Unaltered FEV1 and FVC (0 and 20h post); increased neutrophils in BL and BAL (20h post)	Sequential. N=10, age range 18-40	(Ghio et al., 2012b) Chapel Hill, US-NC
WS: 3h (with exercise), 314 µg/m^3 (PM_1), 1-2.5x10^5 particles/cm^3 (UFP)	Unaltered FEV1 and FVC (0 and 24h post); decreased macrophage, neutrophil and lymphocytes in BL and lymphocytes in BAL (24h post); unaltered CC16 (BL and BAL) (24h post); unaltered $FeNO_{10\ and\ 50}$ (0 and 24h post)	Crossover. N=14, mean age 26, MF	(Muala et al., 2015) Umeå, Sweden
WS: 90 min (with exercise), 250 and 500 µg/m^3 ($PM_{2.5}$)	Unaltered FEV1, FVC and FEV1/FVC (0 and 1h post)	Crossover. N=10, mean age 26, M	(Ferguson et al., 2017) Missoula, US-MT
WS: 2h, 488 µg/m^3 ($PM_{2.5}$), followed by nasal inoculation with a vaccine dose of live attenuated influenza virus	Cytokine levels in nasal lavage (IP10 and IL6) were suppressed by wood smoke exposure and with exposure-sex interaction (48h post)	Parallel groups. N=39, age range 18-40 years old, MF	(Rebuli et al., 2019) Chapel Hill, US-NC
WS: 2h (with exercise), 500 µg/m^3 ($PM_{2.5}$)	Increased percentage of neutrophils in sputum (24h post)	Sequential. N=27, mean age 29, age range 18-45. MF	(Burbank et al., 2019) Chapel Hill, US-NC
DE: 1h, median 4.3x10^6 prticles/cm^3	Increased neutrophils in BAL (18h post)	Sequential. N=8	(Rudell et al., 1990) Umeå, Sweden
DE: 1h (with exercise); 1.4-2.6x10^6 particles/cm^3;	Unaltered FEV1, FVC, FEF_{50}, FEF_{25-75} and VTG; increased R_{aw} and SR_{aw} (0h post)	Crossover. N=15, age range 20-37, MF	(Rudell et al., 1996) Umeå, Sweden
DE: 1h (with exercise), 300 µg/m^3 (PM_{10}), 4.3x10^6 particles/cm^3	Unaltered FEV1 and FVC (0h post); increased neutrophils and IL8 transcription in BL and biopsies and increased ICAM1 and VCAM1 in BL (6h post)	Crossover. N=15, mean age 24, MF	(Salvi et al., 1999) (Salvi et al., 2000) Umeå, Sweden
DE: 2h (with exercise); 108 µg/m^3 (PM_{10})	Unaltered FEV1 and FVC (0h post); increase in airway resistance (0h post); increased neutrophils, IL6 and IL8 in BL (6h post)	Crossover. N=25, mean age 24, MF	(Stenfors et al., 2004) Umeå, Sweden

Table 6. (Continued)

Exposure	Effect	Study and subjects	Reference
DE: 2h (with exercise), 100 µg/m^3 (PM_{10})	Increased neutrophils in BL and biopsies (18h post)	Crossover. N=15, mean age 24, MF	(Behndig et al., 2006) Umeå, Sweden
DE: 1h (with exercise), 300 µg/m^3 (PM_{10})	Unaltered FEV1 and FVC (2h post); increased FeNO 10 and 50 (6h post)	Crossover. N=10, mean age 26, M	(Barath et al., 2013) Umeå, Sweden
DE: Unspecified duration (with exercise), 313 µg/m^3 (PM_{10})	Unaltered FEV1, FVC and VC (2h post)	Crossover. N= 30, age range 21-34, MF	(Langrish et al., 2013) Umeå, Sweden
DE: 1h (with exercise), 159 µg/m^3 (PM_{10}) (biodiesel)	Increased bioactive lipids in BL and BAL (6h post)	Crossover. N=15, mean age 26, MF	(Gouveia-Figueira et al., 2017) Umeå, Sweden
DE: 3h, 276 µg/m^3 (PM_1) 3.9x10^5 particles/cm^3	Decreased PEF and unaltered FEV1 and FVC (0h post); unaltered NL cytokines	Crossover. N=18, mean age 51, MF	(Xu et al., 2013) Lund, Sweden
DE: 2h, 200 µg/m^3 (PM_{10})	Unaltered FEV1 and FVC (0 and 24 h post); increased neutrophils and unaltered IL8 or TNF in sputum (4h post)	Crossover. N=10, mean age 28, MF	(Nightingale et al., 2000) London, UK
DE: 2h, 304 µg/m^3 ($PM_{2.5}$)	Increased pulmonary vascular resistance (2h post); Unaltered FeNO (0h post)	Crossover. N=18; mean age 22.2, M	(Wauters et al., 2015) Brussels, Belgium
DE: 2h (with exercise), 297 µg/m^3 (unspecified PM), 7.3x10^5 particles/cm^3	Unaltered FEV1 and FVC (0 and 4h post). A subsequent exposure to ozone the day after was associated with decline FEV1	Crossover. N=15, mean age 27, MF	(Madden et al., 2014) Chapel Hill, US-NC
DE: 1h, 302 µg/m^3 ($PM_{2.5}$)	Decreased FEV1 change post-exposure to post-exercise challenge	Crossover. N=8, mean age 29, M	(Giles et al., 2012) Vancouver, Canada)
DE: 2h, 300 µg/m^3 ($PM_{2.5}$), 5.4x10^5 particles/cm^3	Increased markers of allergen and non-allergen inflammation in BL, BAL and biopsies (48h post)	Crossover. N=18 (atopic), mean age 28, MF	(Carlsten et al., 2016; Mookherjee et al., 2018) (Hosseini et al., 2016) Vancouver, Canada
DE: 2h, 260 µg/m^3 ($PM_{2.5}$)	Increased amount of neutrophil extracellular traps in BAL (24h post)	Crossover. N=11, mean age 59, MF	(Wooding et al., 2019) Vancouver, Canada
CAP: 2h, range 48-199 µg/m^3 ($PM_{2.5}$)	Unaltered FEV1 and FVC (0 to 20h post). Unaltered sputum cell counts and IL6 (3 and 20h post)	Crossover. N=13, mean age 27, MF	(Urch et al., 2010) Toronto, Canada
CAP: 2h10, 235 µg/m^3 ($PM_{2.5}$)	Unaltered sputum cell counts (24h post)	Crossover. N=35, mean age 27, MF	(Behbod et al., 2013) Toronto, Canada
CAP: 2h (with exercise), 121 µg/m^3 ($PM_{2.5}$)	Unaltered FEV1, FVC and PEF (0h post), increased neutrophils in BL and BAL (18h post)	Parallel groups. N=30, mean age 26, MF	(Ghio et al., 2000) Chapel Hill, US-NC
CAP: 2h (with exercise), 82 µg/m^3 ($PM_{2.5}$)	Unaltered FEV1 and FVC (0 and 18h post); unaltered cell counts, IL6 and IL8 in BAL (18h post)	Crossover. N=23, mean age 25, MF	(Huang et al., 2012) Chapel Hill, US-NC
CAP: 2h (with exercise), 50 µg/m^3 (UFP); 1.2x10^5 particles/cm^3 (UFP)	Unaltered FEV1 and FVC (0 and 18h post); Increased IL8 (but not IL6) in BAL (18h post)	Crossover. N=19, age range 18-35, MF	(Samet et al., 2009) Chapel Hill, US-NC
CAP: 2h (with exercise), 174 µg/m^3 ($PM_{2.5}$)	Unaltered FEV1 and FVC. Unaltered sputum IL6, IL8 and cell counts (0, 4 and 22 h post)	Crossover. N=12, mean age 28, MF	(Gong et al., 2003) Los Angeles, US-CA

Exposure	Effect	Study and subjects	Reference
CAP: 2h (with exercise), 200 µg/m^3 ($PM_{2.5}$)	Unaltered FEV1 and FVC. Unaltered sputum IL6, IL8 and cell counts (0, 4 and 22h post)	Crossover. N=6, mean age 68, MF	(Gong et al., 2004) Los Angeles, US-CA
CAP: 2h (with exercise), 100 µg/m^3 (UFP); 1.5x10^5 particles/cm^3	2% Decreased FEV1 (22h post)	Crossover. N=17, mean age 24, MF	(Gong et al., 2008) Los Angeles, US-CA
Ind: 2h, (6 exposures) from 38-208 µg/m^3 (PM_1) or 3.1x10^5-2.7x10^6 particles/cm^3 (UFP)	Decreased FEV1 after candle burning and frying sausages	Crossover. N=55, mean age 33, MF	(Soppa et al., 2014) Düsseldorf, Germany
Ind: 1h15, 10^5 particles/cm^3 (UFP)	Unaltered FEV1, FVC, Raw, sRaw, ITGV (0h post); Unaltered nasal secretion (0h post)	Crossover. N=23, mean age 44, MF	(Karrasch et al., 2017) Munich, Germany
Ind: 1h, 1.6x10^6 particles/cm^3	Unaltered FEV1 and FVC (0h post); unaltered nasal biomarkers; slight increase in $FeNO_{50}$	Crossover. N=26, mean age 25, MF	(Gumperlein et al., 2018) Munich, Germany

BAL, bronchoalveolar lavage; BL, bronchial lavage; CAP, concentrate ambient particles; CC16, Clara cell protein 16; DE, diesel exhaust; F, females; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; ICAM1, intracellular cell adhesion molecule 1; IL, interleukin; Ind, indoor sources; ITGV, intrathoracic gas volume; M, males; NL, nasal lavage; PM, particulate matter; PEF, peak expiratory flow; PM, particulate matter; Raw, airway resistance, SRaw, specific airway resistance; UFP, ultrafine particles; VC, slow vital capacity; VCAM1, vascular cell adhesion molecule 1; VTG, volume of trapped gas; WS, wood smoke.

Table 7. Systemic inflammation and thrombogenicity in healthy humans after controlled exposures

Exposure	Effect	Study and subjects	Reference
WS: 4h (with exercise), 240-280 µg/m^3, 9.5x10^4-18x10^4 particles/cm^3	Increased SAA (0, 3 and 20h post); decreased IL6 (3h post); increased FVIII (20h post) and FVIII/vWf ratio (0, 3 and 20h post); Unaltered CRP, fibrinogen, FVII, vWf, TNF and cell counts (0, 3 and 20h post)	Sequential. N=13, mean age 34, MF	(Barregard et al., 2006) Borås, Sweden
WS: 3h, *LOW:* 222 µg/m^3 ($PM_{2.5}$), 1.3x10^4 UFP; *HIGH:* 385 µg/m^3, 3.4x10^4 (UFP)	Unaltered IL (-1β, -6, -10, -12, -18), TNF, vWf; P-selectin, E-selectin and ICAM1 (0, 6 and 20h post)	Crossover. N=20 (atopic), mean age 25, MF	(Bonlokke et al., 2014; Forchhammer et al., 2012) Åhrus, Denmark
WS: 3h, *START-UP:* 295 µg/m^3 ($PM_{2.5}$), 1.4 x10^5 particles/cm^3; *BURN-OUT*: 146 µg/m^3 ($PM_{2.5}$), 1.0x10^5 particles/cm^3	Unaltered SAA, CRP, IL6, TNF, P-selectin, ICAM1 and VCAM1 (0, 4 and 20h post); decrease in fibrinogen and platelet counts (20h post for both fires and 4 and 20h post for *burn out* fire); Increased FVII (4 and 47h post); Increased FVIII (20h post)	Sequential. N=13, mean age 34, MF	(Stockfelt et al., 2013) Borås, Sweden
WS: 2h (with exercise), 485 µg/m^3 ($PM_{2.5}$), 4.6x10^4 particles/cm^3	Increased neutrophils in blood (20h post); Unaltered IL6, IL8, TNF and vWf (0 and 20h)	Sequential. N=10, age range 18-40	(Ghio et al., 2012b) Chapel Hill, US-NC
WS: 3h (with exercise), 314 µg/m^3 (PM_1), 1-2.5x10^5 particles/cm^3 (UFP)	Unaltered IL6. TNF and ICAM1 (24 and 44h post)	Crossover. N=14, mean age 26, MF	(Muala et al., 2015) Umeå, Sweden
WS: 1h (with exercise), 1115 µg/m^3 (PM_1)	Unaltered platelets (2, 6 and 24h post)	Crossover. N=16, median age 26, M (firefighters)	(Hunter et al., 2014) Umeå, Sweden

Table 7. (Continued)

Exposure	Effect	Study and subjects	Reference
DE: 1h (with exercise), 300 µg/m^3 (PM_{10}), 4.3x10^6 particles/cm^3	Increased peripheral neutrophils (6h post); increased platelets (6h post)	Crossover. N=15, mean age 24, MF	(Salvi et al., 1999) Umeå, Sweden
DE: 1h (with exercise); 300 µg/m^3, 1.2x10^6 particles/cm^3	Unaltered blood IL6, TNF, CRP, neutrophils and platelets (6h post); Increased blood IL6, TNF and P-selectin; unaltered CRP, ICAM1 and neutrophils (24h post)	Crossover. N=30, age range 20-38, subset of 15 assessed at 24h post mean age 26, M	(Mills et al., 2005; Tornqvist et al., 2007) Umeå, Sweden
DE: 1h (with exercise), 228 µg/m^3 (PM_{10}); 1.2x10^5 particles/cm^3	Unaltered cell counts, TNF, IL6, P-selectin, ICAM1 and CRP (2 and 6h post)	Crossover. N=18, mean age 27, M	(Barath et al., 2010) Umeå, Sweden
DE: 1h (with exercise), 320 µg/m^3, 1.5-2.0x10^5 particles/cm^3 (PM_1)	Unaltered cell counts, TNF, IL6, P-selectin, ICAM1, CRP, tPA and platelets (2, 6 and 8h post); Increased ex-vivo thrombus formation after DE but not after filtered DE	Crossover. N=19, mean age 25, M	(Lucking et al., 2011) Umeå, Sweden
DE: Unspecified duration (with exercise), 313 µg/m^3 (PM_{10})	Unaltered blood cell counts and platelets (2 and 6h post)	Crossover. N= 30, age range 21-34, MF	(Langrish et al., 2013) Umeå, Sweden
DE: 1h (with exercise), 159 µg/m^3 (PM_{10}) (biodiesel)	Alterations in blood bioactive lipids (2, 6 and 24h post)	Crossover. N=15, mean age 26, MF	(Gouveia-Figueira et al., 2018) Umeå, Sweden
DE: 3h, 276 µg/m^3 (PM_1) 3.9x10^5 particles/cm^3	Unaltered blood neutrophils, IL6, IL8, CRP and fibrinogen (0 and 20h post)	Crossover. N=18, mean age 51, MF	(Xu et al., 2013) Lund, Sweden
DE: 2h (with exercise), 348 µg/m^3 ($PM_{2.5}$), 1.2x10^6 particles/cm^3	Unaltered blood neutrophils, IL6, TNF, P-selectin, ICAM1, CRP and platelets (2, 6 and 24h)	Crossover. N=20, age range 21-44, M	(Lucking et al., 2008; Mills et al., 2011b) Edinburg, UK
DE: 2h, 200 µg/m^3 (PM_{10})	Unaltered blood IL6, P-selectin and TNF (0 and 24h post)	Crossover. N=10, mean age 28, MF	(Nightingale et al., 2000) London, UK
DE: 2h, 304 µg/m^3 ($PM_{2.5}$)	Unaltered blood IL8 and E-selectin (0h post)	Crossover. N=12, mean age 23, M	(Wauters et al., 2013) Brussels, Belgium
DE: 2h, 25 µg/m^3 ($PM_{2.5}$)	Unaltered blood CRP (0 and 24h post); Increased fibrinogen (0h post)	Crossover. N=25 (plus 15 smokers), mean age 41, MF	(Tousoulis et al., 2020) Athens, Greece
DE: 2h, 100 and 200 µg/m^3 ($PM_{2.5}$)	Unaltered blood CRP (3 and 22 h post); Unaltered platelets and vWf	Crossover. N=13	(Carlsten et al., 2007) Seattle, US-WA
DE: 2h, 205 µg/m^3 ($PM_{2.5}$), 5.3x10^4 particles/cm^3	Unaltered neutrophils, IL1β, IL6, IL8, IL10, TNF, ICAM1, VCAM1 (7 and 22 h post); Increased platelets (20h post)	Crossover. N=15, mean age 28, MF	(Krishnan et al., 2013) Seattle, US-WA
DE: 2h (with exercise), 297 µg/m^3 ($PM_{2.5}$), 7.3-9.8x10^5 particles/cm^3	Unaltered blood IL1β, IL2, IL4, IL5, IL8, IL10, IL12p70, IL13, IFNγ, TNF (22h post)	Crossover. N=15, mean age 27, MF	(Stiegel et al., 2016) Chapel Hill, US-NC
DE: 2h, 100, 214 and 302 µg/m^3 (mean size<0.04 µm)	Increased blood neutrophils and platelets (18h post 200 µg/m^3 exposure); Unaltered blood CRP	Sequential. N=6, mean age 58, MF	(Tong et al., 2014) Chapel Hill, US-NC
DE: 1h, 295 µg/m^3 ($PM_{2.5}$), 6.8x10^4 particles/cm^3	Increased gene expression of coagulation pathways in PMBC (24h post)	Crossover. N=14	(Pettit et al., 2012)

Exposure	Effect	Study and subjects	Reference
DE: 2h (with exercise), 290 $\mu g/m^3$ ($PM_{2.5}$)	Unaltered blood IL6 and TNF (0, 3 and 24 post)	Crossover. N=27, mean age 28, MF	(Cliff et al., 2016) Vancouver, Canada
DE: 2h (with exercise), 260 $\mu g/m^3$ ($PM_{2.5}$)	Unaltered neutrophil activation marker (0 and 24h post)	Crossover. N=7 never smokers (mean age 56) and N=4 ex-smokers (mean age 66), MF	(Wooding et al., 2019) Vancouver, Canada
DE: 2h (with exercise), 260 $\mu g/m^3$ ($PM_{2.5}$)	Unaltered neutrophil activation marker (0 and 24h post)	Crossover. N=7 never smokers (mean age 56) and N=4 ex-smokers (mean age 66), MF	(Wooding et al., 2019) Vancouver, Canada
DE: 15 min, 325 $\mu g/m^3$ ($PM_{2.5}$)	Unaltered blood cell counts and CRP (0h post)	Crossover. N=15, mean age 45, MF	(Vieira et al., 2016) S. Paulo, Brazil
CAP: 2h, 150 $\mu g/m^3$ ($PM_{2.5}$)	Increased blood neutrophils (0h post); unaltered blood CRP, TNF, IL1β, IL2, IL4, IL6, IL10, IL12, IFNγ, GM-CSF (0 and 24h post)	Crossover. N=31, mean age 27, MF	(Brook et al., 2009) Toronto, Canada
CAP: 2h, median 235 $\mu g/m^3$ ($PM_{2.5}$)	Increased blood neutrophils (24h post); Unaltered IL6 and CRP (3 and 24h post)	Crossover, N=35, mean age 27, MF	(Behbod et al., 2013) Toronto, Canada
CAP: 2h, 136 $\mu g/m^3$ (UFP), $2.3x10^5$ particles/cm^3	Unaltered blood CRP and IL6 (1 and 24h post)	Crossover. N=25, mean age	(Liu et al., 2015) Toronto, Canada
CAP: 2h (with exercise), 121 $\mu g/m^3$ ($PM_{2.5}$)	Unaltered blood CRP, IL6, TNF, FVII, FIX, vWf (0 and 24h post); Increased fibrinogen (18 and 24h post)	Parallel groups. N=15, mean age 25, MF	(Ghio et al., 2003) Chapel Hill, US-NC
CAP: 2h, 278 $\mu g/m^3$ ($PM_{2.5}$), $3.0x10^5$ particles/cm^3	Unaltered blood CRP, ICAM1, VCAM1, IL6, IL8, TNF, fibrinogen, vWf, plasminogen, tPA, PAI1 (0 and 20h post); blood neutrophils were attenuated for participants with fish oil supplementation (20h post)	Sequential. N=29+13, mean age 58, MF	(Tong et al., 2015; Tong et al., 2012) Chapel Hill, US-NC
CAP: 2h (with exercise), 90 $\mu g/m^3$ ($PM_{2.5}$)	Unaltered blood cell counts, CRP, fibrinogen, vWf, FVII, FIX (1 and 18h post)	Crossover. N=23, mean age 25, MF	(Huang et al., 2012) Chapel Hill, US-NC
CAP: 2h, 98 $\mu g/m^3$ (UFP), $1.9x10^5$ particles/cm^3	Increased CRP and SAA (20h post); Unaltered ICAM1, VCAM1, E-selectin, P-selectin, tPA, PAI1, vWf (1 and 20h post); decreased thrombomodulin and plasminogen (20h post)	Crossover. N=34, mean age 48, MF (with metabolic syndrome)	(Devlin et al., 2014) Chapel Hill, US-NC
CAP: 2h (with exercise), 50 $\mu g/m^3$ (UFP), $1.2x10^5$ particles/cm^3	Unaltered blood CRP, fibrinogen, FIX, FVII and vWf (0 and 18h post); Increased D-dimer (0 and 18h post)	Crossover. N=19, age range 18-35, MF	(Samet et al., 2009) Chapel Hill, US-NC
CAP: 2h (with exercise), 174 $\mu g/m^3$ ($PM_{2.5}$)	Increased blood ICAM1 (4 and 22h post), Unaltered SAA, IL6, platelet counts, fibrinogen and vWf (4 and 22h post); time-atmosphere change in FVII (decreased at 4h post)	Crossover. N=12, mean age 28, MF	(Gong et al., 2003) Los Angeles, US-CA
CAP: 2h (with exercie), 200 $\mu g/m^3$ ($PM_{2.5}$)	Unaltered blood neutrophils, platelet counts, fibrinogen, vWf, FVII, FIX (4 and 22h post)	Crossover. N=6, mean age 68, MF	(Gong et al., 2004) Los Angeles, US-CA

Table 7. (Continued)

Exposure	Effect	Study and subjects	Reference
CAP: 2h (with exercise), 100 µg/m^3 (UFP), 1.5x10^5 particles/cm^3	Unaltered blood CRP, ICAM1, VCAM1, E-selectin, fibrinogen, plasminogen, vWf, FVII, FIX and PAI1(4 and 22h post)	Crossover. N=17, mean age 24, MF	(Gong et al., 2008) Los Angeles, US-CA
Ind: 1h15min, 10^5 particles/cm^3 (UFP)	Unaltered blood IL1β, IL5, IL6, IL8, GM-CSF, INFγ and TNF (0h post)	Crossover. N=23, mean age 44, MF	(Karrasch et al., 2017) Munich, Germany

CAP, concentrate ambient particles; CRP, C-reactive protein; F, female; FVII, coagulation factor VII; FVIII, coagulation factor VIII; FIX, coagulation factor IX; GM- CSF, granulocyte-macrophage colony stimulating factor; ICAM1, intercellular adhesion molecule 1; IFNγ, interferon gamma; IL, interleukin; Ind, indoor sources; M, males; PAI1, plasminogen activator inhibitor 1; PM, particle matter; SAA, serum amyloid A; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; UFP, ultrafine particles; VCAM1, vascular cell adhesion molecule 1; vWf, von Willebrand factor; WS, wood smoke.

Table 8. Oxidative stress and genotoxicity in healthy humans after controlled exposures

Exposure	Effect	Study and subjects	Reference
WS: 4h (with exercise), 240-280 µg/m^3, 9.5x10^4-18x10^4 particles/cm^3	Increased urinary excretion 8-isoprostane (20h post); Unaltered 8-isoprostane in EBC (0, 3 and 20h post); Increased MDA in EBC (20h post); Increased levels of *HMOX1* and *hOGG1* expression in blood (20h post); Decreased levels of DNA-SB in PBMC (3 and 20h post); Unaltered DNA-Fpg-sites in PBMC (3 and 20h post); Unaltered 8-oxodG and 8-oxoGua in urine (20h post)	Sequential. N=13, mean age 34, MF	(Barregard et al., 2008; Barregard et al., 2006; Danielsen et al., 2008; Murgia et al., 2016) Borås, Sweden
WS: 3h (with exercise), 224 µg/m^3 ($PM_{2.5}$), 6.7x10^4 particles/cm^3 (UFP)	Unaltered MPO in BL and BAL (24h post); Increased GSH in BAL but not BL (24h post); unaltered *HMOX1* in tissue from biopsies (24h post)	Crossover. N=19, mean age 24, MF	(Sehlstedt et al., 2010) Umeå, Sweden
WS: 3h, *LOW:* 222 µg/m^3 ($PM_{2.5}$), 13.3x10^3 UFP; *HIGH:* 385 µg/m^3, 33.9x10^3 UFP	Unaltered 8-isoprostane in EBC (0 and 6h post); Unaltered *HMOX1* in blood (0, 6 and 20h post); Unaltered DNA-SB, DNA-Fpg-sites in PBMC (0, 6 and 20h post)	Crossover. N=20 (atopic), mean age 25, MF	(Forchhammer et al., 2012; Riddervold et al., 2012) Århus, Denmark
WS: 3h, *START-UP:* 295 µg/m^3 ($PM_{2.5}$), 1.4 x10^5 particles/cm^3; *BURN-OUT*: 146 µg/m^3 ($PM_{2.5}$), 1.0x10^5 particles/cm^3	Decreased 8-isoprostane in urine (20h post and also 3h post for burn-out fire)	Sequential. N=13, mean age 34, MF	(Stockfelt et al., 2013) Borås, Sweden
WS: 3h (with exercise), 314 µg/m^3 (PM_1), 1-2.5x10^5 particles/cm^3 (UFP)	Unaltered MPO in BL and BAL (24h post); unaltered GSSG in BAL in BAL (24h post)	Crossover. N=14, mean age 26, MF	(Muala et al., 2015) Umeå, Sweden
WS: 90 min (with exercise): 250 and 500 µg/m^3 ($PM_{2.5}$)	Increased 8-isoprostane in EBC (1h post); Unaltered MPO in EBC (1h post)	Crossover. N=10, mean age 26, M	(Ferguson et al., 2016) Missoula, US-MT
DE: 1h (with exercise), 300 µg/m^3 (PM_{10}), 4.3x10^6 particles/cm^3	Unaltered GSH in nasal lavage; unaltered MDA in BL, BAL (6h post) and plasma (0 and 6h post)	Crossover. N=15, mean age 24, MF	(Blomberg et al., 1998) Umeå, Sweden

Exposure	Effect	Study and subjects	Reference
DE: 2h (with exercise); 108 µg/m^3 (PM_{10})	Unaltered MPO in BL and BAL (6h post)	Crossover. N=25, mean age 24, MF	(Stenfors et al., 2004) Umeå, Sweden
DE: 1h (with exercise); 300 µg/m^3, 1.2x10^6 particles/cm^3	Increased antioxidant capacity in plasma (24h post)	Crossover. N=15, mean age 26, M	(Tornqvist et al., 2007) Umeå, Sweden
DE: 2h (with exercise), 100 µg/m^3 (PM_{10})	Increased MPO in BL (but not BAL) (18h post); Increased GSH and urate in BAL (but not BL) (18h post)	Crossover. N=15, mean age 24, MF	(Behndig et al., 2006) Umeå, Sweden
DE: 3h, 276 µg/m^3 ($PM_{2.5}$), 3.9x10^5 particles/cm^3	Unaltered expression of *HMOX1* and *hOGG1* in blood (0h post); Unaltered DNA-SB, DNA-Fpg-sites (0h post)	Crossover. N=18, mean age 51, MF	(Hemmingsen et al., 2015a) Lund, Sweden
DE: 2h, 205 µg/m^3 ($PM_{2.5}$), 5.3x10^4 particles/cm^3	Unaltered MPO in blood (20h post)	Crossover. N=15, mean age 28, MF	(Krishnan et al., 2013) Seattle, US-WA
DE: 1h, 295 µg/m^3 ($PM_{2.5}$), 6.8x10^4 particles/cm^3	Increased gene expression of oxidative stress pathways in PMBC (24h post)	Crossover. N=14	(Pettit et al., 2012) Piscataway, US-NJ
DE: 2h (with exercise), 302 µg/m^3 ($PM_{2.5}$), 5.4x10^5 particles/cm^3	GSTT1 null genotype enhanced the allergen-induced inflammation and lung function (post allergen exposure)	Crossover. N=17 (atopic), mean age 28, MF	(Carlsten et al., 2016; Zhang et al., 2016) Vancouver, Canada
CAP: 2h, 149 µg/m^3 ($PM_{2.5}$)	Decreased HDL antioxidant/anti-inflammatory capacity	Crossover. N=30, mean age 28, MF	(Ramanathan et al., 2016) Toronto, Canada
CAP: 2h, 250 µg/m^3 ($PM_{2.5}$)	Unaltered MDA in blood (1 and 21h post); Increased MDA in urine (1 and 21h post); Unaltered 8-oxodG in urine (1 and 21h post); Increased hypomethylation in blood DNA (1h post)	Crossover. N=50 (N=15 in Bellavia et al), mean age 28, MF	(Bellavia et al., 2013; Liu et al., 2015) Toronto, Canada
CAP: 2h, 136 µg/m^3 (UFP), 2.3x10^5 particles/cm^3	Unaltered MDA in blood and urine (1 and 21h post); Increased 8-oxodG in urine (1h post)	Crossover. N=25, MF	(Liu et al., 2015) Torotnto, Canada
Ind: 1h15min, 10^5 particles/cm^3 (UFP)	Unaltered hydrogen peroxide in EBC (0h post); unaltered 8-oxodG in serum	Crossover. N=23, mean age 43, MF	(Karrasch et al., 2017) Munich, Germany
Ind: 1h, 1.6x10^6 particles/cm^3 (UFP)	Unaltered 8-isoprostaglandin in urine (0 and 23h post)	Crossover. N=26, mean age 25, MF	(Gumperlein et al., 2018) Munich, Germany

8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-oxoGua, 8-oxo-7,8-dihydroguanine; BAL, bronchoalveolar lavage; BL, bronchial lavage; CAP, concentrated ambient particles; DE, diesel exhaust; DNA-SB, DNA strand breaks; DNA-Fpg-sites, DNA formamidopyrimide glycosylase sensitive sites; EBC, exhaled breath condensate; F, females; GSH, glutathione; GSSG, glutathione dissulphide; *HMOX1*, gene expression of heme oxygenase 1; Ind, indoor sources; M, males; MDA, malondialdehyde; MPO, myeloperoxidase; OGG1, oxoguanine DNA glycosylase 1; PBMC, peripheral blood mononuclear cells; UFP, ultrafine particles; WS, wood smoke.

Table 9. Vascular function in healthy humans after controlled exposures

Exposure	Effect	Study and subjects	Reference
WS: 3h, 180 µg/m^3 ($PM_{2.5}$)	Unaltered RHI (0h post)	Crossover. N=14, age range 18-25, MF	(Pope et al., 2011) Provo, US-UT
WS: 3h, *LOW:* 222 µg/m^3 ($PM_{2.5}$), 1.3x10^4 UFP; *HIGH:* 385 µg/m^3, 3.4x10^4 (UFP)	Unaltered RHI (6h post)	Crossover. N=20 (atopic), mean age 25, MF	(Forchhammer et al., 2012) Århus, Denmark
WS: 3h (with exercise), 314 µg/m^3 (PM_1), 1-2.5x10^5 particles/cm^3 (UFP)	Increased arterial stiffness, measured AI, AP and pulse wave velocity (1h post)	Crossover. N=14, mean age 26, MF	(Unosson et al., 2013) Umeå, Sweden

Table 9. (Continued)

Exposure	Effect	Study and subjects	Reference
WS: 1h (with exercise), 1115 µg/m^3 (PM_1)	Unaltered arterial stiffness measured AI, AP and pulse wave velocity (0h post); Unaltered FBF during infusions of endothelium dependent and independent vasodilators; Unaltered BP (6 and 24h post)	Crossover. N=16, median age 26, M (firefighters)	(Hunter et al., 2014) Umeå, Sweden
WS: 2h, 46 µg/m^3 (gasifier), 95 µg/m^3 (fan-rocket), 254 µg/m^3 (rocket-elbow), 463 µg/m^3 (three stone fire)	Unaltered diastolic BP; increased systolic blood pressure (24h post, but not before)	Crossover. N=48, mean age 28, MF	(Fedak et al., 2019) Fort Collins, US-CO
DE: 1h (with exercise); 300 µg/m^3, 1.2x10^6 particles/cm^3	Unaltered baseline FBF; DE attenuated FBF during infusions of endothelium-dependent (2-6h post) and independent vasodilators (2-6h and 24h post); unaltered blood pressure (2 and 24h post)	Crossover. N=15, mean age 26, M	(Mills et al., 2005; Tornqvist et al., 2007) Umeå, Sweden
DE: 1h (with exercise), 330 µg/m^3, 1.3x10^6 particles/cm^3	Transient increase in AI immediately after (normalized 30 min post); Unaltered blood pressure	Crossover. N=10, mean age 26, M	(Lundback et al., 2009) Umeå, Sweden
DE: 1h (with exercise), 331 µg/m^3 (PM_{10})	Unaltered 24h-blood pressure; Unaltered plasma ET1 (6 and 24h post); DE increased vascular sensitivity to ET1 (FBF)	Crossover. N=13, mean age 23, M	(Langrish et al., 2009a) Umeå, Sweden
DE: 1h (with exercise), 228 µg/m^3 (PM_{10}); 1.2x10^5 particles/cm^3	DE attenuated FBF during infusions of endothelium-dependent and independent vasodilators (6h post); unaltered blood pressure	Crossover. N=18, mean age 27, M	(Barath et al., 2010) Umeå, Sweden
DE: 1h (with exercise), 320 µg/m^3, 1.5-2.0x10^5 particles/cm^3 (PM_1)	DE attenuated FBF during infusions of endothelium-dependent and independent vasodilators (6-8h post); unaltered blood pressure or arterial stiffness (6-8h post)	Crossover. N=19, mean age 25, M	(Lucking et al., 2011) Umeå, Sweden
DE: Unspecified duration (with exercise), 313 µg/m^3 (PM_{10})	Unaltered baseline FBF; Study 1: local NOS inhibition resulted in unaltered vasoconstriction; study 2: systemic NOS inhibition induced increased arterial stiffness	Crossover. N= 30 (2 parallel studies), age range 21-34, MF	(Langrish et al., 2013) Umeå, Sweden
DE: 2h (with exercise), 348 µg/m^3 ($PM_{2.5}$), 1.2x10^6 particles/cm^3	Unaltered baseline FBF; DE attenuated FBF during infusions of endothelium-dependent and independent vasodilators (6-8h post); Increased systolic BP	Crossover. N=16, age range 18-32, M	(Mills et al., 2011b) Edinburgh, UK
DE: 2h, 303 µg/m^3 (PM_1)	DE attenuated FBF during infusion of endothelium-dependent, but not endothelium independent vasodilators (2h post); decreased systolic BP (2h post)	Crossover. N=12, mean age 23, M	(Wauters et al., 2013) Brussels, Belgium

Exposure	Effect	Study and subjects	Reference
DE: 2h, 25 µg/m^3 ($PM_{2.5}$)	DE decreased FMD and increased pulse wave velocity and AI (0h and 24 h post)	Crossover. N=25, mean age 41, MF	(Tousoulis et al., 2020) Athens, Greece
DE: 2h, 100 and 200 µg/m^3 ($PM_{2.5}$)	Decreased brachial artery diameter (30 min after 200 µg/m^3); Increased plasma ET1 (3h after 200 µg/m^3); Unaltered FMD and BP (30 min post)	Crossover. N=10 (5 for some endpoints), mean age 30, MF	(Peretz et al., 2008b) Seattle, US-WA
DE: 2h, 205 µg/m^3 ($PM_{2.5}$), 0.5x10^5 particles/cm^3	Increased systolic BP but not diastolic (largest increase at 30min and 1h post)	Crossover. N=31, mean age 28, MF	(Cosselman et al., 2012) Seattle, US-WA
DE: 2h, 200 µg/m^3 ($PM_{2.5}$), 7.1x10^5 particles/cm^3	Decreased brachial artery diameter; Unaltered FMD (30 min post)	Crossover. N=21, mean age 29, MF	(Sack et al., 2016) Seattle, US-WA
DE: 2h, 100, 214 and 302 µg/m^3	Unaltered FMD and NMD; Unaltered systolic BP, Increased diastolic BP (2h post); concentration-dependence effects	Sequential. N=6, mean age 58, MF	(Tong et al., 2014) Chapel Hill, US-NC
DE: 30 min (with exercise), 302 µg/m^3 ($PM_{2.5}$), 6.2x10^5 particles/cm^3	Unaltered FMD and BP; Decrased ET1 (2h post)	Crossover. N=18, mean age 25, M	(Giles et al., 2018) Vancouver, Canadá
DE: 21min, 325 µg/m^3 ($PM_{2.5}$)	Unaltered RHI and AI (0h post)	Crossover. N=15, mean age 45, MF	(Vieira et al., 2016) S. Paulo, Brazil
CAP: 2h, 150 µg/m^3 ($PM_{2.5}$)	Unaltered FMD and NMD (0h post); Increased diastolic BP (2h post); Decreased brachial artery diameter (0h post)	Crossover. N=25, mean age 35, MF	(Brook et al., 2002; Urch et al., 2005) Toronto, Canada
CAP: 2h, 149 µg/m^3 (CAP) and 132 µg/m^3 (CAP&O_3)	Increased diastolic BP (2h post); Decreased FMD (24h post, but not at 0h); unaltered NMD and brachial artery diameter; unaltered plasma ET1 (0 and 24h)	Crossover. N=31, mean age 27, MF	(Brook et al., 2009) Toronto, Canada
CAP: 2h, 150 149 µg/m^3 ($PM_{2.5}$)	Increased diastolic BP; Unaltered FMD, NMD and brachial artery diameter (0 and 24h post)	Crossover, N=50, mean age 27, MF	(Brook et al., 2009) Ann Arbor, US-MI
CAP: 2h, 250 µg/m^3 ($PM_{2.5}$)	Increased BP associated with endotoxin and β-glucan	Crossover. N=50, age range 18-64, MF	(Zhong et al., 2015) Toronto, Canada
CAP: 2h, 278 µg/m^3, 3.0x10^5 particles/cm^3	Decreased FMD (0h post); unaltered brachial artery diameter (0 and 24h post); increased ET1 (20h post)	Sequential. N=29, mean age 58, MF	(Tong et al., 2015) Chapel Hill, US-NC
CAP 1.9x10^5 particles/cm^3, 98 µg/m^3 (UFP)	Unaltered BP, brachial artery diameter, AI, FMD and NMD (1 and 20h post);	Crossover. N=34, mean age 48, MF (with MS)	(Devlin et al., 2014) Chapel Hill, US-NC
Ind: 2h, (6 exposures) from 38-208 µg/m^3 (PM_1) or 3.1x10^5-2.7x10^6 particles/cm^3 (UFP)	Increased BP (after toasting bread but not consistently after candle burning and frying sausages); immediate and mostly transient effects on arterial stiffness	Crossover. N=55, mean age 33, MF	(Soppa et al., 2017; Soppa et al., 2019) Düsseldorf, Germany

AI, augmentation index; AP, arterial pressure; BP, blood pressure; CAP, concentrate ambient particles; DE, diesel exhaust; ET1, endothelin 1; F, females; FBF, forearm blood flow; FMD, flow-mediated dilatation; Ind, indoor sources; M, males; MS, metabolic syndrome; NMD, nitric oxide-mediated dilatation; NOS, nitric oxide synthase; RHI, reactive hyperemia index; WS, wood smoke.

Table 10. HRV, arrhythmia and neurotoxicity in healthy humans after controlled exposures

Exposure	Effect	Study and subjects	Reference
WS: 3h, *LOW:* 222 µg/m^3 ($PM_{2.5}$), 13.3x10^3 UFP; *HIGH:* 385 µg/m^3, 33.9x10^3 UFP	Unaltered time and frequency domain HRV (10h post)	Crossover. N=20 (atopic), mean age 25, MF	(Bonlokke et al., 2014) Århus, Denmark
WS: 2h (with exercise), 485 µg/m^3 ($PM_{2.5}$), 4.6x10^4 particles/cm^3	Unaltered time domain HRV (0 and 20h post), HF marginally increased (0h post); Unaltered repolarization variables	Sequential. N=10, age range 18-40	(Ghio et al., 2012b) Chapel Hill, US-NC
WS: 3h (with exercise), 314 µg/m^3 (PM_1)	Unaltered incidence and number of arrhythmias; Decreased time domain HRV (SDNN, RMSSD and pNN50) (1h post); Decreased HF (1h post)	Crossover. N=14, mean age 26, MF	(Langrish et al., 2014; Unosson et al., 2013) Umeå, Sweden
WS: 1h (with exercise), 895 µg/m^3 (PM_1)	Unaltered incidence and number of arrhythmias	Crossover. N=15, mean age 26, M (firefighters)	(Langrish et al., 2014) Umeå, Sweden
DE: 1h (with exercise), 307 µg/m^3, 7.9x10^5 particles/cm^3 (PM_{10})	Unaltered incidence and number of arrhythmias; Unaltered time and frequency domain HRV (2 and 6h post)	Crossover. N=30, age range 20-38, M	(Langrish et al., 2014; Mills et al., 2011a) Umeå, Sweden
DE: 2h (with exercise), 363 µg/m^3, 1.2x10^6 particles/cm^3 (PM_{10})	Unaltered incidence and number of arrhythmias	Crossover. N=16, age range 18-32, M	(Langrish et al., 2014) Edinburgh, UK
DE: 2h, 25 µg/m^3 ($PM_{2.5}$)	Decreased SDNN (2 and 24h post)	Crossover. N=25, mean age 41, MF	(Tousoulis et al., 2020) Athens, Greece
DE: 2h, 102 µg/m^3 and 206 µg/m^3 ($PM_{2.5}$)	Unaltered time domain HRV; Increased HF and decreased LF/HF (3h post 200 µg/m^3); Increased L-DOPA (3h post 200 µg/m^3)	Crossover. N=3, mean age 32, M	(Peretz et al., 2008a; Peretz et al., 2008b) Seattle, US-WA
DE: 2h, 100, 214 and 302 µg/m^3	Unaltered time domain HRV (30 min and 18h post); decreased VLF (18h after 300 µg/m^3); Unaltered QTc	Sequential. N=6, mean age 58, MF	(Tong et al., 2014) Chapel Hill, US-NC
DE: 2h (with exercise), 290 µg/m^3 ($PM_{2.5}$)	Unaltered circulating markers of neurotoxicity (S100b, NSE, BDNF); trend of reduced postural stability in response to exposure	Crossover. N=28, mean age 28, MF	(Cliff et al., 2016; Curran et al., 2018) Vancouver, Canada
DE: 21min, 325 µg/m^3 ($PM_{2.5}$)	Unaltered time and frequency domain HRV; no significand arrhythmias	Crossover. N=15, mean age 45, MF	(Vieira et al., 2016) S. Paulo, Brazil
CAP: 2h, 149 µg/m^3 and 132 µg/m^3 (CAP+O_3)	Dose-response decrease for LF and special dispersion of repolarization when in the presence of CAP+O_3	Crossover. N=23, mean age 32, MF	(Fakhri et al., 2009; Sivagangabalan et al., 2011) Toronto, Canada
CAP: 2h, 238 µg/m^3 ($PM_{2.5}$) and 136 µg/m^3 and 2.3x10^5 particles/cm^3 (UFP)	Fine CAPs was marginally associated with increased blood UCHL1 (21h post). UFP was not significantly associated with changes in any blood or urinary neural biomarker	Crossover. N=55, mean age 28, MF	(Liu et al., 2015) Toronto, Canada
CAP: 2h, 250 µg/m^3 ($PM_{2.5}$)	Decreased LF; borderline decrease of time domain HRV (SDNN, RMSSD) attenuated with B-vitamin supplementation	Crossover. N=10, age range 19-49 (70%<29)	(Zhong et al., 2017) Toronto, Canada

Exposure	Effect	Study and subjects	Reference
CAP: 2h, $2.5x10^5$ particles/cm^3, 159 µg/m^3 (UFP)	Unaltered parasympathetic modulation (HRV and T-wave morphology)	Crossover. N=19, mean age 43, MF	(Breitner et al., 2019) Rochester, US-NY
CAP: 2h, 278 µg/m^3, $3.0x10^5$ particles/cm^3 ($PM_{2.5}$)	Unaltered time domain HRV (0, 20h post); changes in frequency domain HRV, QTc and Tp-Te (and differently for the supplementation regimes)	Sequential. N=29, mean age 58, MF	(Tong et al., 2012) Chapel Hill, US-NC
CAP $1.9x10^5$ particles/cm^3, 98 µg/m^3 (UFP)	Increased LF/HF; decreased HF, increased LF; increase in duration of QTc (1h post)	Crossover. N=34, mean age 48, MF (with metabolic syndrome)	(Devlin et al., 2014) Chapel Hill, US-NC
CAP: 2h (with exercise), $1.2x10^5$ particles/cm^3; 50 µg/m^3 (UFP)	Increased LF and HF; Unaltered time domain HRV; decreased in mean duration and variability of QTc interval duration (18h post)	Crossover. N=19, age range 18-35, MF	(Samet et al., 2009) Chapel Hill, US-NC
CAP: 2h (with exercise), 174 µg/m^3 ($PM_{2.5}$)	Decreased LF/HF (0 and 22h post)	Crossover. N=12, mean age 28, MF	(Gong et al., 2003) Los Angeles, US-CA
CAP: 2h (with exercise), 200 µg/m^3 ($PM_{2.5}$)	Decreased SDNN; increased supraventricular and ventricular ectopic beats	Crossover. N=6, mean age 68, MF	(Gong et al., 2004) Los Angeles, US-CA
CAP: 2h (with exercise), $1.5x10^5$ particle/cm^3, 100 µg/m^3 (UFP)	Decreased LF	Crossover. N=17, mean age 24, MF	(Gong et al., 2008) Los Angeles, US-CA
Ind: 4h, $8.0x10^5$ particles/cm3, 200 µg/m^3 (candle burning); $0.3x10^5$ particles/cm^3, 80 µg/m^3 (terpene-O_3)	Increased HF (after burning candles). Change in HF from burning candles and terpene-O_3 were in different directions	Crossover. N=20, mean age 32, F	(Hagerman et al., 2014) Lund, Sweden

CAP, concentrate ambient particles; DE, diesel exhaust; F, females; HF, high frequency component (0.15-0.4 Hz); HRV, heart rate variability; Ind, indoor sources; LF, low frequency component (0.04-0.15 Hz); M, males; PM, particle matter; pNN50, proportion of successive NN intervals differing by more than 50 milliseconds in total number of NN intervals; QTc, QT interval corrected for heart rate; RMSSD, square root of the mean squared differences of successive NN intervals; SDNN, standard deviation of NN intervals; Tp-Te, interval from peak to end of T-wave; UFP, ultrafine particles; VLF, very-low frequency component (<0.04 Hz); WS, wood smoke.

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