Estimated effects of air pollution and space-time-activity on cardiopulmonary outcomes in healthy adults: a repeated measures study

Author names:
Tom Cole-Hunter\textsuperscript{a,b,c,*}, tcolehunter@gmail.com
Audrey de Nazelle\textsuperscript{d}, anazelle@imperial.ac.uk
David Donaire-Gonzalez\textsuperscript{a}, david.donaire@isglobal.org
Nadine Kubesch\textsuperscript{e}, nadinejanetkubesch@gmail.com
Gloria Carrasco-Turiga\textsuperscript{a}, gloria.carrasco@isglobal.org
Florian Matt\textsuperscript{f}, florian.matt82@googlemail.com
Maria Forast\textsuperscript{a,b,g}, maria.foraster@isglobal.org
Tania Mart\textsuperscript{a}, tania.martinez@isglobal.org
Albert Ambro\textsuperscript{a}, albert.ambros@isglobal.org
Marta Cirach\textsuperscript{a}, marta.cirach@isglobal.org
David Martinez\textsuperscript{a}, david.martinez@isglobal.org
Jordina Belmonte\textsuperscript{b}, jordina.belmonte@uab.cat
Mark Nieuwenhuijsen\textsuperscript{b}, mark.nieuwenhuijsen@isglobal.org

Author affiliations:

\begin{itemize}
\item[a)] Barcelona Institute for Global Health (ISGlobal-CREAL), Barcelona, Spain
\item[b)] Center for Energy Development and Health, Colorado State University, Colorado, USA
\item[c)] Department of Environmental and Radiological Health Sciences, Colorado State University, Colorado, USA
\item[d)] Centre for Environmental Policy, Imperial College London, London, UK
\item[e)] Centre for Epidemiology and Screening, Department of Public Health, University of Copenhagen, Copenhagen, Denmark
\item[f)] Biological Safety & Risk Management Working Group, Institute Straumann AG, Basel, Switzerland
\item[g)] Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland
\end{itemize}
Background: Exposure to air pollution is known to affect both short and long-term outcomes of the cardiopulmonary system; however, findings on short-term outcomes have been inconsistent and often from isolated and long-term rather than coexisting and short-term exposures, and among susceptible/unhealthy rather than healthy populations.

Aims: We aimed to investigate separately the annual, daily and daily space-time-activity-weighted effect of ambient air pollution, as well as confounding or modification by other environmental (including noise) or space-time-activity (including total physical activity) exposures, on cardiopulmonary outcomes in healthy adults.

Methods: Participants (N=57: 57% female) had indicators of cardiopulmonary outcomes [blood pressure (BP), pulse (HR) and heart rate variability (HRV {SDNN}), and lung function (spirometry {FEV₁, FVC, SUM})] measured on four different mornings (at least five days apart) in a clinical setting between 2011 and 2014. Spatiotemporal ESCAPE-LUR models were used to estimate daily and annual air pollution exposures (including PM₁₀, PM_{coarse}, but not Ozone {derived from closest station}) at participant residential and occupational addresses. Participants’ time-activity diaries indicated time spent at either address to allow daily space-time-activity-weighted estimates, and capture total daily physical activity (total-PA), in the three days preceding health measurements. Multivariate-adjusted linear mixed-effects models (using either annual or daily estimates) were adjusted for possible environmental confounders or mediators including levels of neighborhood noise and greenness. Causal mediation analysis was also performed.
separately considering these factors as well as total-PA (as metabolic-equivalents-of-task {METs}). All presented models are controlled by age, height, sex and season.

Results: An increase in 5 µg/m³ of daily space-time-activity-weighted PM\textsubscript{Coarse} exposure was statistically significantly associated with a 4.1% reduction in total heart rate variability (SDNN; \( p = 0.01 \)), and remained robust after adjusting for suspected confounders [except for occupational-address noise (\( \beta = -2.7, p = 0.20 \)]. An increase in 10 ppb of annual mean Ozone concentration at the residential address was statistically significantly associated with an increase in diastolic BP of 6.4 mmHg (\( p < 0.01 \)), which lost statistical significance when substituted with daily space-time-activity-weighted estimates. As for pulmonary function, an increase in 10 µg/m³ of annual mean PM\textsubscript{10} concentration at the residential address was significantly associated with a 0.3% reduction in FVC (\( p < 0.01 \)) and a 0.5% reduction in SUM (\( p < 0.04 \)), for which again significance was lost when substituted for daily space-time-activity-weighted estimates. These associations with pulmonary function remained robust after adjusting for suspected confounders, including annual Ozone, as well as total-PA and bioaerosol (pollen and fungal spore) levels (but not residential address greenness {\( \beta = -0.22, p = 0.09; \beta = -0.34, p = 0.15 \), respectively}). Multilevel mediation analysis indicated that the proportion mediated as a direct effect on cardiopulmonary outcomes by suspected confounders (including total-PA, residential-greenness, and occupational-noise level) from primary exposures (including PM\textsubscript{10}, PM\textsubscript{Coarse}, and O\textsubscript{3}) was not significant.

Conclusion: Our findings suggest that increased daily space-time-activity-weighted PM\textsubscript{Coarse} levels significantly affect cardiac autonomic modulation (as reduced HRV). Additionally, increased annual levels at the residential address of Ozone and PM\textsubscript{10} significantly increase diastolic BP and reduce FVC/SUM, respectively, among healthy adults. These associations typically remained robust when adjusting for suspected confounders. Occupational-address noise and residential-neighborhood greenness levels, however, were seen as mediators of HRV and FVC/SUM, respectively. Total-PA was not seen as a mediator of any of the studied outcomes.

KEY WORDS

Air pollution; Noise; Physical activity; Greenness; Cardiovascular health; Lung function
ABBREVIATIONS

BP, blood pressure; HR, heart rate (pulse); FEV₁, forced expiratory volume in first second; FVC, forced vital capacity; SUM, largest sum of FEV₁ and FVC; PA, physical activity; PM₁₀, particulate matter of <10 µm diameter; PM₉·₅, particulate matter of 10 to 2.5 µm diameter; PM₂·₅, particulate matter of <2.5 µm diameter; NO₂, nitrogen dioxide; NOₓ, nitrogen oxides; O₃, ozone.

1. Introduction

Ambient air pollution exposure has been well-established as an adversary to public health (WHO Regional Office for Europe 2015), especially airborne particulate matter (PM) of a size considered inhalable (Kim et al. 2015), through long-term studies. It is known to exacerbate underlying conditions of the cardiovascular (Hoek et al. 2013) and pulmonary (Langrish et al. 2012; Qian et al. 2013) systems. Short-term effects of exposure in healthy people, however, are less studied and have been shown as inconsistent in association (Panasevich et al. 2009), particularly for measures such as heart rate variability (HRV; reflecting acute changes in cardiac autonomic modulation, with reduced variability indicating heightened cardiovascular disease risk) (Cole-Hunter et al. 2016; Weichenthal 2012). Moreover, it is believed that the major impact of air pollution exposure may be through eliciting subclinical and asymptomatic effects traditionally not easily detected or reported for investigation (WHO Regional Office for Europe 2006).

Existing studies have shown daily variation in exposure to ambient PM₁₀ and PM₂·₅ (as respirable), and gaseous nitrogen dioxide (NO₂) and Ozone (O₃), to be linked to acute pulmonary (Ostro et al. 2001) and cardiovascular (Qian et al. 2013; Künzli et al. 2010; Chuang et al. 2007) health risk outcomes. Moreover, levels considered generally safe by regulatory authorities have been suggested to also increase the daily (Beelen et al. 2014) and even hourly (Wellenius et al. 2012) risk of adverse health outcomes. As well as variations in time, it is important to investigate more finely the ambient co-exposures and space-time-activity behaviors that occur in an urban environment, for the
consideration of modification or modulation effect. This may be better done in real-world conditions rather than controlled human exposure studies.

For example, exposure to PM in combination with Ozone (O₃) has been seen to increase diastolic blood pressure (BP; as with reduced HRV, such a change indicating a heightened risk) among healthy adults (Brook et al. 2009).

Noise, as a different form of pollution emitted with traffic, also needs to be considered in the complex, urban ambient mix. Health effects of ambient noise have been observed in combination with associated traffic-related air pollution components, confounding associations with health outcomes (Beelen et al. 2008; Kluizenaar et al. 2007; Leon Bluhm et al. 2007). Other confounders may exist in an urban (built) environment, including public space (such as parks, or green spaces promoting greenness) and transport infrastructure. A consequence of urban greenness can be natural sources of aerosols (‘bioaerosols’), of which daily level variations have been associated with adverse pulmonary outcomes (e.g. reduced lung function), after adjusting for air pollutants, in some populations (Zhang et al. 2012; DellaValle & Triche 2012; Carracedo-Martinez et al. 2008). In addition, real-world (complex) exposures in experimental studies with traffic-related air pollution among healthy adults have shown mitigations in cardiovascular health outcomes through intermittent, moderate physical activity emulating active mobility (Matt et al. 2016; Cole-Hunter et al. 2016; N. Kubesch et al. 2015). Considerations of the above exposures combined have been made in a recent health impact assessment which relies on evidence produced by experimental and epidemiology studies, which in turn rely on exposure-assessment methods such as modelling (Mueller et al. 2017).

A number of studies have used land-use regression modeling to estimate ambient air pollution exposure concentrations, including that of PM₂·₅ and NO₂, at the residential addresses of study participants (Henderson et al. 2007; Eeftens, Tsai, et al. 2012; Wang et al. 2013; Hoek et al. 2008). Noise exposure levels have also been modelled (Xie et al. 2011), and links to cardiovascular health outcomes have been made also considering air pollution co-exposure (Beelen et al. 2008). A recent review, however, has called for more personal methods (Steinle et al. 2013) such as time-activity diaries to determine micro-environment exposure sources and periods such as commuting (Morabia et al. 2010) or co-inhabiting with environmental tobacco smoke (Aquilina et al. 2010). Another recent review has called for more studies using traffic-related pollution
indicators to properly assess confounding of health effects by noise or air pollutants (Tétreault et al. 2013).

Accordingly, the current study aimed to augment evidence on the effects of annual and daily space-time-activity-weighted estimates of ambient air pollution, as well as consider confounding or mediating effects of co-exposures including neighborhood noise, daily bioaerosol and total physical activity (alongside other space-time-activity) levels, on short-term intermediary cardiopulmonary health indicators (outcomes) among healthy adults.

2. Methods

2.1. Study Design and Protocol

The current analysis makes use of data collected in two separate (but methodologically comparable) experimental case-crossover studies, within the TAPAS (de Nazelle et al. 2011) and EXPOsOMICS (Vineis et al. 2016) projects, which ran from 2011 to 2014. As part of these experimental studies, repeated measures were taken of a set of baseline health markers on four occasions (sessions) by each participant at the same study clinic. Each of these measures took place in the morning between 07:00 to 08:00 h to minimise diurnal variation. The four sessions of a participant took place at least five days apart on either a Tuesday, Wednesday or Thursday to avoid weekend-influenced environmental quality parameters, e.g. air quality due to traffic congestion. The period of participation per participant lasted from one to four months, depending on availability of participants, study personnel and equipment.

See Figure S1 for a flow chart of the study design.

Participants provided their home and work addresses and were asked to fill out time-activity diaries for the three days prior to attending each session. Information on activity, time and location from these diaries were combined with spatially and temporally resolved air pollution maps to reconstruct home-based and space-time-activity based exposure (and co-exposure) models assessing both annual exposures and exposure in the three days prior to each session.
2.2. Participants

Inclusion criteria for participation were to be: aged 18–60 years; non-smoking (since at least one year prior to the study); living with non-smokers; of normal weight (BMI 18.5–30; not underweight or clinically obese); not pregnant (self-reported); not knowingly needing medication (except oral anti-contraceptives) during the last three months, during the study, nor foreseen in the near future, including anti-allergic treatments (local and systemic, antihistaminic or corticosteroid); not having any previously diagnosed chronic medical conditions (e.g. asthma or diabetes); not having symptoms of an acute pulmonary infection in the last 14 days, and; performing spirometry above the Lower Limit of Normal for FEV₁ and >0.70 for FEV₁/FVC ratio according to the ERS/ATS COPD Guidelines (Celli & Macnee 2004).

A blood sample given during each clinical visit was considered (e.g. eosinophil concentrations within normal range, 0.05–0.5x10³ / µl) for current pulmonary infections. See Table 1 (Results) for participant characteristics.

2.3. Study location

The study was performed in Barcelona, Spain. This study area, along with the research unit site (clinic at the Biomedical Research Park of Barcelona, PRBB) have been described previously (Matt et al. 2016; Cole-Hunter et al. 2016). Participant residential addresses, as well as various air pollutant, noise, and bioaerosol (pollen and fungal spore) measurement sites, were in Barcelona city and the greater surrounding region. Pollen sampling occurred at a single trap in the centre of the city and was analysed at the Institute of Environmental Science and Technology, Autonomous University of Barcelona (ICTA-UAB). See Figure 1a, and Figure S2, for geographical context of the study sites.

2.4. Exposure measures

2.4.1. Time-activity diary

Participants were required to complete an intensive time-activity diary detailing (per 30-minute periods) their activity and location for three days prior to performance of health measurement sessions. Questions included the time spent indoors and outdoors and the specification of activities, the time and location spent performing
physical activity (PA: METs; Ainsworth et al. 2011), and irritant exposures (e.g. environmental tobacco smoke, ETS). This diary also included information on the mode of transport which participants used in the three days prior to health measurement and to arrive at the site of measurement. Transport modes were categorized, considering together for example public transport as metro and bus use and walking and cycling as active transport.

The template for this diary can be made available upon request from the corresponding author.

2.4.2. Air pollution and noise

Exposure estimates of air pollution and noise were based on modeled levels at participant residential and occupational addresses during the year of and three days prior to health measurements.

Briefly, air pollution exposure estimates at participant addresses for two exposure periods were created: annual estimates at the residential address during the year of study, and; daily estimates, at the residential address or weighted for time spent at both the residential and occupational address (with the latter approach described in a following paragraph), during the three days preceding the health measurements. This was limited to addresses that had air pollution (PM$_{10}$, PM$_{coarse}$ (PM$_{10-2.5}$), PM$_{2.5}$, NO$_2$, NO$_x$) estimates created by the ESCAPE project method of the Barcelona region (Beelen et al. 2013; Eeftens, Beelen, et al. 2012). For the annual estimates, a spatial (annual mean) model created in 2007 for the ESCAPE project was weighted to 2009 using the ratio of 2011 and 2007 annual means from a single regional air pollution monitoring station (Vall d’Hebron <http://www14.gencat.cat/icqa/AppJava/start.do>). For the daily estimates, daily mean values were used to further temporally-weight the 2009 weighted estimates from nearest available air pollution monitoring stations. See Figure 1b for an illustrative example of PM$_{2.5}$ (as PM$_{10}$ maps were not available, although PM$_{2.5}$ is highly-correlated here with PM$_{10}$ ($r^2=0.73$; Table S1)).

Ozone (O$_3$) data for the Barcelona province was obtained online from the Department of Territory and Sustainability (Generalitat de Catalunya 2016) as the
ESCAPE model (used for other pollutants as described above) does not calculate O₃. Annual average O₃ values (averaged from January to December, regardless of study participation month) were assigned to participant addresses from the closest reference station according to study participation year (2011, 2013 or 2014).

For daily time-weighted air pollution estimates attributed to a participant based on time-activity-reported (diary) location (i.e. time spent at residential or occupational address), when both addresses had assignable values then the sum of the two were weighted as a proportion of the day (e.g. 8 hours at occupation plus 14 hours at residence equals 22 hours {or occupational 36% + residential 64% = 100%}) attributable out of 24 hours in a day) was created. No attempt was made to attribute exposure to time spent at addresses other than residence or occupation, so not all time-activity-weighted values summed completely to 24 hours but instead were a proportional value of the estimates available at the residential or occupational address.

A reasonable amount of modelled air pollution data was expected to be missing due to the way in which it is collected; the ESCAPE land-use regression model estimations are restrained spatially to geocodable addresses within the area that it covers (Figure S2), and; temporal (e.g. daily) weighting of estimations are dependent on air monitoring station data being available for that time period. When occupational address values were unavailable (e.g. missing station data, no occupational address {or address outside of modelled area}) then only residential address values were assigned for the 24-hour period so that all participants had complete (100%) values. These daily values were summed to make three-day totals and then three-day means. Annual air pollution estimates were simply spatially-weighted according to the residential address as participant time-activity for this time period was not known, however expected to predominantly be at the residence. As with air pollution, noise levels were estimated for all participant residential and occupational addresses where data was available from the Barcelona strategic noise map developed in the year 2007 (Ajuntament de Barcelona 2010) derived under the EU Directive 2002/49/EC for noise mapping, based upon previous work (Ministere de L’Environnement, 1980) and described in a previous publication (Cole-Hunter et al. 2015). Briefly, the model considers: the number of light vehicles, the number of heavy vehicles, the speed of circulation, the street
slope, the type of pavement of the street, and reflections of the building on either side of the road. The EU indicator used in the current study, $L_{DEN}$ (day-evening-night noise levels, in decibels {dB}), was calculated as the geometric mean of the day, evening, and night-averages, with 5 dB and 10 dB penalties for the evening and night periods, respectively, (according to the EU Directive 2002/49/EC), for a given address.

While participant addresses were not necessarily checked for changes between sessions (i.e. moved residence/occupation), the assigning of annual and daily exposure estimates were done per session. While a participant may have moved residence/occupation during their participation, this was not expected (within less than four months maximum).

Attribution of model estimates was performed in ArcGIS (ESRI® ArcMap TM 10.0, ArcGIS Desktop 10 Service Pack 4 and Spatialite 4.1.1).
Figure 1 – Study areas: (a) topography of Barcelona, and; (b) air quality of Barcelona (2009 annual-spatial ESCAPE-LUR model for PM$_{2.5}$); including participant addresses and sampling sites.

PRBB = research unit (health measurement) site. XAC-ICTA-UAB = pollen analysis site.

2.4.3. Bioaerosols

Daily bioaerosol (pollen and spore) counts for Barcelona were provided by the Catalan Network of Aerobiology at the Institute of Environmental Science and Technology, Universitat Autònoma de Barcelona (XAC ICTA-UAB).

Concentrations of pollen and fungal spores taxa were assessed according to the Spanish Network of Aerobiology norms (Galan et al. n.d.) and the minimum requirements of the European Aerobiology Society (Galan et al. 2014). Tree species included Birch (*Betula*), Cypress family (*Cupressaceae*), Ashes (*Fraxinus*),Privet
(Ligustrum), Olive (Oleaceae), Palm (Areceaceae), Pine (Pinus), Plane (Platanus) and Oak (Quercus). The sole shrub species was Hazelnut (Corylus). Herb species included Mugwort (Artemisia), Grass (both cereal and wild varieties; Poaceae), Plantain (Plantago), Knotweed (Polygonaceae), Goosefoot (Chenopodiaceae/Amaranthaceae) and Nettle (Urticaceae). The sole fungal species was Alternaria.

Daily regional values (not spatially-relevant to participant addresses), of the individual species and total counts, were initially included in basic models as the primary exposure. Only total counts were included in final models as a sensitivity analysis (confounding exposure) due to insignificant associations in basic models.

2.4.4. NDVI

Residential and occupational addresses were assigned Normalised Difference Vegetation Index (NDVI) values (calculated as continuous variables averaged over an area: minimum; 5th-percentile;mean; standard deviation; median; 95th-percentile; maximum) within 100, 300 and 500m radius buffers. These values, corresponding to Spring (mid-April, as the greenest part of the year) were obtained from Landsat 8 TM at 30 x 30 m resolution (U.S. Geological Survey 2013). Details of this measurement method have been given previously, particularly for Barcelona city (Gascon et al. 2016). Attribution of these values was performed in ArcGIS (ESRI® ArcMap TM 10.0, ArcGIS Desktop 10 Service Pack 4 and Spatialite 4.1.1).

2.5. Health measures

All study participants were asked to abstain from alcohol for 24 hours and from caffeinated drinks for at least four hours prior to health measurement sessions. Furthermore, volunteers were asked not to perform heavy exercise during the preceding 48 hours of each session. All measurements were taken in an atmosphere-controlled clinic at the PRBB (Figure 1).

2.5.1. Cardiovascular health

Blood pressure (BP, diastolic and systolic) and pulse (heart rate, HR) were measured by trained technicians using an automated BP monitor (Omron Healthcare, Japan).
Participants were instructed to sit quietly for 10 minutes before BP was measured from the left upper arm. Up to three BP measurements were taken with a pause of two minutes in-between; if the difference of either systolic or diastolic readings were \( \geq \) five mmHg, a third measurement was performed. The value used for analysis is the average of the two accepted readings.

Holter monitors (ModelCardioLight, Gem-Med, ESP) were used to collect five-minute moving averages of heart rate variability (HRV) after a ten-minute rest period. Raw data was processed to give the following measures, as previously described (Cole-Hunter et al. 2015): time-domain (standard deviation of normal-to-normal intervals (SDNN); root mean square of successive differences in adjacent NN intervals (RMSSD), and; frequency-domain [low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.40 Hz)] power, plus the ratio of LF to HF (LF:HF).

2.5.2. Pulmonary function

Following cardiovascular health measurements, pulmonary function (forced expiratory volume in first second \( \{\text{FEV}_1\} \), forced vital capacity \( \{\text{FVC}\} \), and largest sum of \( \text{FEV}_1 \) and \( \text{FVC} \) \( \{\text{SUM}\} \)) was tested by trained technicians using a portable computerised spirometer (EasyOne, NDD Medical, Switzerland) according to standards of the American Thoracic Society and European Respiratory Society (Miller et al. 2005). The process of selecting maneuvers has been described previously by two associated studies (Matt et al. 2016; N. J. Kubesch et al. 2015).

2.6. Data analysis

Health measurements were modelled as an outcome with annual and daily exposure estimates adjusted for expected confounders including ambient co-exposures and space-time activities. Firstly, annual (spatial, at residence) and daily (spatiotemporal, proportional mean of three days prior to health measurement; at residence and occupation, if data available) exposure estimates were included separately in basic models. Other environmental factors (e.g. noise, pollen) were then considered in adjusted (e.g. co-exposure/ ‘complex’) models, along with adjustments for activity-related exposures (e.g. PA, ETS).
Figure 1a/b (and Figure S2) was produced using ArcGIS (ESRI® ArcMap TM 10.0, ArcGIS Desktop 10 Service Pack 4). Description and modeling of data were performed (and Figure S3 created) using STATA/SE (v14.2 for Windows). Causal mediation analyses were performed (and Figures 3 to 5 were produced) using R [v3.3.1 for Windows (R Core Team 2016)] in RStudio [v1.0.136 for Windows (RStudio Team 2016)].

2.6.1. Preparing data

Two participants were involved in both the first and second exposure study (TAPAS and EXPOsOMICs; previously described) and so maintained the same ID for both data sets to account for their additional repeated measures (i.e. a total of eight, rather than four measures as with all other participants {only participating in one of the two studies}).

All air pollution values except those for PM$_{2.5\text{abs}}$ were converted from absolute to standard increments as per the ESCAPE method (Beelen et al. 2014): PM$_{10}$ and NO$_2$ as 10 µg/m$^3$; PM$_{\text{Coarse}}$ and PM$_{2.5}$ as 5 µg/m$^3$, and; NO$_x$ as 20 µg/m$^3$. PM$_{2.5\text{abs}}$ was left as an absolute value.

O$_3$ was converted from µg/m$^3$ to ppb to relate to, and therefore augment evidence from, previous work (Jerrett et al. 2009) [1.0 µg/m$^3$ = 0.506 ppb, at 24°C, 1.004 atm]. Atmospheric pressure and temperature averages were obtained to assist in accurate conversion (Moreno-Grau et al. 1998).

Daily bicycle use, as a time-activity exposure, was categorized to “yes” (≥0.5 hours) or “no” (0 hours) since absolute (hourly, continuous) values were considered too low for statistical power purposes. The same approach was used for other time-activities. The primary exposures for analysis purposes, derived from participant time-activity diaries besides time spent at residence and occupation, was the daily amount of METs (physical activity) and ETS (second-hand smoke). All activities reported in time-activity diaries were converted to METs and summed to a daily total amount.

Pollen data remained as provided by XAC ICTA-UAB, as mean daily concentrations (counts per cubic metre; pollen/m$^3$, spores/m$^3$).
2.6.2. Describing data

Prior to modelling, participant and environment descriptive data was checked for normality using Shapiro-Wilk normality tests. This determined if either an ANOVA or Kruskal-Wallis test should be used for each variable (i.e. if normally or abnormally distributed, respectively) to determine significant variance between sex, and so on. Pearson’s pairwise correlation coefficients were obtained prior to performing basic mixed effect models (including single outcome and single exposure {annual/daily estimation} plus confounders), between days comparing for space-time-activity values.

2.6.3. Modeling data

2.6.3.1. Variable selection for preparing models

Regression coefficients were first obtained as outputs from separate linear mixed effects models for the association of each available participant characteristic (age, height, weight, BMI, sex) and each exposure variable [air pollution (NO₂, NOₓ, O₃, PM₁₀, PMcoarse, PM₂·₅), noise, fungal and pollen spores, weather (regional temperature, humidity and air pressure), PA, neighborhood-greenness and neighborhood-noise] with the individual health outcomes. Personal characteristics and exposure variables with an unadjusted-regression coefficient with a p-value <0.1 were further selected as covariates to be considered in adjusted models and to explore modification by co-exposure variables on the association between air pollutants and the health outcomes. As the participant characteristics of height, weight and BMI are expected to be highly-correlated within an individual, model output is used to indicate which one to select for adjustment in models and avoid over-adjustment of coefficient. The final models were obtained using a step-wise backwards elimination process in which only covariates with coefficients having p-values <0.05 finally included. This method of inclusion allows us, considering the bivariate analyses, to select those variables that become predictive in the presence of the rest of the possible predictive variables.
2.6.3.2. Adjusting models

Final adjusted models for each health outcome (i.e. heart rate variability, blood pressure, and pulmonary function) were controlled for age, sex, height, and season (suggestive of meteorological and bioaerosol levels) (here onwards referred to as “model”).

Estimated ambient air pollution levels were considered (in separate models) in three ways (e.g. shown in supplementary Figure S3, from left to right), as:

1. Annual (2009) average spatial value at residential address only;
2. Daily-weighted (2011 or 2013/2014, depending on participation date) spatial value at residential address only, and;
3. Daily space-time-activity-weighted total of the time spent at residential and (if available) occupational address (as reported by a participant’s time-activity diary).

2.6.3.3. Testing models with sensitivity and mediation analyses

Sensitivity analyses were performed by adjusting models for other factors that may have confounded model outcomes. Factors included transport mode on the morning of health measurement, in addition to PA and ETS levels, as well as other specific irritants during the immediate (three) days prior to measurement. Commute mode in the three days prior to health measurement were considered as a total time spent in-commute when adjusting models; if a participant used more than one mode then the mode occupying the highest proportion of time was only considered; if the times spent in more than one mode were equivalent, then the physically-active mode (e.g. bicycling) was solely considered. Modes were investigated individually in this way, and then categorically, derived into active, private motorised and public motorised transport mode groups to increase power.

To assess potential causal mechanisms (or estimate causal mediation effect by primary exposure on a modelled outcome), causal mediation analysis was performed on variables that, when included as an adjustment to models, changed the Beta coefficient by 10% or more [e.g. three-day total physical activity level (total-PA), residential-neighborhood greenness (NDVI) and
occupational-address noise (L<sub>DEN</sub>). Using a previously-suggested approach for multilevel mediation analysis (Bauer et al. 2006), we considered the average results of causal mediation, including ACME (average causal mediation effect, which is the average indirect effect), ADE (average direct effect) and Proportion Mediated. This was done using the ‘lme4’ and ‘mediation’ packages of R (Bates et al. 2015). The library was conceived for clinical trials, however also concedes for epidemiological studies with continuous variables. The results of this analysis are to be interpreted as the proportion mediated (expected difference in the outcome) by one exposure or environmental factor from a primary exposure of interest.
3. Results

3.1. Descriptive analyses

3.1.1. Participants

Initially, a total of 62 participants were recruited having satisfied inclusion criteria upon screening. Three participants were excluded from the study after the first health measurement session had shown underlying health issues (e.g. sub-normal pulmonary function) pertaining to exclusion criteria. One participant only completed one study session, and so was excluded from all analyses. Further, one spirometric session of another participant did not fulfill reproducibility criteria and so this single participant-session data point was excluded from all analyses. Another participant exceeded BMI range pertaining to exclusion criteria and so was excluded also. Finally, a total of 57 participants performed satisfactory health measurement sessions on four different occasions (excluding the one spirometric session) of at least five days apart. This gave a total of 231 participant-session datasets.

When stratifying participants by sex, we observed that males and females were not significantly different in age, however males had a significantly-higher mean body mass index (BMI) and height than females (Table 1). As predicted, absolute pulmonary function as well as resting (baseline) systolic BP and HR values were significantly different across sex: females, compared to males, on average measured significantly lower in FEV1, FVC, and systolic BP, however significantly higher in baseline heart rate (Table 1).

When running basic models with separate participant characteristics, model output indicated that height was more strongly associated than BMI with all related health outcomes.

| Table 1 – Participant characteristics and cardiopulmonary baseline health measures: of total group and according to sex |
| Measure | All\(^*\)[n=231 (N=57); 100\%] | Sex diff.\(^#\) | Female\(^+\)[n=107 (N=27); 54\%] | Male\(^+\)[n=120 (N=30); 46\%] |
| Age (years) | 34 ± 8 (21–52) | n.s | 34 ± 8 (24–52) | 34 ± 8 (21–52) |
**BMI** (kg/m²) 23.1 ± 3.0 (18.2–29.6) ** 22.1 ± 3.1 (18.2–29.6) 24.1 ± 2.5 (19.0–28.4)

**Height** (cm) 168 ± 22 (150–187) ** 161 ± 6 (150–172) 174 ± 6 (155–187)

**Cardiovascular health measure**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>68 ± 8 (51–89)</td>
<td>**</td>
<td>71 ± 6 (55–84)</td>
</tr>
<tr>
<td>BP Diastolic (mmHg)</td>
<td>67 ± 8 (51–89)</td>
<td>n.s.</td>
<td>67 ± 8 (53–89)</td>
</tr>
<tr>
<td>BP Systolic (mmHg)</td>
<td>100 ± 11 (75–131)</td>
<td>*</td>
<td>95 ± 10 (75–121)</td>
</tr>
</tbody>
</table>

**Pulmonary function measure**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ (L)</td>
<td>3.5 ± 0.7 (2.3–5.1)</td>
<td>**</td>
<td>3.0 ± 0.4 (2.3–4.0)</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>4.4 ± 1.0 (2.8–6.4)</td>
<td>**</td>
<td>3.6 ± 0.6 (2.8–4.9)</td>
</tr>
<tr>
<td>SUM (L)</td>
<td>8.1 ± 1.9 (5.1–12.6)</td>
<td>**</td>
<td>6.5 ± 1.0 (5.1–9.7)</td>
</tr>
</tbody>
</table>

Values presented as Mean ± Standard Deviation (Range); ANOVA / Krallis test for difference, between sex; n, number of measurements; N, number of participants; BMI, body mass index; FEV₁, forced expiratory volume in first second; FVC, forced vital capacity; SUM, largest sum of FEV₁ plus FVC; BP, blood pressure; HR, heart rate. To indicate sex differences, ANOVA or Kruskal-Wallis test was performed with normally or abnormally distributed variables, respectively:* p < 0.05, ** p <0.01, “n.s.” = p ≥ 0.05. Participants had HR and BP measured prior to spirometry performance.
3.1.2. Space-time exposure weightings

We could not fully weight daily exposure estimation for time spent at residential and occupational addresses due to incomplete data availability. All residential addresses were geocodable and thus modeled air pollution (i.e. all pollutants except for O₃) exposure estimates could be applied; however, many occupational addresses were not geocodable and thus such data were missing for 22 (of 57) participants (and hence respective missings seen in Table 2). When using annual estimates, the range of exposure was much narrower than when using either daily or daily space-time-activity-weighted estimates. The median of exposure was higher for PM₁₀, and lower for O₃, with annual estimates than with daily space-time-activity-weighted estimates. Both of these annual estimates, however, remained within the range of their respective daily estimates. See supplementary Figure S3.

Ozone data was infrequently unavailable for the Barcelona province, and therefore not attributable to the residential and/or occupational addresses of participants for 11 of 227 cases (i.e. total participant-days). Similarly with noise estimates; only 36 of 57 residential addresses were modelled, while only 21 of 57 occupational addresses were modelled (or the participant did not have an occupation). See Table 2.

The level of noise (Lデン) at residential/occupational address, and greenness (as 5th-percentile of NDVI within 100m buffer surrounding residential/occupational address) exposure varied across participant addresses. PA and ETS varied greatly across participants and were not typically correlated between study days (r² <0.6. Pollen concentrations were available for most days (223 of 227), and again varied greatly between them. See Table 2.

See Table S1 for correlations between exposure estimations.

<table>
<thead>
<tr>
<th>Table 2 – Environmental exposure descriptives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Air pollution, annual</td>
</tr>
<tr>
<td>NO₂</td>
</tr>
<tr>
<td>Residence</td>
</tr>
<tr>
<td>Occupation</td>
</tr>
<tr>
<td>NOx</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td><strong>Residence</strong></td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>O₃</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PM₂·₅</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PM₁₀</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PM_{Coarse}</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Greenness, lower-5^{th}%</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Noise, L_{DEN}</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Pollen, 3-day-total</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Time-activity,</strong></td>
</tr>
<tr>
<td><strong>MET</strong></td>
</tr>
<tr>
<td><strong>ETS</strong></td>
</tr>
</tbody>
</table>

Abbreviations: NDVI = normalized digital vegetation index; lower-5^{th}% = lower fifth percentile; L_{DEN} = daily weighted-average of noise; dB(A) = A-weighted decibels; p/m³ = pollen per cubic metre; MET = metabolic equivalents of task; ETS = environmental tobacco smoke.
Due to the large number of exposures and outcomes involved in this analysis, only the exposure set and outcomes that showed significant associations (or evidence suggesting so) are presented in the following sub-sections (and supplementary material). All pollutants and all outcomes were modelled, however results (for brevity) are not shown (but can be requested from the corresponding author).

### 3.2.1. Adjusted models

When adjusting models for the season of health measurement, or separately for the day of week (either Tuesday, Wednesday or Thursday) of health measurement, model estimates of health outcomes remained robust (results not shown). Moreover, models adjusted for transport mode (e.g. walking and bicycling as active transport) use, as a proxy for transport-related exposure, in the three days prior to health measurement did not show significant associations between transport mode use and any health outcomes (results not shown). Furthermore, including transport mode/s used to arrive at the site of health measurement as a sensitivity analysis did not alter association between any exposure and health outcome.

#### 3.2.1.1. Cardiovascular health

Annual PM\textsubscript{Coarse} increments of 5 \(\mu g/m^3\) were not observed as significantly associated with SDNN \((p=0.091)\). The same exposure estimate when weighted for daily space-time-activity, however, was significantly (negatively) associated with a reduction in total heart rate variability (HRV) of 4.35 \% (SDNN, as proxy; \(p=0.011\)). See Figure 2 and Table S3.
Figure 2 – Annual (H-A) and three-day-average (H-D, H-W-D) PM\textsubscript{Coarse} models of overall heart rate variability; unadjusted (m0) and adjusted (m1–m4)

H-A = annual estimate at residential address; H-D = three-day average estimate at residential address prior to outcome measurement; H-W-D = three-day average estimate weighted for time at residential and occupational address prior to HRV (as standard deviation of N-N interval, SDNN) outcome measurement.

SDNN = Standard deviation of normal-to-normal intervals (indicating overall heart rate variability)

Models (x-axis sub-labels):

- m0 = PM\textsubscript{Coarse} (Residential-Occupational-Daily, 5 µg/m\textsuperscript{3}) + age, sex, height, season;
- m1 = m0 + O\textsubscript{3} (Residential-Annual, 10 ppb);
- m2 = m0 + Residential- neighborhood Greenness (NDVI);
- m3 = m0 + PA (Total);
- m4 = m0 + Occupational-address noise (L\textsubscript{DEN})

Annual Ozone (O\textsubscript{3}) increments of 10 ppb were observed as significantly (positively) associated with an increase in diastolic blood pressure of 6.42 mmHg ($p=0.003$) (Figure 3 {and Figure S4a, Table S5a}).
Daily space-time-activity-weighted estimates did not show signification associations with any cardiovascular health outcomes besides SDNN.

Heart rate (pulse) was not significantly associated (although positively trending) with annual or daily space-time-activity-weighted O3 exposure estimate increments.

3.2.1.2. Pulmonary function

Separate components of air pollution were seen to affect pulmonary function at annual mean concentrations when adjusting for indicated personal and environmental factors, respectively.

Annual particulate matter (PM10) exposure estimate increments of 10 µg/m³ were observed as significantly negatively-associated with FVC ($p=0.003$) and SUM ($p=0.009$), and approaching significance of negative association with FEV1 ($p=0.055$) (Figure S4b, Table S5b).

Similarly, annual respirable PM (PMCoarse) exposure increments of 5 µg/m³ were observed as significantly negatively-associated with FVC ($p=0.002$) and SUM ($p=0.008$), but not FEV1 ($p=0.074$).

When daily levels of both PM10 and PMCoarse were weighted for space-time-activity the significance of association with pulmonary function in models was lost (Figure S4b, Table S5b).

3.2.2. Complex models

3.2.2.1. Cardiovascular outcomes

Further adjusting models of daily space-time-activity-weighted PMCoarse with daily weighted-average noise estimates at the occupational address led to a loss of significance of association with total HRV (SDNN, as proxy) (-2.75%; $p=0.20$) (Model m4; Figure 2, Table S3). This was not the case, however, with noise estimates at the residential address.
Adjusting models of annual O₃ with annual PM₁₀ did not substantially alter associations with cardiovascular outcomes – diastolic BP remained significantly positively-associated with an increase of 7.60 mmHg (Model m1; Figure 3, Table S4a).

Adjusting models with residential-neighborhood greenness (as the lower 5th percentile of NDVI within a 500 m radius buffer around the residential address) did not alter cardiovascular outcome associations with annual O₃ (Models m2; Figure 2, Table S5).

The daily weighted-average estimate of noise at the occupational address, however, did alter associations of diastolic BP with annual O₃ (Model m4; Figure 3, Table S4a).

Total-PA levels were not seen to substantially alter associations between annual O₃ and any cardiovascular outcomes (Model m3; Figure 3, Table S4a).
3.2.2. Pulmonary outcomes

When including annual O₃ (increments of 10 ppb) estimates with the annual PM₁₀ (increments of 10 µg/m³) estimates in models (Model m1; Figure 4, Table S4b), significant negative associations remained for both FVC (-0.24%; \( p=0.025 \)) and SUM (-0.41%; \( p=0.039 \)).

When including residential-neighborhood greenness in models of PM₁₀, significance of association was lost with FVC (-0.22%; \( p=0.088 \)) and SUM (-0.34%; \( p=0.148 \)) (Models m2; Figure 4, Table S4b). PM₉ₐᵣ₂, however, remained significantly negatively-associated with FVC (-0.15%; \( p=0.033 \)) when including residential-neighborhood greenness in models.

When adjusting models of PM₁₀ with either total-PA levels or total pollen concentrations, significant associations remained between PM₁₀ and both FVC and SUM (Models m3 and m4; Figure 4).
3.2.3. Causal mediation effects analyses

The adjusted models showed some co-exposures to be confounding effects of primary exposures on health outcomes, although none to be statistically significantly so. When investigating this confounding as mediation or moderation, the following observations were made. The proportion mediated effect on SDNN by total-PA
from daily space-time-activity-weighted PM$_{\text{Coarse}}$ was not suggestive (p = 0.98), nor was diastolic BP by total-PA from annual O$_3$ (p = 0.40). Similarly, the proportion mediated effect on FVC by total-PA from annual PM$_{10}$ was not significant (p = 0.37). See supplementary Figure S5a, S6a and S7a, respectively.

There was, however, some suggestion of mediation by co-exposures of occupational-address noise and residential-neighborhood greenness. Occupational-address noise was suggested to mediate (and antagonise) the effect on SDNN by daily space-time-activity-weighted PM$_{\text{Coarse}}$, but not significantly so (Figure S5b). Nor was occupational-address noise observed as a significant mediator of the effect on diastolic BP by daily space-time-activity-weighted PM$_{\text{Coarse}}$ (Figure S6b). Similarly, residential-neighborhood greenness was suggested to mediate the effect on FVC by residential-annual PM$_{10}$, but not significantly so (p=0.57) (Figure S7b).

### 4. Discussion

**Summary**

To our knowledge, this is the first study using repeated clinical measures of cardiopulmonary health or function among healthy adults in association with a combination of long (annual) and short-term (daily) exposures and co-exposures of multiple pollutants and time-activities. It is also the first study to weight daily exposure levels according to a time-activity diary indicating time spent at either the residential or occupational address. By doing this, we observed that when daily estimates of coarse (respirable) particulate matter (PM$_{\text{Coarse}}$) were weighted for time spent at either address daily, the negative association between annual estimates and total heart rate variability became statistically significant. The same model outcome was not seen with annual estimates of PM and other cardiovascular parameters or pulmonary function – significantly reduced FVC was only seen with annual and not daily space-time-activity-weighted estimates of PM$_{10}$. We also saw that cardiopulmonary outcomes were not mediated by total physical activity (as metabolic equivalent of task, MET) levels in the three days prior to health measurements, nor significantly by other suspected environmental confounders.
Participant characteristics including age, sex, and height were controlled for in models as they were observed separately to affect some cardiopulmonary outcomes. This has been seen previously in healthy individuals (Hankinson et al. 1999). Sex played the largest influence, with females having lower PEF yet higher FEV1/FVC compared to males. Individuals with a higher height also typically had a higher PEF. Older individuals of the current study had a lower FEF25-75\% in agreement with previous studies of healthy individuals (Chaunchaiyakul et al. 2004). Season was also controlled for. The indication that height was more controlling than BMI for health outcomes, and therefore not controlled for in final models, was observed previously in a related study (Matt et al. 2016).

Participant residential and occupational neighborhoods, and reported levels of time-activity in the three days immediate to cardiopulmonary outcome measurements, were suspected to confound the effect of pollution exposures. Multilevel mediation analysis, however, did not indicate this with our models – the proportion mediated as a direct effect on cardiovascular outcomes by occupational-address noise level, and on pulmonary outcomes by residential-neighborhood greenness, and either by total daily PA was not significant. PA was especially suspected as the benefit obtained from it is believed partly due to a reduction in BP (Ellekjær et al. 2000; Lee et al. 1999) and maintenance of cardiovascular fitness in healthy people (Cheng et al. 2003). PA obtained from active mobility may be particularly complex to consider due to elevated exposure to PA and traffic-related pollution (Cole-Hunter et al. 2012; Knibbs et al. 2011), although both experimental and epidemiological studies have shown benefits from the former outweighing detrimental effects of the latter (Matt et al. 2016; Andersen et al. 2015).

Outcomes

Cardiovascular

The finding of daily PM\textsubscript{Coarse} concentration estimates affecting cardiac autonomic regulation (HRV) is arguably the most pertinent of this study, illustrating the accuracy of weighting annual and even daily modelled estimates by time-activity diary-contrived information. Particles this coarse (2.5-10 µm) are considered inhalable (and elicit symptoms in asthmatic children), but are expected to settle in the upper airways without reaching the blood-gas barrier and thus cardiovascular system (Kim et al.
This size fraction is attributed to re-suspended road dust (from vehicle exhaust) but also pollen, and expected to travel for shorter distances than finer PM (PM$_{2.5}$) and so be more spatially-relevant (Srimuruganandam & Nagendra 2012). A controlled human exposure trial among healthy adults had seen that while HRV was not associated with PM$_{2.5}$, it was statistically so with PM$_{Coarse}$ (Samet et al. 2007). One previous study among a community-based elderly population had found that exposure to PM$_{2.5}$ (at equivalent average concentrations of $\sim$15 µg/m$^3$) led to reduced heart rate variability over a period of hours (Gold et al. 2000). While Gold and associates findings suggested that O$_3$ confounded this outcome, we did not observe similarly in our daily-weighted PM$_{Coarse}$ models when adjusting for annual average O$_3$ at the residential address. In adult-onset diabetics, Zhao and associates found that PM smaller than the coarse fraction only were significantly associated with BP (Zhao et al. 2015). Despite several previous studies performed, limited evidence has been seen for effects of black carbon (which near traffic-related emission sources can range down to ultrafine in diameter $\{PM_{0.1}\}$) on heart rate variability among health adults (Janssen et al. 2012). A meta-analysis, not reporting outcomes from PM$_{Coarse}$, did report PM$_{2.5}$ to significantly reduce HRV (Pieters et al. 2012).

Cardiovascular outcomes have been seen from modelled, short-term spatially-variable ambient concentrations of PM and other pollutants (Künzli et al. 2010; Panasevich et al. 2009), even at levels considered to be low or acceptable by regulators (Wellenius et al. 2012), suggesting that modelled PM exposures are adequate for investigating health outcomes. Controlled, shorter-term (2-hour) exposure of real-world relevant PM$_{2.5}$ levels has been shown to transiently raise diastolic blood pressure (BP), suggesting a mechanism which links traffic-related air pollution and cardiovascular disease (Urch et al. 2005). While the levels of PM$_{2.5}$ observed in our study did not indicate an association with cardiovascular outcomes (results not reported), our findings that instead concentrations of PM$_{Coarse}$ is associated with HRV may be due to limitations in different models of PM speciation used to estimate exposures.

We did not observe NO$_2$ to be associated with any outcomes measured (results not reported), which is inconsistent with previous study findings (Qian et al. 2013). We did, however, see an association between O$_3$ and diastolic BP which remained when adjusting for PM$_{10}$. Previously, PM, and not O$_3$, has been seen to increase diastolic BP in an experimental study with healthy adults (Brook et al. 2009), possibly due to
imbalancing autonomic regulation (reflected by HRV) or increased peripheral vascular resistance (Bartoli et al. 2009). Similarly, PM has previously been associated with an increase, while O₃ was associated with a decrease, in systolic BP among adult-onset diabetics (Hoffmann et al. 2012). The association observed in the current study for O₃ was lost when adjusting for daily weighted-average noise at the occupational address, which suggests confounding (or potentially over-adjusting of models). Evidence shows a diverse degree of mutual confounding when adjusting for residential noise and/or air pollution in cardiovascular health studies (in general, not only mortality), which is typically small, but depends on the study area (Tétreault et al. 2013; Foraster 2013).

Pulmonary

In models, a significant decrease in pulmonary function [forced vital capacity (FVC), and highest sum of forced expiratory volume in first second {FEV1} plus FVC (SUM)] was observed in healthy participants that lived at addresses with higher annual average estimates of PM₁₀. This observation remained robust when adjusting for annual O₃, and when considering the total amount of physical activity as well as environmental tobacco smoke exposure levels in the three days prior to pulmonary function testing. While healthy adults are less studied than those diseased, the latter are known to be more susceptible (and likely to respond) to acute exposures. Previously, daily exposure to ambient PM (both coarse and fine) has been seen to affect health outcomes in susceptible individuals such as through the exacerbation of asthma leading to hospital admission (Ostro et al. 2001; Patel & Miller 2009). Similar associations have been seen in another analysis of the same study sample, although this was reported in percent rather than absolute change (Matt et al. 2016). While we did not see daily NO₂ exposure to affect pulmonary outcomes, traffic-related NO₂ and PM are known to be highly-correlated close to the emission source (Beckerman et al. 2008), therefore PM effects may also be confounded with NO₂.

Further to anthropogenic air pollution, natural sources of aerosols (‘bioaerosols’) such as pollen and spores at elevated ambient levels have been associated with adverse pulmonary outcomes. We did not see any association between daily city-centre levels of pollen and any cardiopulmonary outcomes, perhaps due to the lack of spatial variability and the outcome measurements being made among healthy adults. Previously, pulmonary symptoms have been observed as positively associated with daily pollen
levels, after adjusting for air pollutants (including O3 and PM), among susceptible
groups including outpatients (Zhang et al. 2012) and asthmatic children (DellaValle &
Triche 2012). One study found a lag of two days between elevations in pollen levels and
pulmonary-associated medical emergency calls (Carracedo-Martinez et al. 2008),
suggesting that our three-day-total level should have been sufficiently adequate to
investigate short-term associations.

In addition to pollutants and bioaerosols, PA levels were suspected to confound health
measurements. Numerous literature associating PA levels with pulmonary function exist
but focus typically on elite athletes, the elderly, ex-smokers or chronic disease patients.
PA has been shown to mitigate pulmonary function decline that occurs with aging in
otherwise-healthy individuals (Cheng et al. 2003; Pelkonen et al. 2003). While PA has
also been shown as positively-associated with FVC, a lack of consistency in association
seen among previous literature has been noted (Twisk et al. 1998). More recent
(experimental) studies with healthy adults have shown an attenuation of pulmonary
function effects due to air pollution exposure with moderate physical activity performed
(Matt et al. 2016; N. J. Kubesch et al. 2015). The current study, however, did not
observe an attenuating (moderating) effect of three-day total PA levels on pulmonary
function in PM models. We are reporting effects of this total PA on long-term markers
of health, contrary to said previous studies which have looked at short-term impacts of
combined effects of PA and air pollution in an experimental short-term (two hour)
exposure setting. We are thus reporting a very novel result on the combined effects of
PA and air pollution, addresssing for the first time the effects of habitual exposures on
the reported intermediary health markers. Our results suggest to agree with a recent
long-term (epidemiological) study among elderly adults which showed an attenuation of
benefits from PA on respiratory mortality in high versus low air pollution (NO2)
exposure levels at the residential address (Andersen et al. 2015). This is a highly under-
studied area although we believe of increasing interest for the environmental health
community because short-term health effects of ambient air pollution exposure such as
those studied have been seen as inconsistent (Panasevich et al. 2009), and the World
Health Organisation believes that most effects are subclinical and symptomatic (WHO
Regional Office for Europe 2006). While we did not detect significant associations
between short-term (daily time-weighted) exposures and pulmonary function outcomes,
this is the first time that healthy individuals are suggested to have a measurable
reduction in pulmonary function from long-term (annual) exposure.

**Limitations** include the fact that not all participant occupational addresses could be
geocoded for exposure attribution of air pollution levels; some participants either did
not have an occupation, or did not have one fixed or geocodable occupational address.
This limited the power of analyses due to a reduced sample size of exposure data. This
also applied for analyses with noise co-exposure. While residential-neighborhood noise
is the most common noise exposure considered in the literature, the current study
variable was separated from analysis with occupational-address levels due to the high
number of missing cases of the latter. Therefore, the same number of observations did
not exist across models (m0 to m4) to compare coefficients (and their large changes
with model adjustment) reliably. This is highlighted by the very wide confidence
intervals after model adjustment for occupational-address noise. It should also be noted
that noise estimates from models represent long-term means of day-time, night-time or
24h (weighted) noise levels, so they do not capture the temporal change in the three last
days seen with air pollution estimates. For these reasons, noise was only considered as a
co-exposure for confounding or mediating air pollution effects. Air pollution models
were not adjusted for any meteorological components such as precipitation or wind
which may influence actual levels. While pollen data could be given for individual days,
levels could not be spatially-weighted (assigned to geocoded addresses) due to
measurements only being taken in one location (Barcelona city center) and models for
extrapolation not existing. Additionally, participant addresses were not necessarily
checked for changes between sessions (i.e. moved residence/occupation) when
assigning annual exposure estimates, however this was not expected to have happened
(within less than four months participation maximum) and assignments were made per
session.

**Strengths** of the current study include the broad array of environmental exposures
considered, particularly space-time activity such as PA performance and ETS exposure,
to elucidate confounding or mediating/ moderating effects of such factors on health
outcomes among healthy adults. To assign environmental exposures and associated
health outcomes to individuals, the method of land-use regression modeling used in the
current study has been shown previously as effective for ambient air pollution (Eeftens,
Tsai, et al. 2012; Wang et al. 2013), as well as when incorporating meteorology (Su et
al. 2008) and noise (Xie et al. 2011) for investigation of association with cardiovascular and pulmonary outcomes. In the current study, estimates were modelled in a similar way to previous work by Hoffmann and associates (Hoffmann et al. 2012). Moreover, ambient air pollution was converted into incremental (relatable) units of exposure based upon previous work (Jerrett et al. 2009), useful for future studies such as meta-analyses. Further, time spent in major microenvironments by participants including the residential and occupational address, to which these modeled ambient levels apply, was recorded and used to time-weight exposure attributable to health outcomes.

*Future research* may include a longer period monitored by time-activity diary prior to health outcome measurement, such as up to one week or more, especially if chronic rather than acute exposures are believed to have a greater influence on some health outcomes such as lung function. In addition to a longer monitoring period, exposure estimate models could be improved by the recent availability of cheap, portable, and accurate sensors contributing to data assimilation techniques. Further, such improved models could consider meteorological components such as precipitation, and wind direction and speed, which may influence exposure levels of air pollution and bioaerosols.

**Conclusion / Recommendations**

In conclusion, this study has highlighted the influence of various ambient environmental factors on specific cardiopulmonary health outcomes among even healthy adults, and that daily time-weighted model estimates may be precise enough to investigate some outcomes such as heart rate variability. Therefore, it is suggested important to collect data of ambient air pollution as well as space-time-activity information (particularly physical activity) prior to baseline health measures.

Implications for public/environmental health policy are suggested to be the following. As we have unveiled impacts on subclinical markers among healthy adults which are thought to be on the pathway to the development of chronic diseases due to long term exposures (Kim et al. 2015; Langrish et al. 2012), we recommend closer monitoring of such markers to better understand and eventually prevent these diseases. Despite concerns of potential increased inhalation during urban PA, our results provide support for the promotion of active travel (i.e. cycling or walking for transport; as we did not observe any worsening of subclinical markers with increased PA levels) within cities.
ACKNOWLEDGEMENTS

This study used data from experimental studies of both the TAPAS (<http://www.tapas-program.org/>) (funded by the Coca-Cola Foundation, AGAUR, and CREAL) and EXPOsOMICS (<http://www.exposomicsproject.eu/>) (funded by the 7th Framework Programme for Research and Technological Development of the EU {grant number FP7 GA 308610}) projects. Further, this work is contributing to the ICTA 'Unit of Excellence' (MinECo, MDM2015-0552). These funding bodies had no role in the study design, study procession, data analysis and interpretation, nor the decision to submit this paper for publication.

The authors would like to thank the ESCAPE project (funded by European Community’s Seventh Framework Program {FP7/2007–2011; grant number FP7 GA 211250) for use of their 2007 air pollution and noise models.

REFERENCES


DellaValle, C. & Triche, E., 2012. Effects of ambient pollen concentrations on
frequency and severity of asthma symptoms among asthmatic children.


Leon Bluhm, G. et al., 2007. Road traffic noise and hypertension. Occupational and
environmental medicine, 64(2), pp.122–6. Available at:


Patel, M. & Miller, R., 2009. Air pollution and childhood asthma: recent advances and


R Core Team, 2016. R: A language and environment for statistical computing. Available at: https://www.r-project.org/.


Wellenius, G. a et al., 2012. Ambient air pollution and the risk of acute ischemic stroke.


**SUPPLEMENTARY MATERIAL**

**FIGURES**

*Figure S1 – Flow chart for study design*
Figure S2 – Residential and Occupational addresses of participants in Barcelona and greater surroundings; visualising the extent of the 2009 annual-spatial ESCAPE-LUR model for PM$_{2.5}$.

Figure S3 - Participant exposure estimates of air pollution: (a) PM$_{10}$, (b) PM$_{\text{Coarse}}$, (c) PM$_{2.5}$, (d) NO$_2$, (e) NOx, and (f) Ozone; annual and daily estimates adjusted for time spent at residential, occupational or both address/es.

Figure S4 – Comparison of (a) cardiovascular and (b) pulmonary outcomes from exposure attributions: single-pollutant [(a) O$_3$, (b) PM$_{10}$] models (spatially and spatiotemporally) controlled for age, sex, height, season.

Figure S5 - Proportion mediated effect by (a) daily physical activity level and (b) occupational-address noise level on SDNN from PM$_{\text{Coarse}}$ exposure.

Figure S6 – Proportion mediated effect by (a) daily physical activity level and (b) occupational-address noise level on diastolic BP from Ozone (O$_3$) exposure.

Figure S7 – Proportion mediated effect by (a) daily physical activity level and (b) residential-neighborhood greenness on FVC from PM$_{10}$.

Tables

Table S1 – Correlation of all exposure estimates to those single-pollutant exposure estimates used in final models (PM$_{10}$, Ozone).

Table S2a – Outcomes of mixed effects models for cardiovascular health parameters and air pollution estimates [Figure S3a of sup material].

Table S2b – Outcomes of mixed effects models for pulmonary health parameters and air pollution estimates [Figure S2b of sup material].

Table S3 – Estimates for PM$_{\text{Coarse}}$ (5 µg/m$^3$) models, adjusted for PM$_{10}$ (10 µg/m$^3$), greenness, time-activities, and noise [Figure 3 of manuscript].

Table S4a – Estimates for O$_3$ (annual-residential, 10 ppb) models, adjusted for PM$_{10}$ (annual-residential, 10 µg/m$^3$), greenness, time-activities, and noise [Figure 4 of manuscript].
Table S4b – Estimates for PM$_{10}$ (annual-residential, 10 µg/m$^3$) models, adjusted for O$_3$
(annual-residential), greenness, time-activities, and noise [Figure 3 of manuscript]