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Summary

Each year, five million premature deaths are due to a lack of physical activity. Active mobility (i.e. walking and cycling as a means of transport) has been introduced as an accessible solution to increase daily physical activity levels because it may be easier to integrate into daily routines compared to sports and exercise. The PASTA project (Physical Activity through Sustainable Transport Approaches) studied the benefits and risks of active mobility based on data from seven European cities (Antwerp, Barcelona, London, Örebro, Rome, Vienna and Zurich).

Active mobility or physical activity in urban outdoor settings may put individuals at higher risk of air pollution exposure. Moreover, higher ventilation rates during physical activity may exacerbate the adverse effects on health. Worldwide, air pollution accounts for over four million annual deaths. Therefore, it has become an important public concern whether the health benefits of physical activity in an urban environment outweigh the risks.

Various health impact assessments reported that the health benefits of physical activity on mortality and morbidity outweigh the risks of air pollution exposure on a population level. Less is known about the long- and short-term interaction on a subclinical, individual level. These physiological responses may impact the built-up of physical activity health benefits and the development of chronic conditions. Clearly, this is important information for the successful implementation of active mobility and urban physical activity programs.

Therefore, the aim was to estimate the subclinical cardiorespiratory responses to real-world physical activity, air pollution and their interaction in healthy adults.

We designed a study within the PASTA project to obtain a comprehensive view on the cardiorespiratory responses to physical activity and air pollution (chapter 1). A set of non-invasive outcomes was selected and related to continuous information on real-world physical activity and air pollution (cardiorespiratory outcomes: heart rate variability (HRV), retinal vessel

diameters, fractional exhaled nitric oxide (FeNO), lung function). We monitored 122 individuals in three European cities (Antwerp: 41; Barcelona: 41; London: 40) during one week which was repeated in three different seasons. This allowed to approximate their long-term lifestyle to estimate the subchronic cardiorespiratory effects. Both physical activity and air pollution were measured on a personal level with wearable sensors to limit exposure misclassification. Black carbon (BC) was used as a proxy to study the health effects of air pollution and was assessed with the microAeth. Daily physical activity patterns were monitored with the SenseWear armband. However, no standard of good practice is available to assess physical activity, so we compared the results of two frequently used measurement techniques: the SenseWear, a wearable sensor, and the GPAQ (Global Physical Activity Questionnaire), a questionnaire.

Table 1 contains the main results, conclusions and perspectives summarized per chapter and compared to the state of the art. Overall, we found statistically significant acute and long-term responses to habitual physical activity and air pollution exposure in healthy individuals in an urban environment.

1) Assessment of free-living physical activity (chapter 2): GPAQ estimates were lower compared to the SenseWear, yet they were significantly correlated. Estimates of vigorous-intensity physical activity specifically were highly similar. The differences between all variables were reproducible across repeated measurements.
2) Long-term respiratory effects (chapter 3): Weekly physical activity improves lung function (FEV_1, FEV_1/FVC and FEF_{25-75}) at low BC concentrations. The beneficial effect decreased with increasing yearly, average BC levels.
3) Short-term cardiorespiratory effects (chapter 4): Sympathetic tone dominated with both acute physical activity (increased LF/HF) and BC (decreased SDNN, rMSSD and HF). No responses of the retinal microvasculature were observed. Regarding respiratory effects, physical activity acted as bronchodilator (FEV_1 and FEV_1/FVC), while lung function decreased with BC (PEF). The interaction suggested counteracting of the adverse BC effect by physical activity (FEV_1 and FVC).

Table 1 Summary of this doctoral thesis.

	Previous research	Our results	Conclusions and perspectives
Assessment of PA in free-living conditions (Chapter 2)	No standard of good practice exists. Two established techniques: • WHO validated **questionnaires** (e.g. GPAQ) for PA surveillance • **Wearables** (e.g. SenseWear) provide an objective and detailed view into daily activity patterns.	• GPAQ estimates < SenseWear • Magnitude of the difference depends on the PA intensity and personal characteristics. • Reproducible differences between GPAQ and SenseWear across repeated measurements.	**Good practice proposed:** to use the GPAQ in addition to a wearable of choice. The wearable provides objective estimates of daily time-activity patterns while the GPAQ offers a reference to compare results of different studies.
Long-term cardiorespiratory effects of PA and AP (Chapter 3)	• **Cardiovascular markers**: no interaction effects reported yet. • The **respiratory mortality** decrease associated with PA is smaller in high compared to low AP.	• No long-term effects on **cardiovascular markers** • The long-term beneficial effect of additional PA on **lung function** decreased in elevated yearly average AP concentrations.	• **Cardiovascular markers:** no long-term interaction between PA and AP responses. • **Lung function:** health benefits of PA may decrease in high AP. Further research should complement spirometry with other respiratory measures.
Short-term cardiorespiratory effects of PA and AP (Chapter 4)	• **Cardiovascular markers**: (1) HRV response to interacting PA and AP is unclear, (2) PA counterbalances the BP increase associated with AP, (3) beneficial pulse wave velocity increase disappears after PA in high AP (vulnerable individuals). • **Lung function:** (1) PA counterbalances AP hazards, (2) beneficial PA effects disappear in high AP (vulnerable individuals).	• **Cardiovascular markers**: (1) HRV decreased with both PA and AP, but there was no interaction effect, (2) the retinal vessel diameters did not change. • AP/PA were associated with a **lung function** decrease/increase respectively. A significant interaction pointed towards a protective effect of PA to the adverse respiratory effects of AP.	• More research needed to elucidate the short-term combined effects of PA and AP on **cardiovascular markers**. • Evidence tends towards a short-term protective effect of PA on AP associated **lung function** decreases in healthy adults (contrary to the long-term changes).

All results concern healthy individuals unless specified otherwise. AP = air pollution. PA = physical activity. BP = blood pressure. FeNO = fractional exhaled nitric oxide. GPAQ = global physical activity questionnaire. HRV = heart rate variability.

This is the first study simultaneously executed in multiple European cities that integrated effects of air pollution and physical activity to assess both the long- and short-term independent and combined impact on subclinical, cardiorespiratory markers. Based on the results, we propose caution for respiratory health with physical activity in polluted areas. Regarding short-term cardiorespiratory effects, no clear harmful effects were observed in healthy individuals. We recommend for further research to expand on our research design and focus on time- and location-specific responses to environmental and lifestyle triggers. This will aid the urban design process to improve public health. It is also recommended to further characterize the cardiorespiratory responses to physical activity and air pollution to enhance the physiological interpretation.

Samenvatting

Jaarlijks sterven vijf miljoen mensen aan de gevolgen van te weinig fysieke activiteit. Actieve mobiliteit (wandelen en fietsen om ergens naartoe te gaan) is makkelijk te integreren in de dagelijkse planning en daarom een interessante oplossing om meer te bewegen. Het PASTA project ('Physical Activity through Sustainable Transport Approaches') bestudeerde de voor- en nadelen van actieve mobiliteit op basis van data uit zeven Europese steden (Antwerpen, Barcelona, Londen, Oerebro, Rome, Wenen en Zurich).

Actieve mobiliteit of fysieke activiteit in stedelijke buitenlucht gaat vaak gepaard met een verhoogde blootstelling aan luchtvervuiling. Daarnaast kan een snellere ademhaling tijdens fysieke activiteit de negatieve gevolgen van luchtvervuiling voor gezondheid versterken. Luchtvervuiling is wereldwijd verantwoordelijk voor vier miljoen overlijdens per jaar. Hieruit volgt de maatschappelijke vraag of de gezondheidsvoordelen de risico's van fysieke activiteit in de stad compenseren.

Verschillende kosten-batenanalyses rapporteerden reeds dat de positieve impact van fysieke activiteit op mortaliteit en morbiditeit groter is dan de risico's van luchtvervuiling. Hoe de effecten elkaar op lange en korte termijn beïnvloeden op een subklinisch en individueel niveau is minder duidelijk. Dergelijke fysiologische responsen beïnvloeden de opbouw van gezondheidsvoordelen van beweging en kunnen bijdragen aan de ontwikkeling van chronische aandoeningen. Deze informatie is belangrijk voor de succesvolle implementatie van projecten die actieve mobiliteit of fysieke activiteit in de stad willen bevorderen. Het doel van dit doctoraat is daarom: het bepalen van de cardiorespiratoire effecten van dagdagelijkse fysieke activiteit, luchtvervuiling en hun interactie in gezonde volwassenen.

Onze studie kaderde in het PASTA project en verschaft een overzicht over de cardiorespiratoire responsen geassocieerd met beweging en luchtvervuiling (hoofdstuk 1). Een set niet-invasieve merkers werd gerelateerd aan continue informatie over dagdagelijkse fysieke activiteit en blootstelling aan luchtvervuiling (merkers: hartritmevariabiliteit (HRV), retinale microvasculaire diameters, fractie uitgeademde stikstofoxide (FeNO), longfunctie). 122

deelnemers werden tijdens een volledige week opgevolgd in drie Europese steden (Antwerpen: 41; Barcelona: 41; Londen: 40). Dit werd herhaald in drie verschillende seizoenen waardoor de lange termijn levensstijl benaderd kon worden om de subchronische cardiorespiratoire effecten te onderzoeken. Zowel fysieke activiteit als luchtvervuiling werden gemeten op een persoonlijk niveau met draagbare sensoren. Roet (Black Carbon – BC) werd gebruikt om de gezondheidseffecten van luchtvervuiling te analyseren en werd gemeten met de microAeth. Dagdagelijkse beweging werd gemonitord met de SenseWear armband. Voorlopig bestaat nog geen standaard methode om fysieke activiteit te meten. Daarom werden de resultaten van twee vaak gebruikte technieken vergeleken: de SenseWear, een draagbare sensor, en de GPAQ, een vragenlijst.

Tabel 1 vergelijkt wat reeds geweten is met de belangrijkste resultaten, conclusies en perspectieven uit dit proefschrift. Statistisch significante effecten werden geobserveerd van zowel onmiddellijke als lange termijn fysieke activiteit en luchtvervuiling bij gezonde volwassenen in een stedelijke omgeving.

1) Meten van fysieke activiteit tijdens dagelijkse routines (hoofdstuk 2): GPAQ metingen waren kleiner dan die van de SenseWear armband, de resultaten waren wel significant gecorreleerd en zeer gelijkaardig voor beweging met een hoge intensiteit. Over de herhaalde metingen heen werden reproduceerbare verschillen waargenomen.
2) Lange termijn respiratoire effecten (hoofdstuk 3): Wekelijkse fysieke activiteit geeft een betere longfunctie (FEV_1, FEV_1/FVC en FEF_{25-75}) bij lage BC concentraties. Dit voordelige effect is kleiner bij een hogere jaargemiddelde BC.
3) Korte termijn cardiorespiratoire effecten (hoofdstuk 4): Het sympathisch zenuwstelsel domineerde bij fysieke activiteit (hogere LF/HF) en BC (lagere SDNN, rMSSD en HF). De microvasculatuur van de retina vertoonde geen effecten. Bij de respiratoire merkers vonden we dat beweging de luchtwegen verwijdde (FEV_1 en FEV_1/FVC), terwijl longfunctie daalde met BC (PEF). De interactie suggereert dat fysieke activiteit beschermt tegen de risico's van BC (FEV_1 en FVC).

Tabel 1 Samenvatting van dit proefschrift.

	Voorgaand onderzoek	Resultaten	Conclusies en perspectieven
Meten van FA tijdens de dagelijkse routine (Hoofdstuk 2)	Geen standaardmethode beschikbaar. Twee vaak voorkomende technieken: • WHO gevalideerde **vragenlijst** (e.g. GPAQ) voor FA supervisie. • **Draagbare sensoren** (e.g. SenseWear) voorzien een objectieve en gedetailleerde inkijk in dagelijkse activiteitspatronen.	• GPAQ waarden < SenseWear • Grootteorde van de verschillen hangt af van de FA intensiteit en persoonlijke kenmerken. • Reproduceerbare verschillen tussen de GPAQ en SenseWear over de herhaalde metingen heen.	**Goede praktijk:** gebruik de GPAQ samen met een sensor die aan de voorwaarden van de studie voldoet. De sensor geeft objectieve inzichten in het dagelijkse FA patroon terwijl de GPAQ als referentie dient om resultaten over studies heen te vergelijken.
Lange termijn cardiorespiratoire effecten van FA en LV (Hoofdstuk 3)	• **Cardiovasculaire merkers**: nog geen interactie-effecten gerapporteerd. • De daling in **respiratoire mortaliteit** geassocieerd met FA is kleiner in hoge LV in vergelijking met lage LV.	• Geen lange termijn effecten op **cardiovasculaire merkers** • Het lange termijn voordelige effect van FA op **longfunctie** daalde in verhoogde jaarlijks gemiddelde LV concentraties.	• **Cardiovasculaire merkers:** geen lange termijn interactie tussen FA en LV responsen. • **Longfunctie:** Gezondheidsvoordelen van FA kunnen dalen in hoge LV. Verder onderzoek moet spirometrie metingen aanvullen met andere respiratoire merkers.
Korte termijn cardiorespiratoire effecten van FA en LV (Hoofdstuk 4)	• **Cardiovasculaire merkers**: (1) hoe HRV responsen op FA en LV elkaar beïnvloeden is onduidelijk, (2) FA biedt tegenwicht aan de BD stijging geassocieerd met LV, (3) voordelige stijging in pulse wave snelheid verdwijnt na FA in hoge LV (kwetsbare groepen). • **Longfunctie:** (1) FA beschermt tegen LV risico's, (2) voordelige effecten van FA verdwijnen in hoge LV (kwetsbare groepen).	• **Cardiovasculaire merkers**: (1) HRV daalde met zowel FA als LV, maar er was geen interactie-effect, (2) diameters van de retinale microvasculatuur veranderden niet. • LV/FA was geassocieerd met respectievelijk een daling/stijging in **longfunctie**. Een significante interactie suggereerde een tegengewicht van FA voor de nadelige respiratoire effecten van LV.	• Meer onderzoek is nodig om de korte termijn gecombineerde effecten van FA en LV op **cardiovasculaire merkers** te bevestigen. • Huidige kennis suggereert een korte termijn beschermend effect van FA op de **longfunctie** daling geassocieerd met LV in gezonde volwassenen (tegengesteld aan de lange termijn veranderingen).

Alle resultaten betreffen gezonde individuen tenzij het anders gespecifieerd werd. LV = luchtvervuiling. FA = fysieke activiteit. BD = bloeddruk. FeNO = fractie uitgeademde stikstofoxide. GPAQ = 'global physical activity questionnaire'. HRV = hartritmevariabiliteit.

Dit is de eerste studie die simultaan werd uitgevoerd in verschillende Europese steden om de onafhankelijke en gecombineerde impact van korte en lange termijn fysieke activiteit en luchtvervuiling op subklinische, cardiorespiratoire merkers te onderzoeken. Onze studie stelt voor om voorzichtig om te springen met respiratoire gezondheid bij beweging in een vervuilde, stedelijke omgeving. In verband met korte termijn effecten werden bij gezonde individuen voorlopig geen duidelijke schadelijke effecten gerapporteerd. We raden verder onderzoek aan om onze studie uit te breiden en te focussen op tijds- en locatie-specifieke responsen op een combinatie van omgevings- en levensstijlfactoren. Dit zal de rol van stedelijk ontwerp voor de verbetering van de volksgezondheid versterken. Daarnaast wordt aangeraden om in toekomstig onderzoek de cardiorespiratoire responsen verder te karakteriseren om hun fysiologische interpretatie te verbeteren.

Abbreviations

AB²C	Activity-Based modeling framework for Black Carbon exposure assessment
AM	Active Mobility
AP	Air Pollution
BC	Black Carbon
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
CO	Carbon Monoxide
COPD	Chronic Obstructive Pulmonary Disease
CRAE	Central Retinal Arteriolar Equivalent
CRVE	Central Retinal Venular Equivalent
CRP	C-Reactive Protein
DAG	Directed Acyclic Graph
DLW	Doubly Labelled Water
EE	Energy Expenditure
eNOS	endothelial Nitric Oxide Synthase
ERS	European Respiratory Society
ESCAPE	European Study of Cohorts for Air Pollution Effects
FEATHERS	Forecasting Evolutionary Activity-Travel of Households and their Environmental RepercussionS
FeNO	Fractional exhaled Nitric Oxide
FOXP3	Forkhead Box p3 Promoter
FEF_{25-75}	Forced Expiratory Flow at 25% to 75% of the FVC
FEV_1	Forced Expiratory Volume in the 1st Second
FVC	Forced Ventilation Capacity
GPAQ	Global Physical Activity Questionnaire
GPS	Global Positioning System
H_2O_2	Hydrogen peroxide
HF	High Frequency
HIA	Health Impact Assessment
HRV	Heart Rate Variability
ICC	Intraclass Correlation Coefficient
IL	InterLeukin
iNOS	inducible Nitric Oxide Synthase
IPAQ	International Physical Activity Questionnaire
IQR	InterQuartile Range
LF	Low Frequency
MET	Metabolic Equivalent of Task
MVPA	Moderate- to Vigorous-intensity Physical Activity
MESA	Multi-Ethnic Study of Atherosclerosis
MS	Mean Squared differences
NO	Nitric oxide
NO_2	Nitrogen dioxide
O_3	Ozone
ONA	Optimized Noise-Reduction Algorithm
PA	Physical Activity
PARROTS	Personal digital assistant system for Activity Registration and Recording of Travel Scheduling

PASTA	Physical Activity through Sustainable Transport Approaches
PDA	Personal Digital Assistant
PEF	Peak Expiratory Flow
PM	Particulate Matter
rMSSD	root Mean Square of Successive Differences in adjacent NN intervals
ROS	Reactive Oxygen Species
SB	Sedentary Behaviour
SD	Standard Deviation
SDNN	Standard Deviation of Normal-to-Normal intervals
SO_2	Sulfur Dioxide
TAPAS	Transportation, Air pollution and Physical Activities
TNF	Tumor Necrosis Factor
UFP	UltraFine Particles
UZA	University Hospital Antwerp
VOC	Volatile Organic Components
WBC	White Blood Cell
WHO	World Health Organization

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General introduction

Problem statement

Worldwide, 30% of the population is physically inactive resulting in approximately five million premature deaths annually.[1,2] Moreover, physical inactivity contributes to the development and progression of type 2 diabetes, cardiovascular diseases, cancer, dementia and depression.[3] Therefore, promotion of active mobility (walking and cycling as a means of transport) has recently been introduced as an innovative and accessible measure to increase physical activity.[4,5] It requires less time and motivation compared to sports and exercise and has the potential to reach all parts of society.[6] Unfortunately, implementation of successful active mobility measures is very challenging on all policy levels.[4,5] This is due to a lack of (1) understanding of available measures, and (2) the political and administrative conditions necessary to successfully implement them in different places. This is where the PASTA project comes in (Physical Activity through Sustainable Transport Approaches). PASTA aimed to promote active mobility projects and to develop guidelines on how to successfully implement these measures based on experiences from seven European cities (Antwerp, Barcelona, London, Örebro, Rome, Vienna and Zurich).

However, when physical activity is promoted in urban outdoor settings, individuals may be more exposed to air pollution. Air pollution accounts for over four million annual deaths globally and contributes to the onset and development of cardiovascular and respiratory diseases.[7–9] Higher ventilation rates during physical activity may result in increased alveolar deposition of particulate matter which could exacerbate the adverse physiological effects of air pollution.[8,10,11] As a result, it has become an important public concern whether physical activity is more harmful than beneficial in polluted, urban areas. Factual knowledge on this topic is necessary to increase awareness and effectuate interdisciplinary collaborations between stakeholders in the public health and transport domain.

Various health impact assessments (HIA) have reported that the overall health benefits of physical activity outweigh the risks of air pollution exposure on a population level.[12,13] However, the estimated health impact is based on studies that independently assessed the benefits of physical activity and the risks of air pollution and only used mortality or established morbidity as an outcome. Consequently, less is known about the long- and short-term interaction between the physiological responses provoked by physical activity and air pollution. This may have a significant impact on the onset and development of disease and consequently the implementation of active mobility projects.

One cohort study integrated the measurement of physical activity and air pollution and found no modification effect of air pollution on the relationship between physical activity and all-cause mortality.[14] However, the benefits of physical activity on respiratory mortality seemed to be attenuated in elevated air pollution concentrations. In air pollution epidemiology, few panel studies directly assessed the effect of physical activity in order to disentangle the short-[11,15–18] and long-term[19,20] responses to physical activity and air pollution. The majority of these investigations used a scripted design which limits our view on the independent and combined effects of physical activity and air pollution in real-world conditions.

Quantification of physical activity under real-world conditions is an important challenge in epidemiological research.[21,22] It has been documented that self-reported tools for the assessment of physical activity are prone to error (e.g. recall bias and social desirability).[23–25] During the last decade, development of wearable devices allowed for a detailed assessment of daily physical activity patterns. Nevertheless, a standard of good practice to measure physical activity levels is not yet available.[22] Similarly, quantification of air pollution exposure in daily life also remains challenging. Measurements of ambient air pollution concentrations at the home location differ considerably from those where time-activity patterns are taken into account.[26] For this reason, it is recommended to assess exposure personally, especially when pollutants that are highly variable in space and time are of interest.[27–29] Thus, both the physical activity and air pollution exposure variables in epidemiological models are often inaccurate which may lead to the assignment of wrong health effects or insufficient strength of a relationship.

Objectives

The framework for this PhD was the European PASTA project (Health Innovation call of the Seventh Framework Program funded by the European Commission) which aimed to improve health by increasing physical activity levels through the promotion of active mobility. The PASTA consortium conducted a large empirical study where self-reported data was collected in seven European cities (Antwerp, Barcelona, London, Örebro, Rome, Vienna and Zurich). Online surveys were distributed and asked about the physical activity and active mobility levels of the urban citizens.

PASTA defined active mobility as: "All regular physical activity undertaken as a means of transport. It includes travel by foot, bicycle and other vehicles which require physical effort. Use of public transport is also included in the definition as it often involves some walking or cycling to pick-up and from drop-off points. It does not include walking, cycling or other physical activity that is undertaken for recreation." However, public health concerns over the balance between health benefits and risks of physical activity in elevated urban air pollution concentrations cover all forms of physical activity.

As a result, we aimed to estimate the independent and combined effects of total physical activity and air pollution exposure on subclinical, cardiorespiratory outcomes in healthy adults based on personal, real-world measurements in an urban environment.

State of the art

Assessment of physical activity and air pollution

Large-scale epidemiological studies have had a major role in establishing the health effects of physical activity and ambient air pollution on health.[7,30,31] However, an accurate assessment of both factors under real-world conditions remains a major challenge.[21,32]

Panel studies allow a greater control over exposure metrics because they are conducted in smaller groups of volunteers (ranging from ten to over 100 participants) exposed to either controlled or ambient environments.[30] In air pollution epidemiology, the number of panel studies increased during the last 20 years. Recently, researchers started adding physical activity monitoring to the study design to disentangle responses associated with physical activity and air pollution.[16]

In the remainder of this chapter different techniques to measure physical activity and air pollution will be summarized.

Physical activity

Evidence on the health benefits of physical activity has been growing since the 1970s.[33] At the same time, new technologies were developed that reduced the amount of physical activity needed in daily life.[1] Today, the resulting physical inactivity epidemic is responsible for over five million deaths per year. This highlights the importance of surveillance instruments for the collection of data to demonstrate the need for action. Early efforts to establish such instruments focused on occupational or leisure-time physical activity only which is insufficient to obtain information about the total physical activity level. Only about 20 years ago, an international group of researchers developed a standardized instrument to collect internationally comparable data on physical activity levels: the International Physical Activity Questionnaire (IPAQ).[34] Another established self-reported surveillance tool is the Global Physical Activity Questionnaire (GPAQ), developed by the World Health Organization (WHO).[23,35]

The IPAQ and the GPAQ capture information on domain-specific physical activity duration and intensity. The results of both questionnaires have been validated and compared to each other with data from different countries.[23,34] Overall, a good reproducibility of the IPAQ and GPAQ estimates and a fair agreement with pedometer or accelerometer results was reported. In addition, an acceptable level of association between the IPAQ and the GPAQ was observed with Spearman's rho ranging from 0.45 to 0.57.[23] The validation studies indicate that both instruments perform well. Drawbacks of the IPAQ compared to the GPAQ are (1) the high burden of filling out the longer version, and (2) the short IPAQ does not cover multiple physical activity domains such as transport and leisure time. Self-reported tools to collect data on physical activity levels are practical, budget-friendly and have a low participant burden which makes them easy to apply in large-scale epidemiological studies. Nevertheless, they are prone to sources of error such as recall bias and social desirability.[23–25]

The gold standard to accurately assess free-living energy expenditure objectively is doubly labelled water (DLW).[36] Being physically active results in an increase in energy expenditure above resting levels.[37] Therefore, measurements of energy expenditure are often used to quantify physical activity. In DLW, hydrogen and oxygen atoms have been replaced with an isotope of these elements for tracing purposes. After administering a DLW dose, isotope elimination rates are measured through regular sampling of saliva, urine, or blood. This is an expensive and labor-intensive technique that cannot be used in a large number of participants. In addition, this method does not allow to differentiate between light-, moderate- and vigorous-intensity physical activity or to identify time-activity patterns.

Recently, wearable sensors have been developed that register physiological responses such as heart rate and/or mechanical body movements.[38] These devices enabled an objective and detailed view into people's daily activity patterns.[22] Hence, they have become a key element in panel studies examining effects of physical activity. The SenseWear armband and the Actigraph are two wearable physical activity monitors that have been widely used by researchers.[39] The Actigraph is a hip-worn accelerometer providing 'counts' that are converted to estimates of energy expenditure.[40] On the other hand, the SenseWear is a multi-sensor device, worn on the upper arm, that uses pattern recognition algorithms of various activities to estimate energy expenditure.[38,40,41] Although estimates of total energy expenditure of both the Actigraph and the SenseWear correlated well with DLW[36,40,42–44], each wearable device has specific performance characteristics.[45]

Estimates from the Sensewear may be more accurate to monitor upper body or cycling movements compared to hip-worn accelerometers because they are based on pattern recognition.[38,41,46–48] The Actigraph has been shown to underestimate energy expenditure during moderate-to-vigorous physical activity (MVPA) while the SenseWear overestimates and underestimates energy expenditure during respectively moderate- and vigorous-intensity physical activity.[41,49] A recent study compared the SenseWear armband, its previous version and the Actigraph to indirect calorimetry. The authors reported that all devices underestimate energy expenditure at high intensities, and the most recent version of the SenseWear provided the best available estimate.[50]

A standard measurement technique to assess physical activity in panel studies remains absent.[22] Nevertheless, an accurate and reproducible measurement of physical activity is a crucial element to investigate its relationship with health.

The challenge to quantify physical activity levels continues to grow since health promotion measures focus on lifestyle changes where physical activity is integrated into daily routines.[51] This is why promotion of active mobility has become an important approach to increase physical activity levels.[52] Both the IPAQ and GPAQ can be used to collect the self-reported, average volume of walking and/or cycling undertaken as a means of transport per day. For this purpose, the GPAQ proved to be adequately reliable.[23] On the other hand, the use of wearable sensors to quantify cycling remains challenging.[41,46,50,53] Accelerometers, such as the Actigraph, are often worn on the hip which hampers detection of movements involved in cycling. Consequently, estimates of energy expenditure during cycling improved with the use of pattern recognition followed by estimation of activity-specific energy expenditure, the algorithms used by the

SenseWear.[38] Although, the SenseWear armband still underestimates energy expenditure during cycling, it has been reported to provide the best available estimate.[50,54] The most recent validation study by Lopez et al. (2017)[50] reported a mean absolute error of 19% to 35% during cycling for the most recent version of the SenseWear, compared to 32% to 74% for the Actigraph. The overall mean absolute error was 20% for the SenseWear and 28% for the Actigraph.

In the transport sector, travel diaries are commonly used to capture information on trip purpose, mode, duration and time of day.[55] Multiple travel diaries exist such as those based on the KONTIV design[55] (i.e. respondent-oriented with clear questions; used in the PASTA project). Compared to questionnaires, travel diaries offer a more detailed view into an individual's daily transport planning. However, they also require an active involvement of the participant which often results in poor data quality (e.g. non-participation or drop-out, gaps and overlays).[32] As a response to this problem, electronic diaries became available with built-in consistency checks and user guidance to enhance data quality. To further enhance the data quality and decrease user burden, the PARROTS (Personal digital assistant system for Activity Registration and Recording of Travel Scheduling) tool was developed.[56] This is an automated, mobile activity-travel diary that respondents complete on a personal digital assistant (PDA). It uses global positioning system (GPS) data, complemented with user-provided information on activity-travel behaviour. Both planned and executed activities are captured resulting in the collection of both decision and scheduling information. This enables the development of activity-based models such as the FEATHERS model (Forecasting Evolutionary Activity-Travel of Households and their Environmental RepercussionS).[56] These models predict time-activity patterns in order to forecast travel demand and can be complemented with estimations of air pollution concentrations to model an individual's exposure.

Air pollution

This paragraph is based on:

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Ambient air pollution has been identified as one of the major risk factors for ill health, annually causing a loss of over four million lives worldwide.[7] Both natural and anthropogenic factors determine air quality, yet human activities are the main cause of pollution. The most important constituents of air pollution are nitric oxides (NO and NO_2), carbon monoxide (CO), sulfur dioxide (SO_2), ozone (O_3), particulate matter, and black carbon (BC).[57] Particulate matter (PM) is often expressed in terms of its specific size fractions with PM_{10} and $PM_{2.5}$ referring to particles with a diameter smaller than 10 and 2.5 µm respectively. Globally, combustion of fossil fuels from industry and traffic are the major source of particulate matter. Traffic emissions also contribute to levels of NO_2 and BC. BC is a major element of soot, an incomplete combustion product from diesel exhaust.[26,58] Most BC particles have a diameter smaller than 1 µm. Due to their penetration capacity, such small particles are believed to be more harmful than particles of larger sizes.[59] Hence, BC is a valuable marker to study physiological effects of traffic-related air pollution.[60]

Similar to the assessment of physical activity, the measurement of air pollution exposure at an individual level remains challenging.[10] In large-scale epidemiological studies, the assessment often relies on monitoring data from official stations.[30] This induces exposure measurement error as the concentrations of fixed monitoring sites are poorly correlated with true personal exposure. Especially when pollutants with a high spatiotemporal variability are of interest, such as those related to traffic, the problem of exposure misclassification needs to be addressed.[61] An accurate determination of exposure is necessary to estimate precise effect sizes in exposure-response relationships. [27,62]

The concentration of air pollution on a specific location results from the combination of emissions and their dispersion.[32] Dispersion modelling is a technique based on a dense network of samplers and has been used to account for this spatial variability.[63] Another technique to model air quality with a high spatial resolution is land use regression. This method uses linear regression models to estimate air pollution concentrations based on predictor variables such as traffic, land use and meteorology.[63] The ESCAPE project (European Study of Cohorts for Air Pollution Effects) developed land use regression models to estimate annual NO_2 and $PM_{2.5}$ concentrations at the home location of members of multiple cohorts.[64] A major advantage of these models is that they allow a harmonized estimation of air pollution concentrations over different European study areas. However, these models don't take time-activity patterns into account which have a significant impact on the level of personal air pollution exposure as shown by Dons et al. (2011).[26]

Hence, Dons et al. (2013) developed the AB²C model (Activity-Based modeling framework for Black Carbon exposure assessment).[65] This model estimates personal exposure to BC of the Flemish population by including information on time-activity patterns into the prediction. It integrates different models into one framework including hourly land use regression models and FEATHERS. FEATHERS is an activity-based model to predict the time and duration of specific activities based on characteristics of the study sample.[56] In a validation study, the AB²C model performed well in determining the population's average exposure during 24 hours and almost all individual measurements were within the predicted range. However, the predicted intra-individual variability was large compared to the inter-individual variability.

Personal monitoring of exposure to air pollution can be done with both passive and active samplers. Passive samplers, such as for the measurement of NO_2 concentrations, are easy to use as they are small, cheap and don't require power.[66] However, they cannot be used when information about air pollution concentrations with a high time resolution needs to be collected. Active samplers measure air pollution concentrations with a higher temporal resolution, but are often expensive, too complex and too large to use for mobile, personal measurements.[32] Examples are the DUSTTRAK aerosol monitor for the measurement of particulate matter and the P-TRAK ultrafine particle counter to assess ultrafine particles (UFP). Although still expensive, the microAeth AE51 (AethLabs) is a small, easy to use active sampler with a small form factor. Hence, this device enables the personal measurement of exposure to BC during daily activities in panel studies.[60,67] This results in more accurate measurements compared to modelling, limiting exposure misclassification. The microAeth measures the BC concentration based on the optical absorbance of light transmitted through a fiber filter matrix where particles accumulate.[68] Concentrations can be assessed with a time resolution of up to one second, yet integration over longer time intervals increases the measurement's reliability. Post-processing of the raw data is required, therefore, among others, an online algorithm is available to smoothen the BC data (Optimized Noise-Reduction Algorithm (ONA); developed by the US Environmental Protection Agency).

Higher ventilation rates during physical activity may increase pulmonary uptake and deposition of air pollutants.[10,69] This has become a major public concern since urban physical activity is promoted[4,5], but we created cities that are hotspots of poor air quality.[70] Therefore, an individual's dose of air pollution may be important to consider in dose-response relationships. To estimate the inhaled dose, information on minute ventilation is needed.[69,71,72] Minute ventilation is defined as the volume of air inhaled or exhaled per minute. It can be calculated by multiplying breathing frequency and tidal volume, the volume of air displaced between an inhalation and exhalation. The gold standard to measure ventilation directly is ergospirometry. This method uses a facemask to measure nose and mouth inhalation simultaneously, and registers minute ventilation and heart rate at increasing intensities.[71] A portable facemask was used by Int Panis et al. (2011)[69] to assess minute ventilation in the field, but this is uncomfortable and may affect breathing itself.[10] Ergospirometry also allows to define the relationship between heart rate and minute ventilation for each individual.[71] While such calibrations allow to estimate minute ventilation during scripted activities or in free-living conditions, they are labour-intensive and difficult to scale. Therefore, Dons et al. (2017) evaluated five methods to estimate the inhaled dose based on the results of personal measurements of BC, physical activity and heart rate during real-life daily routines.[10] Inhaled air pollution doses were estimated with (1) fixed ventilation rates per activity type, and formulas based on (2) energy expenditure, (3) heart rate, (4) breathing rate and (5) a combination of heart and breathing rate.

When the inhaled dose was estimated over longer time periods, calculations based on fixed ventilation rates corresponded well to those of continuous methods. However, substantial differences were observed when the results were compared per activity. Thus, individual variation and changes in minute ventilation were better reflected with continuous measures. Formulas of continuous methods based on heart rate may provide unexpected results when the heart rate is below 100.[73] In this range, the influence of the sympathetic nervous system may be high compared to the individual's activity state. In addition, results from methods based on breathing rate were rather uncertain due to the characteristics of selected measurement devices. Methods based on both heart and breathing rate provided high inhaled doses in comparison to the other estimates. The heart- and breathing rate monitor was also considered more burdensome by the volunteers, compared to wearing an accelerometer or carrying a device. Moreover, some formulas required individual calibration which makes them impractical to use in large panel studies. Since no golden standard is available to assess the inhaled air pollution dose, the choice of a suitable method in future studies will depend both on the size and the objectives of the study. When the size of the study allows it and air pollution and energy expenditure are monitored at a similar, high temporal resolution, continuous methods using energy expenditure are preferred to calculate inhaled dose.

Summary

- Both the accurate assessment of personal, free-living physical activity and air pollution remain a major challenge
- The IPAQ and GPAQ have been established as a response to the need of a standardized instrument for physical activity surveillance. The WHO developed the GPAQ and validated both the IPAQ and GPAQ. Both instruments provide reliable results and comparable estimates.
- Wearable sensors have become a key element in physical activity research as they provide an objective and detailed view into daily activity patterns. However, no standard wearable device has been identified to monitor physical activity.
- Promotion of active mobility has become an important approach to increase physical activity levels, resulting in a quest for tools to accurately quantify these activities. Travel diaries offer a detailed view into an individual's activity and travel plans. Since manual travel diaries require a lot of input from the participant, automated, electronic tools have been developed based on GPS data. This information allows to construct activity-based models.
- Air pollution assessment based on fixed monitors or models that estimate exposure at the home location don't take time-activity patterns into account. Therefore, they don't provide an accurate measure of personal exposure.
- A modeling framework that includes both an activity-based model and an hourly land use regression model may partly address this issue.
- A more accurate solution is the personal measurement of exposure to air pollution, which is preferred in panel studies. This can be done with the microAeth, a user-friendly, mobile device to measure BC concentrations. BC has been identified as a valuable marker to assess the health effects of traffic-related air pollution.
- Physical activity is associated with higher ventilation rates which affects an individual's inhaled air pollution dose. This may be important to consider in dose-response relationships. Therefore, Dons et al. (2017) compared the results of different previously published formulas to calculate inhaled dose.[10] The authors concluded that the best available methods are those based on continuous measurements of energy expenditure.

Physiological responses to physical activity and air pollution

Physical activity

Physical activity involves a myriad of acute physiological responses to increase blood flow to the working muscle (Figure 1).[74] Withdrawal of parasympathetic tone and sympathetic activation will result in the release of norepinephrine and epinephrine to elevate heart rate. This will increase cardiac output and systolic blood pressure, but no significant changes occur in diastolic blood pressure. Moreover, the overall increase in sympathetic tone to the vasculature results in vasoconstriction. This is blunted by local vasodilators, released in the exercising muscle. The respiratory system also responds to acute exercise with an immediate increase in ventilation.[74,75] Parasympathetic withdrawal will induce bronchial smooth muscles relaxation and bronchodilation.

Physical activity also provokes an anti-inflammatory response; the cytokine cascade induced by exercise differs from that of infections.[3,76] The first cytokine in the circulation during exercise is interleukin (IL) 6, followed by IL-10 and IL-1ra. Exercise-induced IL-6 exposes anti-inflammatory characteristics: it inhibits IL-1 and tumor necrosis factor (TNF) α. Even with moderate-intensity exercise, IL-6 is released from the contracting skeletal muscle. Its concentration increases in an exponential fashion and is related to e.g. exercise intensity, duration, and endurance capacity.[3] IL-10 and IL-1ra appear in the circulation after exercise and mediate anti-inflammatory effects. IL-10 inhibits the production of pro-inflammatory cytokines IL-1a, IL-1b and TNF-α; IL-1ra inhibits signaling transduction through the IL-1 receptor complex. Moreover, exercise probably also suppresses TNF-a via epinephrine, an IL-6-independent pathway. Consequently, regular exercise protects against (diseases associated with) chronic low-grade systemic inflammation which is involved in the pathogenesis of insulin resistance, atherosclerosis, neurodegeneration and tumour growth.[3]

Oxidative stress is also reduced by physical activity with beneficial effect on endothelial function.[77] The endothelium is the inner layer of the vascular wall. In healthy individuals, the middle layer of the vascular wall consists of smooth muscle cells and the outer layer of collagen. Physical activity will increase superoxide dismutase activity that reduces oxidative stress by converting reactive oxygen species (ROS) to hydrogen peroxide (H_2O_2).[78,79]Aerobic exercise and H_2O_2 will activate kinases to increase nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS).[80] NO will be incorporated by smooth muscle cells to mediate vasodilation.

The cardiovascular and autonomic nervous system adapt to regular physical activity resulting in a lower resting heart rate and increased vagal tone or parasympathetic activity.[74,81] Regular physical activity will enhance NO production resulting in improved endothelial function (vasodilation and anti-inflammatory properties).[82,83] In addition, the vascular wall will adapt to increase the lumen. Regular physical activity will also improve strength and endurance of the respiratory muscles. However, exercise induces smaller changes on the respiratory compared to the cardiovascular system because it is already equipped to deal with the demands of high-intensity activities.[74,75]

Air pollution

It is hypothesized that air pollution causes acute, subclinical changes through three mechanisms (Figure 1):[8]

1) Air pollution activates autonomic reflexes through pulmonary receptors which changes the autonomic input to the heart and vessels. This gives a change in heart rate, heart rate variability (HRV) and blood pressure.
2) Deposited air pollution particles may enter the systemic circulation provoking an inflammatory and oxidative stress response.
3) Particles that enter the lung can induce a pulmonary inflammatory response and oxidative stress.[8,84,85] Consequently, pulmonary cells may secrete chemokines and cytokines in the systemic circulation. This mechanisms is referred to as systemic overspill.

Air pollution inhalation induces parasympathetic withdrawal and increased sympathetic tone leading to heart rate elevations and HRV reductions.[8] This mediates a blood pressure increase, arrhythmias and vasoconstriction.[86] In addition, inhalation of particles provokes a pulmonary and systemic inflammatory and oxidative stress response which may lead to airway resistance and the development of endothelium dysfunction. Bronchoconstriction occurs due to smooth muscle contraction and excessive mucous secretion.[84,85] This induces coughing and chest tightness. The systemic acute phase response provoked by alveolar deposition and systemic overspill involves e.g. C-reactive protein (CRP), TNF-α and IL-1b and may induce activation of endothelial cells and leukocytes.[8] Oxidative stress reduces NO bioavailability resulting in an impairment of the endothelium-dependent vasodilation or endothelium dysfunction preventing the vasculature to adequately dilate.[86] Responses may differ depending on variations in the pollutant chemistry and exposure duration or concentration.[8] However, these mechanisms may be able to trigger acute pulmonary or cardiovascular events in susceptible individuals.[87,88]

Chronic inflammation or oxidative stress as a response to air pollution exposure may result in the development of pulmonary and cardiovascular diseases.[89] To protect the vascular wall, the structure will change due to proliferation of smooth muscle cells and deposition of elastin and collagen. As a result, blood pressure will increase and atherosclerotic plaque formation will be initiated. Potential consequences are thrombosis, arrhythmia, myocardial infarct or stroke.[8]

The interaction between physical activity and air pollution

Physical activity in polluted air involves higher ventilation rates potentially resulting in a higher inhaled pollutant dose.[10] With higher ventilation rates, breathing also switches from predominantly nasal to oral.[11] As the nose plays an important role in particle removal, this potentially increases the deposited fraction as well. Consequently, if the adverse effects of air pollution were proportional to the increased dose, they would amplify after exercise. Despite widespread public concern about whether physical activity in polluted cities is more harmful than beneficial, the volume of scientific evidence on the interacting physiological responses is limited. Physical activity and air pollution affect similar biological systems which may result in additive, synergetic or antagonistic effects (Figure 1). Physical activity could enhance the adverse response to air pollution or have a protective effect. On the other hand, air pollution potentially reduces the health benefit of physical activity. The following chapter summarizes findings of previous studies that examined how the effects of physical activity and air pollution interact to affect cardiorespiratory health and physiology.

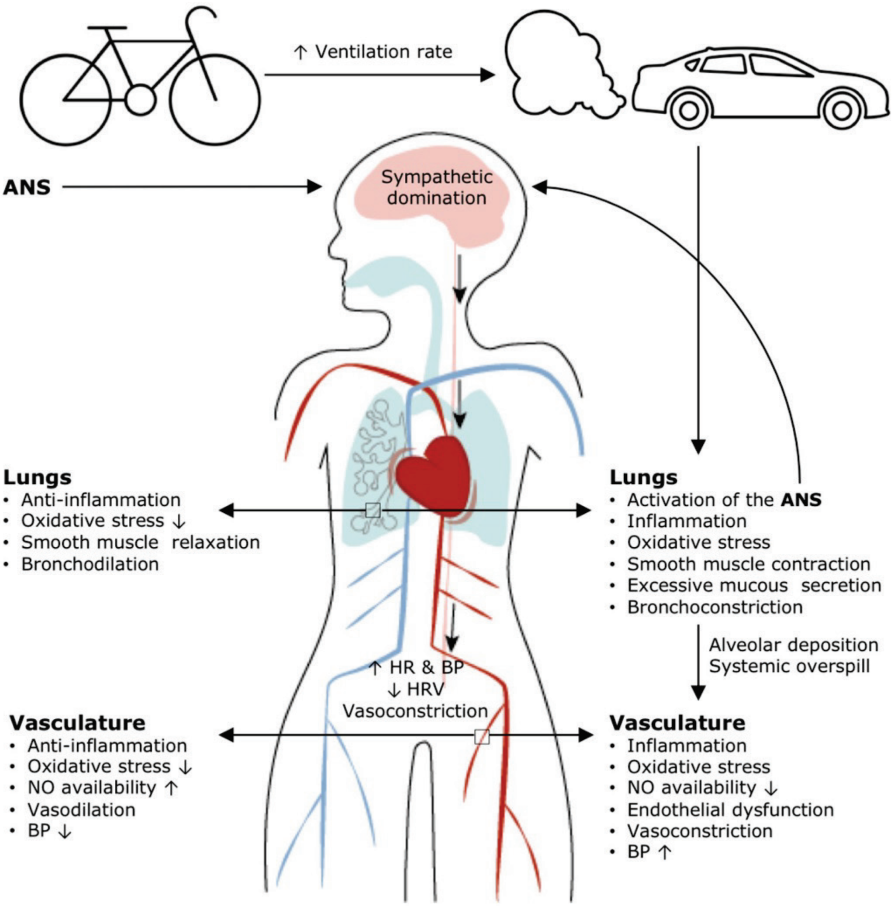


Figure 1 Overview of the physiological responses to physical activity (left) and air pollution (right). ANS = autonomic nervous system; BP = blood pressure; HR = heart rate; HRV = heart rate variability

Evidence on the cardiorespiratory effects of physical activity, air pollution and their interaction

To estimate whether the health benefits of physical activity during active mobility outweigh the risks of air pollution exposure and crashes, various HIAs have been carried out.[13] A review by Mueller et al. (2015), as part of the PASTA project, found that the benefit-risk ratios ranged from minus two to 360 indicating that active mobility is beneficial to health. These calculations are based on the results of large-scale epidemiological studies where the effects of physical activity and air pollution were studied independently. In addition, such studies focus on established adverse health effects such as mortality, hospital admission or health costs.[13,30] Hence, early changes on the pathway to disease are overlooked.

The Danish Diet, Cancer and Health Cohort is the only available cohort study that integrated the assessment of physical activity and air pollution to study the combined effects on all-cause and cause-specific mortality. The researchers did not find a modification effect of air pollution on the relationship between physical activity and all-cause mortality.[14] However, they did observe greater reductions in respiratory mortality with cycling in low to moderate compared to high air pollution exposure. This indicates a reduced benefit of long-term physical activity due to enhanced air pollution concentrations. Panel studies may complement such large-scale epidemiological studies with clues for mechanistic effects as they enable researchers to study specific subclinical health outcomes.[30]

A recent study assessed the acute cardiovascular and respiratory responses after a walking tour in high versus low air pollution concentrations in 135 older adults with and without pre-existing COPD or ischemic heart disease.[90] They observed increased cough, sputum, shortness of breath and wheeze after the walk in high compared to low air pollution concentrations. In addition, they found that walking increased lung function and decreased arterial stiffness on the less polluted site. These beneficial effects did not appear after walking in a polluted environment, indicating that air pollution attenuates the cardiorespiratory benefits of physical activity in people over 60 years old with pre-existing medical conditions. McCreanor et al. (2007) conducted the same experiment in 60 asthmatics and also found that lung function decreases were more severe after walking in a polluted environment.[91] Such results need to be complemented with studies of physiological responses in healthy volunteers. This contributes to the evidence base on markers of early physiological changes associated with the combination of physical activity and air pollution and may inform prevention of chronic conditions.

The remainder of this paragraph is devoted to (1) the evidence on long- and short-term subclinical responses to physical activity, air pollution and their interaction in healthy adults; and (2) the measurement techniques to quantify these responses.

Long-term subclinical cardiorespiratory effects

Only few studies examined the long-term interaction between physical activity and air pollution on subclinical markers.[19,20,92,93] Zhang et al. (2017) assessed the effects on white blood cell (WBC) count as a marker of systemic inflammation in a cohort of over 350 000 Taiwanese adults.[19] Independent effects were reported: the number of white blood cells decreased with physical activity and increased with air pollution. It was hypothesized that physical activity induces an anti-inflammatory response that reduces inflammation provoked by air pollution, but no interaction between habitual physical activity and yearly average $PM_{2.5}$ exposure was observed.[19]

Lovinsky et al (2017) suggested lower FOXP3 (forkhead box p3 promoter) methylation as a pathway for better lung function in active compared to non-active children (of which ca. 50% had asthma).[92] Physical activity increases activity of the T regulatory cell pathway, marked by FOX3P demethylation. This pathway suppresses pro-allergic immune responses which is associated with better lung function. They also assessed whether air pollution modified the relationship. FOXP3 demethylation was higher in active compared to non-active children in high air pollution concentrations only. The authors speculate that regular physical activity induces an enhanced anti-inflammatory response which may increase during physical activity in polluted air to counterbalance the chronic effect of airway inflammation. However, when the relationship between physical activity and lung function was assessed directly, better lung functions were observed in active versus non-active children exposed to the lowest air pollution concentrations. For children exposed to the highest air pollution concentrations, they found lower lung functions in active compared to non-active children. These results align with a review of previous work where it was reported that lung function decreased with daily training in high particulate matter environments.[94]

The same group of children was also studied by Lovinsky et al. (2016) where they found that fractional exhaled nitric oxide (FeNO) was 20% lower in active compared to non-active children.[20] FeNO is the concentration of NO in exhaled air.[95] It marks eosinic airway inflammation and is used to diagnose respiratory diseases. They hypothesized that physical activity protects against airway inflammation, assessed with FeNO, and that air pollution reduces this protective effect. When the analysis was stratified based on air pollution exposure, lower FeNO concentrations in active versus non-active children were only observed in lower air pollution concentrations. However, the interaction effect was not significant, so the authors didn’t observe a formal difference in the FeNO response to physical activity in high and low air pollution concentrations. Bos et al. (2011) included two groups of participants that completed a training program in an urban or rural environment.[93] The authors found that FeNO significantly increased after training in the urban environment while it didn’t change after training in a rural environment with lower air pollution concentrations. However, a formal test to assess if there was a difference between the FeNO responses was not reported. Lovinsky et al. (2016) and Bos et al. (2011) assessed FeNO responses to habitual physical activity in high and low air pollution which may yield information about long-term or subchronic effects. Both studies indicate either an inverse or no relationship between regular physical activity and FeNO. This response might depend on air pollution concentrations with higher FeNO levels in more polluted sites.

To our knowledge, no other studies are available where the long-term or subchronic combined effects of physical activity and air pollution on subclinical markers have been examined in a healthy study population. The described studies are also relatively recent, marking increased interest in this research question.

Short-term subclinical cardiovascular effects

Table 2 contains the results of studies that investigated the short-term, subclinical responses to physical activity in polluted air in healthy adults. A number of studies were part of the TAPAS project (Transportation, Air pollution and Physical ActivitieS) in Barcelona.[15–17,96] To our knowledge, this is the only project available that used four scenarios (rest/physical activity in combination with high/low air pollution) in order to disentangle the short-term effects of physical activity and air pollution on cardiorespiratory markers.

Jacobs et al. (2010) and Kubesch et al. (2015) used blood samples to investigate systemic inflammation.[17,97] The measured markers were plasma IL-6, IL-8, IL-10, TNF-α, CRP, platelet function, Clara cell protein in serum and total and differential blood cell counts. IL-6 has both pro- and anti-inflammatory characteristics and it is known as an anti-inflammatory myokine (a cytokine originating from muscles) produced during physical activity.[3] Kubesch at al. (2015) observed increased IL-6 concentrations with physical activity, but Jacobs et al. (2010), where participants cycled for a shorter period, didn't. It is generally accepted that anti-inflammatory IL-6 stimulates the release of other anti-inflammatory cytokines such as IL-10 which was not observed by Kubesch et al. (2015).[76] Possibly, the time period between physical activity and blood withdrawal was too short to detect secondary cytokines of the anti-inflammatory pathway induced by physical activity. Results of both Jacobs et al. (2010) and Kubesch et al. (2015) illustrated that air pollution might provoke a systemic inflammation response (Jacobs: increase in %neutrophils after cycling in high air pollution which was not observed in a clean room, Kubesch: increased neutrophils and leucocytes).[8,17,97] Other pro-inflammatory markers that have been associated with air pollution in previous studies are TNF-α, CRP, platelet function, clara protein and IL-6.[8,97] Blood samples were also used to analyze the concentration of brain-derived neurotrophic factor (BDNF).[98] BDNF plays an important role in the molecular mechanism through which physical activity enhances neural plasticity and cognition; enabling the brain to remember and recover from injury.[99] Bos et al. (2011) found a beneficial BDNF increase with physical activity that was not observed in polluted air.[98]

HRV has also been used to study the effects of physical activity in polluted air.[96,100,101] HRV informs about the autonomic control of the heart and is represented by the variation in the intervals between consecutive heartbeats.[102] The heart is electrically stimulated by the brain via the autonomic nervous system where sympathetic and parasympathetic tone interact and modulate heart rate. When sympathetic activity increases, heart rate rises and the variability between beats decreases. Weichenthal et al. (2011) reported short-term HRV decreases associated with air pollution.[100,101] The authors addressed the HRV responses to continuous pollutant concentrations during cycling only, so the study design did not allow to estimate the modification effect of physical activity on the association between air pollution and HRV. Cole-Hunter et al. (2016) assessed both physical activity and air pollution and reported larger HRV decreases during rest compared to physical activity on sites with high traffic related air pollution. Contrary, on locations with low traffic related air pollution, greater HRV reductions were observed after exercise.[96] Hence, more evidence is needed to elucidate the interaction effect between air pollution and physical activity on HRV.

Air pollution is associated with systemic inflammation markers and it is also hypothesized that oxidative stress induced by air pollution reduces NO bioavailability. However, NO availability is enhanced during physical activity, potentially provoking a defense mechanism in an inflamed environment.[78,79,103] Consequently, it is hypothesized that physical activity protects the vasculature against the detrimental effects of air pollution. This mechanism is supported by results of the TAPAS project where the increase in systolic blood pressure associated with air pollution was attenuated after physical activity.[16] Weichenthal et al. (2014) assessed microvascular function as endothelial-dependent vasodilation in response to flow mediated dilation. They observed a decrease in microvascular function after cycling in increased air pollution concentrations.[101] However, whether the responses differed during rest or if air pollution modified the relationship between physical activity and microvascular function was not analysed. More recently, retinal imaging has been applied to assess microvascular responses.

The retina is an inner membrane of the eye which converts incoming light into a neural signal that is further processed in the visual cortex of the brain. Retinal image analysis allows to determine the diameters of the central retinal vessels. These vessels supply the inner retina with blood and branch from the ophthalmic artery, which in turn branches from the internal carotid artery.[104] Retinal vessels lack autonomic innervation, but demonstrate myogenic and metabolic autoregulatory characteristics.[104,105] Consequently, local vasoactive agents such as NO dominate regulation. Retinal vessel caliber has been associated with different cardio- and cerebrovascular risk factors in large cohorts.[106–109] Regarding the pathophysiology, narrowed arteriolar lumen is related to elevated blood pressure and endothelial dysfunction.[110] Due to altered hemodynamics and vascular remodeling, this may result in hypertension. On the other hand, studies have shown that venular dilation is a predictor of stroke and associated with endothelial dysfunction and inflammation. Only few studies looked at the short-term effects of physical activity and air pollution on retinal vessel diameters. One study in cardiac rehabilitating patients observed immediate arteriolar widening after exercise.[111] Also, Adar et al. (2010) reported short-term arteriolar narrowing associated with particulate matter exposure in older adults.[112] Smaller studies reproduced this short-term observation in younger adults.[113,114] The evidence on responses in retinal venules is less consistent. However, wider retinal venules have been associated with both air pollution and low physical activity levels in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort.[112,115] There are no studies available that simultaneously assessed the effects of physical activity, air pollution and their interaction on markers of the retinal microvasculature.

Short-term subclinical respiratory effects

Some studies addressed respiratory symptoms of exercise in polluted air with questionnaires.[84,116] These questionnaires capture information about offensive odor detection, dust or soot observation, eye, nose and throat irritation, coughing and/or phlegm production, chest tightness and wheezing. Strak et al. (2010) distributed the questionnaires among their participants, yet did not analyze the collected data due to the small amount of reported changes.[116] Cole-Hunter et al. (2013) used a similar questionnaire and reported more offensive odor detection, dust or soot observation and nasopharyngeal irritation on the high air pollution site.[84]

The nasal cavity and respiratory tract are amongst the first targets of inhaled pollutants. Consequently, markers or respiratory airway inflammation and oxidative stress have been found in nasal lavage fluid, sputum and exhaled breath condensate.[30] Sputum has been analyzed by Cole-Hunter et al. (2013), but the inflammation markers did not differ between cycling in high and low air pollution concentrations.[84] In other studies, biomarkers of nasal lavage fluid, sputum and exhaled breath condensate have been shown to increase with air pollution exposure.[30] Though, these markers have not been used to disentangle the effects of physical activity and air pollution.

Increased FeNO is also identified as a marker of airway inflammation and has been observed to increase with air pollution in previous studies.[117,118] However, due to the vasodilating effect of NO, Kubesch et al. (2015) observed that short-term physical activity was related to FeNO increases.[17] The authors reported no effects of air pollution and no interaction between physical activity and air pollution on FeNO.

Another marker to measure the combined, short-term effects of physical activity and air pollution is lung function.[15,17,84,100,116,119] During physical activity, physiological changes occur to limit airway resistance[75], while exposure to air pollution has been associated with impaired lung function.[120–122] As part of the TAPAS project, independent short-term effects of physical activity and air pollution on lung function were observed.[17] In addition, a similar study did report interaction effects on respiratory parameters.[15] The researchers concluded that physical activity had an immediate, protective effect on the adverse respiratory responses to air pollution.

Finally, pulmonary inflammation and oxidative stress provoked by inhalation of particulate matter may result in airway resistance affecting exercise performance.[75,94] Giles et al. (2014) assessed the respiratory and metabolic responses to low- and high-intensity cycling in high diesel exhaust concentrations and filtered air.[11] Presence of diesel exhaust significantly increased oxygen demands (minute ventilation (Ve), oxygen consumption (VO_2), CO_2 production (VCO_2)) of low-intensity cycling which may induce reduced performance. This was not observed in high intensity cycling, indicating a protective effect of high intensity exercise on oxygen availability.

Table 2 Literature overview (chronological) of short-term physiological effects of air pollution and physical activity in healthy adults.

Author	Year	Sample size	Age	Time window	Pollutant	Independent variables	Outcomes	Result
Strak[116]	2010	12	23-57 range	1 hour 6 hours	PM_{10} Soot PNC High VS low AP	AP; no modifier	LF FeNO	No associations; trend towards ↑ LF with PNC (1 hour time window)
Jacobs[97]	2010	38	43±9 mean±SD	30 min	High VS low AP	Pre-post PA; modifier: AP	FeNO Blood inflam. markers[B]	No difference +3.9%[D] neutrophils in high AP VS no change in clean room ($p_{interaction}$=0.004); No other differences
Bos[98]	2011	38	43±9 mean±SD	30 min	High VS low AP	Pre-post PA; modifier: AP	BDNF	+14.4% in clean room[D] VS +0.5% in high AP ($p_{interaction}$ not reported)
Weichenthal[100]	2011	42 14 with asthma	19-58 range	1 hour 2 hours 3 hours 4 hours	UFP $PM_{2.5}$ BC O_3 NO_2 VOC	AP; no modifier	HRV LF	Few significant effects where HRV ↓ after exposure during cycling Overall, no associations
Cole-Hunter[84]	2013	35	39±11 mean±SD	30 min to 1 hour	High VS low AP	PA and AP in separate models; no modifier	LF Sputum Resp. symp.[A]	No difference No difference More odour detection, dust or soot observation and nasopharyngeal irritation in high AP

Author	Year	Sample size	Age	Time window	Pollutant	Independent variables	Outcomes	Result
Jarjour[119]	2013	15	23-48 range	ca. 40 min 4 hours	High VS low AP	PA and AP in separate models; no modifier	LF	No difference
Weichenthal[101]	2014	53	18-44 range	2 hours **3 hour FU**	UFP $PM_{2.5}$ BC O_3 NO_2	AP; no modifier	HRV Sys. BP Microvasc. function[C]	Overall, few significant effects ↓ HRV with $PM_{2.5}$ +2.5% with O_3 [D,E] -4.9% with UFP [D,E]
Giles[11]	2014	18	25±6 mean±SD	2 hours	Diesel exhaust	PA (categorical), AP and their interaction	Respiratory and metabolic responses to PA	<u>Overall PA:</u> -0.02 RER[D] +0.9 RPE_{lungs}[D] +0.6 RPE_{legs}[D] in high VS low AP <u>High-intensity PA:</u> No difference in responses between high and low AP <u>Low-intensity PA:</u> V_E, VO_2 and VCO_2 are significantly greater in high VS low AP
Kubesch[16]	2014	29	21-53 range	2 hours	High VS low AP	PA (categorical), AP and their interaction	Sys. BP	Ref.=low AP & rest High AP & PA: -1.4[D] Low AP & PA: -3.1[D] High AP & rest: 0.38 ($p_{interaction}$=0.08)

Author	Year	Sample size	Age	Time window	Pollutant	Independent variables	Outcomes	Result
Kubesch[17]	2015	28	21-53 range	2 hours	High VS low AP	PA (categorical), AP and their interaction		Overall, no interactions
							LF	+34mL FEV_1 with PA[D] -0.003 FEV1/FVC with AP[D]
							FeNO	+0.88ppb with PA[D]
							Blood inflam. markers[B]	+52.7% IL-6 with PA[D] +18.7% neutro. with PA[D] +4.5% leucocytes with AP[D]
Matt[15]	2016	30	19-57 range	2 hours	High VS low AP	PA (categorical), AP and their interaction	LF	+48.5mL FEV1 with PA[D] Sig. positive interaction for PEF and FEV_1/FVC
Cole-Hunter[96]	2016	28	21-52 range	2 hours	High VS low AP	AP; modifier: PA	HRV	<u>High AP site:</u> No association with AP Effect modification by PA: HRV ↓ with AP is larger during *rest* (most $p_{interaction}$ < 0.05) <u>Low AP site:</u> ↓HRV with AP Effect modification by PA: HRV ↓ with AP is larger during *PA* (some $p_{interaction}$ < 0.05)

When exposure windows are shown in bold, the reported results refer to this exposure window. LF = Lung function. Resp. symp. = Respiratory symptoms. Sys. = systolic. BP = Blood pressure. Microvasc. = Microvasculature. FU = Follow up. Inflam. = Inflammation. Neutro. = neutrophils. RER = Respiratory Exchange Ratio. RPE = Rate of Perceived Exertion. Ve = Minute ventilation. VO_2 = Oxygen consumption.VCO_2 = CO_2 production. Sig. = significant. PA = Physical activity. AP = Air pollution.
[A]Questionnaire (based on information from the American Thoracic Society) to report offensive odour detection (and dust or soot observation), eye, nose and throat irritation, coughing and/or phlegm production, chest tightness and/or wheezing, on a five-grade scale (1 = Very Low, 5 = Very High). [B]IL-6, leukocytes count, neutrophil count and % neutrophils. Additional markers were measured in both studies that used this outcome, yet none of them showed significant effects. [C]Measured as reactive hyperemia index. [D]Statistically significant with p at least <0.05. [E]Expressed as the change per interquartile increase in the pollutant.

The measurement of subclinical markers

To study subclinical changes in epidemiological studies, biomarkers in blood samples provide valuable information on the biological mechanisms. However, the use of blood samples requires extensive training of field workers and careful sample handling.[30] In addition, concentrations of circulating biomarkers are low, fluctuate over time and their analysis is subject to limited assay sensitivity. Therefore, circulating biomarkers may fail to provide accurate and robust effect estimates. Also, drawing blood is invasive which makes volunteers rather reluctant to participate.

Samples that can be collected non-invasively are: (1) sputum, nasal lavage fluid and exhaled breath condensate that contain markers of airway inflammation[30]; (2) buccal cells, oral epithelial cells with fast turnover collected by swabbing the inside of the mouth, which are used to assess DNA damage and FOXP3 methylation.[92] Analysis of biomarkers in these samples still requires laboratory tests that are expensive and time- and labor intensive.[30] Therefore, the use of non-invasive measurements that enable direct readout (or minimal post-processing) is preferred during the field work of panel studies.

We identified a number of non-invasive and direct readout markers that could be used in panel studies: HRV, blood pressure, flow-mediated dilation, retinal microvasculature, FeNO and lung function. Non-invasive measurements of health outcomes are of particular interest to use in epidemiological field studies. They facilitate an increased panel size since more volunteers might be willing to participate.

HRV can be measured with a heart rate monitor. Based on continuous heart rate measurements, time-domain and frequency-domain measures are calculated. Time domain measures include the standard deviation of normal-to-normal intervals (SDNN) and root mean square of successive differences in adjacent NN intervals (rMSSD). Frequency-domain measures are high frequency activity (HF, 0.15 to 0.40 Hz), low frequency activity (LF; 0.04 to 0.15 Hz) and their ratio. SDNN estimates overall HRV while rMSSD and HF have been linked to parasympathetic activity specifically.[102] LF is regulated by both the parasympathetic and sympathetic systems and LF/HF informs us about the degree of domination by the sympathetic system. No standard measurement method is available for the assessment of HRV in epidemiological studies.[123]. As a result, different studies use different time intervals to calculate HRV markers which complicates its interpretations and the comparability of results.

Blood pressure is a clinically established marker assessed with a blood pressure monitor that provides the results via direct readout. Markers of blood pressure are systolic, diastolic and mean arterial pressure (calculated as diastolic pressure plus 1/3th of the difference between systolic and diastolic pressure).

Flow mediated dilation is induced by occlusion of the brachial artery and marks endothelial-dependent vasodilation as a measure of microvascular dynamics.[30,101] Due to the mechanical force on the vasculature, NO production and release is stimulated. A reactive hyperemia index is calculated to identify the response time of the endothelium. Despite its potential to reveal insights on the interacting responses of the vasculature to physical activity and air pollution, its use in the field is difficult (requires immobile, specialized equipment).[30]

The retinal microvasculature, a marker of structural microvascular changes, can be visualized fast and non-invasively with static retinal imaging. Via image analysis, markers of the central retinal vessel diameters are calculated: the CRAE represents the central retinal arteriolar equivalent and the CRVE the central retinal venular equivalent. Manual analysis of these images requires a substantial amount of time, yet development of automated image analysis software is in progress.

FeNO is measured with a user-friendly, specialized device that has a small form factor. NO-free air is inhaled through the device and exhaled with a constant flow of 50 mL/s. After the measurement, the device immediately provides the results. The use of FeNO is recommended in the diagnosis of eosinophilic airway inflammation which occurs in lung diseases such as asthma.[95] In this case, NO originates from the airway epithelium where it is produced by inducible NOS (iNOS) which is upregulated when inflammation occurs.[95,124] Consequently, some studies reported that FeNO increases are also related to air pollution exposure.[117,118] However, the physiological role of NO is complex: it acts as a vasodilator, bronchodilator, neurotransmitter, and inflammatory mediator. For this reason, the origin and function of FeNO in healthy individuals is less clear.

Lung function is measured by a spirometry test; easy to use in the field and provides the results directly.[30] Lung function markers include forced ventilation capacity (FVC), forced expiratory volume in the 1st second (FEV_1), the Tiffeneau index (FEV_1/FVC), forced expiratory flow at 25% to 75% of the FVC (FEF_{25-75}) and peak expiratory flow (PEF).[125] FVC is a structural marker which gives an indication of the lung volume. FEV_1, FEV_1/FVC, FEF_{25-75} and PEF mark the airflow resistance which illustrates that these parameters are rather functional.[30,75] Clinically, spirometry is used to measure lung volumes, capacities and flow rates as an aid to diagnose respiratory diseases such as asthma and COPD.[74]

Summary

- Various HIAs reported that the benefits of active mobility outweigh the risks. However, whether the risks of air pollution modify the beneficial effect of physical activity remains unclear.
- The Danish Diet, Cancer and Health Cohort observed a smaller reduction in respiratory mortality associated with cycling in high compared to low air pollution environments.
- Evidence on subclinical response to the combination of physical activity and air pollution may yield important insights in the prevention and development of cardiorespiratory diseases.
- In vulnerable individuals, the acute beneficial effects of physical activity disappeared after walking in a polluted environment. Consequently, air pollution may affect the built-up of the benefits of regular physical activity. Additional evidence in healthy adults is needed.
- **Long-term subclinical effects of physical activity and air pollution in healthy adults:**
    - Physical activity/air pollution respectively decreased/increased WBC count as a marker of systemic inflammation. There was no interaction effect.
    - Evidence on the effects of physical activity, air pollution and their interaction on airway inflammation (marker: FeNO) and lung function is inconclusive.

      FeNO: An inverse or no relationship has been reported between regular physical activity and FeNO. This response might depend on air pollution concentrations with higher FeNO levels in more polluted sites.

      Lung function: The beneficial effect of regular physical activity on lung function may decrease in chronically elevated air pollution concentrations.

- **Short-term subclinical effects of physical activity and air pollution in healthy adults:**
    - **Blood samples**: Inflammatory markers were assessed in different studies. Only the beneficial BDNF increase was smaller after physical activity in a polluted environment compared to filtered air. This also indicates that air pollution might affect the built-up of physical activity benefits.
    - **HRV:** Only one study actively assessed the interaction between physical activity and air pollution on HRV where the direction of the interaction effect was unclear.
    - **Blood pressure**: Increases associated with air pollution are counterbalanced by physical activity which indicates a protective effect of physical activity on the vasculature. This complements the observation in vulnerable participants by Sinharay et al. (2017) where air pollution modified the relationship and attenuated the physical activity benefit.
    - **Lung function**: Two similar studies assessed the interaction between physical activity and air pollution on lung function. One study didn't observe an interaction effect. The other one observed potential counterbalancing of the air pollution effects by physical activity. This is also complements the observation in vulnerable participants by Sinharay et al. (2017).
- Only studies that used a design where all combinations of rest, physical activity, high and low air pollution are included allow to fully disentangle the responses to physical activity and air pollution. If physical activity is done in polluted versus clean air, and no effects of resting are taken into account, this restricts the observation of a protective effect of physical activity level.
- Non-invasive and direct read-out subclinical markers are preferred in the fieldwork of epidemiological studies.

Chapter 1 – Physical Activity through Sustainable Transport Approaches (PASTA): A protocol for a real-life monitoring campaign

This chapter is based on:

Dons E, Götschi T, Nieuwenhuijsen M, de Nazelle A, Anaya E, Avila-Palencia I, Brand C, Cole-Hunter T, Gaupp-Berghausen M, Kahlmeier S, **Laeremans M**, Mueller N, Orjuela JP, Raser E, Rojas-Rueda D, Standaert A, Stigell E, Uhlmann T, Gerike R, Int Panis L, 2015. Physical Activity through Sustainable Transport Approaches (PASTA): protocol for a multi-centre, longitudinal study. BMC Public Health, 15(1126).

Gerike R, de Nazelle A, Nieuwenhuijsen M, Int Panis L, Anaya E, Avila-Palencia I, Boschetti F, Brand C, Cole-Hunter T, Dons E, Eriksson U, Gaupp-Berghausen M, Kahlmeier S, **Laeremans M**, Mueller N, Orjuela JP, Racioppi F, Raser E, Rojas-Rueda D, Schweizer C, Standaert A, Uhlmann T, Wegener S, Götschi T, on behalf of the PASTA consortium, 2016. Physical Activity through Sustainable Transport Approaches (PASTA): a study protocol for a multicentre project. BMJ Open, 6(1).

Introduction

Physical inactivity has emerged as a leading risk factor for non-communicable diseases.[7] Active mobility, namely walking and cycling for transport solely or in combination with public transport, is well suited for integrating physical activity into daily routines. In contrast to sports or exercise, active mobility can be convenient in that it serves the dual purpose of being a mode of transport and physical activity. Active mobility is economically affordable for most people, so it provides an equitable and accessible form of physical activity. As such, it has the potential to reach population groups that are unresponsive to the appeals and benefits of leisure time physical activity. In addition to the direct health benefits of physical activity, an increase in active mobility may lead to city-wide improvements in air quality and additional population health benefits.

When gathering data on issues related to physical activity and active mobility, an important limitation is the typical cross-sectional design of travel and physical activity surveys. Therefore they do not allow the analysis of mobility behaviour of a cohort over a longer time period or identify causal influence.[126] In contrast, numerous research questions – for instance, how do active mobility measures influence the behaviour of inhabitants over time?; or does physical activity from active mobility substitute for physical activity from other domains?; - require repeated measurement in a multi-period design where comparable data are gathered at regular or irregular intervals. Repeated measures of active mobility and physical activity are also warranted to derive robust estimates of long term average behaviour since both active mobility and physical activity show substantial temporal variability.

Active mobility, however, is also associated with certain health risks. The higher minute ventilation during physical activity increases the inhaled dose of traffic-related air pollutants.[10,69] Health risks associated with elevated exposure to air pollution are demonstrated, but evidence is mainly from long-term studies estimating air pollution exposure on the home address.[121] Short-term health effects from exposure to traffic-related air pollution are usually studied in a small number of people in one place in a crossover design using scripted routes and repeated measures.[100,101,116]. Some studies assessed the interaction between physical activity, air pollution exposure and health outcomes.[15–17,19,96]. However, the evidence is incomplete and multiple study designs have been used: a broad range of exposure and outcome variables with different combinations of exposures for the analysis of modification effects. In addition, study samples are often small which hampers the reproducibility of the results. It follows that the available evidence doesn't allow to draw robust conclusions on the interaction effects between air pollution and physical activity on subclinical markers.

Physical Activity through Sustainable Transport Approaches (PASTA) is a collaborative research project that aims to better understand active mobility. The PASTA project addressed the outlined gaps by conducting a longitudinal study to look into correlates of active mobility, their interrelation with overall physical activity and exposure to air pollution.

Study design

The PASTA project combined a longitudinal web-based survey with smaller studies to collect objective data with wearable sensors. The survey collected data on physical activity and travel behaviour in over 12,500 volunteers living or working in seven European cities: Antwerp, Barcelona, London, Örebro, Rome, Vienna, Zurich.

This chapter elaborates on the protocol for the PASTA panel study: a real-life monitoring campaign where cutting edge technologies were applied in subsamples pulled from the survey respondents in Antwerp, Barcelona and London.

The PASTA survey was available online from November 2014 until January 2017. The PASTA panel study took place between February 2015 and March 2016.

Recruitment

A standardized recruitment strategy was developed for the longitudinal survey using an opportunistic approach.[5] Most participants were recruited through workplaces and direct mailing (e.g. via the bike to work initiative), project outreach activities like street recruiting , presence at events and web presence including social media activity (Twitter and Facebook).[55,127]

From the survey sample, we aimed to recruit 120 healthy adults to participate in the panel study. This number corresponds to 40 in each city and was decided upon based on a literature review of previous panel studies (Table 2, p. 31). Studies where 40 volunteers were recruited already observed significant physiological changes, while the execution of the study was feasible for field workers.[15–17,96]

The online survey included a question whether participants were interested in participating in a real-world monitoring campaign. Willing respondents received an additional questionnaire to check their eligibility for participation. Eligible participants were healthy, non-smoking, between 18 and 65 year olds with a self-reported BMI below 30 and no cardiovascular, respiratory or neurological condition. Sex, age, physical activity and active mobility behaviour of willing and eligible respondents was known based on their answers to the PASTA survey. This information enabled us to compose a balanced study sample. Selected volunteers were contacted personally with further information about the study design and those still willing to participate were enrolled. In the end, 122 volunteers participated in the panel study (Antwerp: 41 participants, Barcelona: 41 participants, London: 40 participants).

Monitoring devices

Participants were equipped with objective monitoring devices during one week while pursuing their regular activities (Figure 2): (1) a microAeth BC aerosol monitor (AethLabs, USA); (2) a SenseWear (BodyMedia, USA) for physical activity; (3) a GPS (I-GOTU GT-600); (4) a smartphone (Samsung Galaxy SII, Korea) with the ExpoApp installed which was developed by ISGlobal (one of the partners in the PASTA consortium). The ExpoApp assessed geolocalization and accelerometry simultaneously. In addition, we asked the participants to wear the Zephyr BioHarness (Zephyr, USA) on two days during waking hours for the continuous measurement of breathing rate, heart rate, and HRV. The measurement week was repeated in three contrasting seasons by every volunteer (mid-season, summer, winter) to address temporal variability in physical activity habits.

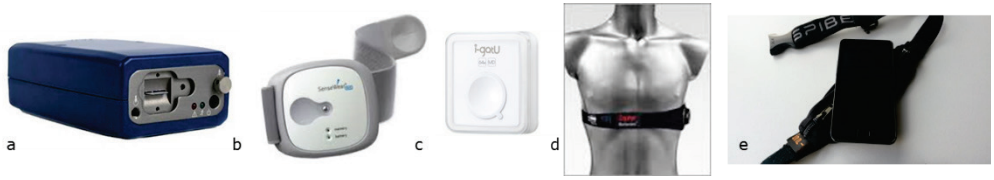


Figure 2 Wearable devices used for tracking in the PASTA panel study. (a) microAeth; (b) SenseWear armband; (c) I-GOTU GT-600 GPS; (d) Zephyr BioHarness; (e) Smartphone with ExpoApp

Subclinical health evaluations

At the beginning and at the end of the measurement week, participants visited the study center (one in each city) to evaluate a set of subclinical health outcomes in a controlled setting (Figure 2d and Figure 3).

The outcomes were selected after a literature review of the cardiorespiratory markers used in studies where the combined effects of physical activity and air pollution were of interest, with non-invasive and direct read-out measures given preference. The final selection included both cardiovascular and respiratory parameters: (1) HRV is measured by the Zephyr BioHarness; (2) Blood pressure (measured by the Omron M10-IT, the Netherlands); (3) The retinal microvasculature which is visualized noninvasively with fundus photography (Canon CR-2 Retinal Camera, Hospithera, Belgium). The CRAE and CRVE were assessed through image analysis software (iFlexis, VITO, Belgium); (4) FeNO is measured with the NIOX VERO (Aerocrine, Sweden); (5) Lung function was assessed with the EasyOne spirometer (ndd Medizintechnik AG, Switzerland). Moreover, on the fourth day of the measurement week, participants assessed their blood pressure and HRV themselves at home. Participants were asked to refrain from vigorous-intensity activities, caffeine, alcohol and foods that are high in nitrates during four hours before the biomarker assessments. Finally, during the participant's last visit to the research center, weight, body mass index (BMI) and body fat were measured with a body composition monitor (model BF511, Omron, Japan).

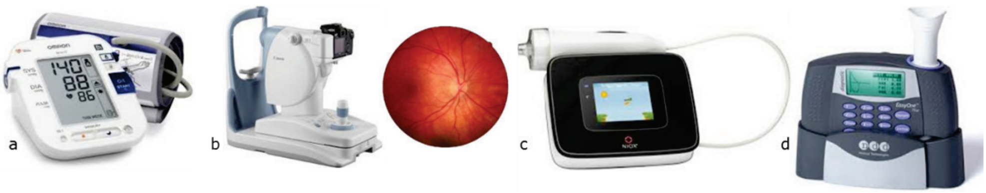


Figure 3 Devices to assess the subclinical health outcomes in the PASTA panel study. (a) Omron M10-IT blood pressure monitor; (b) Canon CR-2 Retinal Camera with retinal image; (c) NIOX VERO to assess FeNO; (d) EasyOne spirometer

Questionnaires

During each study visit, participants completed a survey about their behaviour and wellbeing during the previous week. It contained questions about their disease status, medications or food supplement intake, and included the Global Physical Activity Questionnaire (GPAQ). On the fourth measurement day, the measurements of HRV and blood pressure at home were also complemented with a questionnaire.

The surveys were implemented on the online PASTA platform. This is an online web application, with a responsive design approach (i.e. the questionnaire can be completed across a wide range of devices – from mobile phones and tablets to desktop computers). The PASTA platform is implemented in PHP (a popular general-purpose scripting language, especially suited for web development) with a PostgreSQL back-end database. All content was developed in English and translated into Dutch, Catalan and Spanish by native speakers.

The measurement week: overview

Each participant repeated the measurement week in three different seasons: in the mid-season (autumn or spring), in the summer and in the winter. The course of a measurement week is illustrated in Figure 4 and summarized below:

- Day 0, late afternoon: Kick-off session to evaluate the subclinical outcomes in the following order: (1) HRV (while the participants filled out the kick-off questionnaire), (2) blood pressure, (3) retinal microvasculature, (4) FeNO and (5) lung function. The continuous measurements started during late afternoon, at the end of this session.
- During the whole week, participants behaved as a mobile lab and measured their exposure to BC, tracked their physical activity level and geolocation and collected data with the ExpoApp.
- Day 1 and 6: Participants also measured their heart rate continuously, during waking hours with the Zephyr BioHarness.
- Day 4, late afternoon: Participants assessed their HRV and blood pressure themselves (at home) at approximately the same time as the kick-off session.
- Day 7, late afternoon: Finish line session where we also evaluated the subclinical outcomes in the same order and at approximately the same time as the kick-off session. Participants again filled out a finish line questionnaire during the HRV measurement.

Finally, during the kick-off and finish line session of the participants' last measurement week, body measures were evaluated as well.

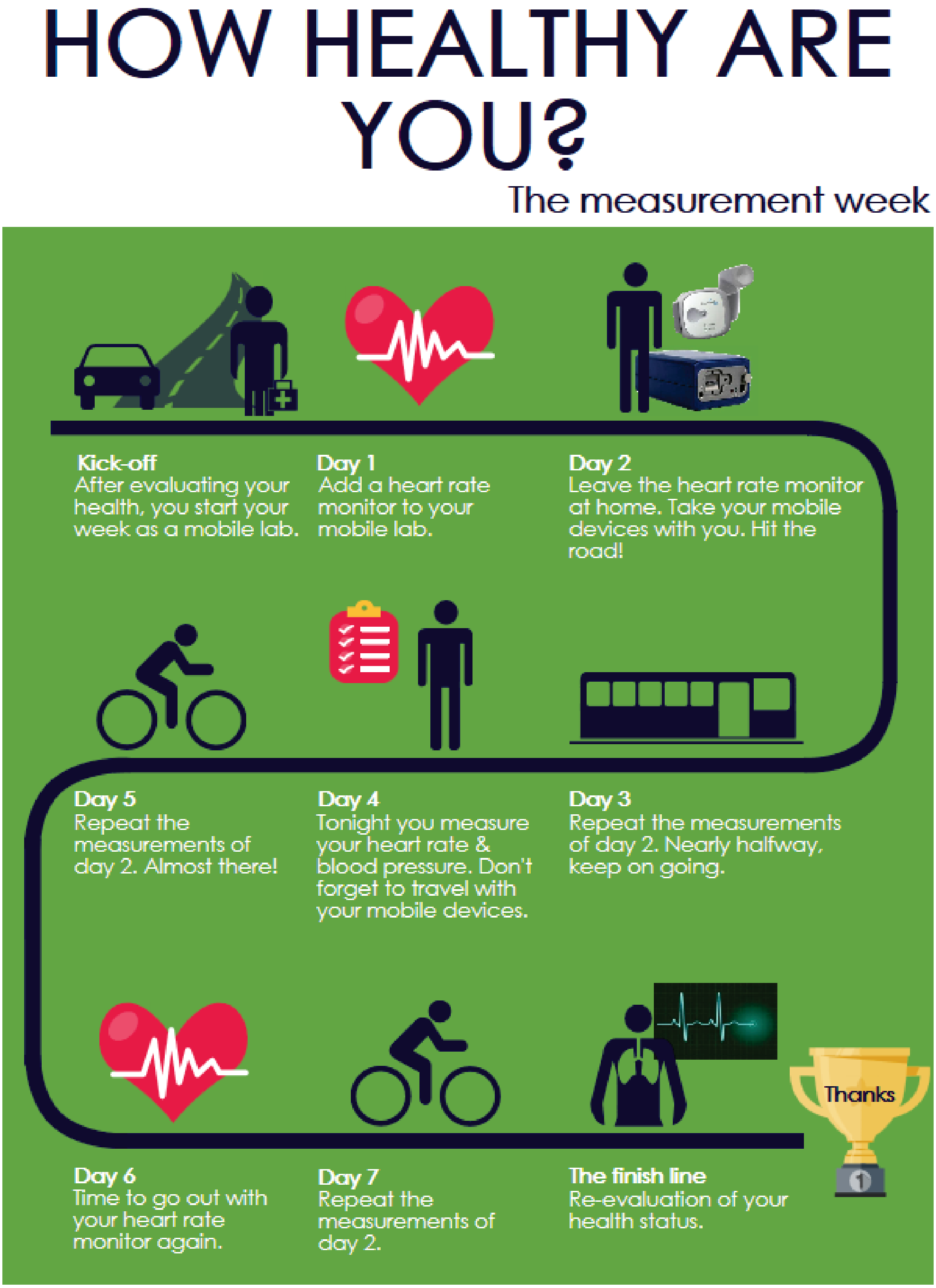


Figure 4 Graphical representation of the measurement week

Data analysis coordination

The data analysis plan for the PASTA panel study involved coordinated procedures for planning, organizing and documenting research. GitHub was used as a practical tool for exchange of R scripts, version control, issues and milestones between researchers (https://github.com/). All data used for the panel study was anonymized before further analysis.

Data analysis for this PhD includes (1) the comparison of results from different physical activity measurement techniques: the GPAQ and the SenseWear armband, and (2) estimation of the long-and short-term combined effects of physical activity and BC, assessed with the SenseWear armband and microAeth, on all subclinical outcomes excluding blood pressure. Researchers from ISGlobal (a partner within the PASTA consortium) will analyze the effects on blood pressure and validate the data collected with the ExpoApp. Future research will handle continuous HRV data collected with the Zephyr BioHarness (Hasselt University, Belgium) and data from the GPS for the development of novel air pollution exposure assessment techniques (Imperial College, London).

Raw data was processed so all researchers performed their final analysis on the same cleaned dataset. Raw data of the microAeth was smoothened with the Aethalometer Optical Noise-Reduction Averaging (ONA) program. The following measurements were excluded from the analysis: (1) an error codes that marked to change the filter ticket or that the flow was out of range, (2) a flow equal to zero, and (3) unrealistic BC values (below -50,000 ng/m^3 and above 250,000 ng/m^3). HRV was calculated over a time window of 20 minutes and was not determined in the following situations: (1) sessions where no full 20 minute interval was recorded, (2) a heart rate of zero during at least 10 minutes of the interval, (3) measurements where heart rates had unacceptable physiological values (less than 25 bpm or more than 200 bpm), and (4) a mean heart rate over 120, since this is unlikely to mark a resting state. For the retinal vessel diameters, blurry pictures were excluded if the borders of the microvasculature could not be distinguished during image analysis. the EasyOne spirometer assessed the quality of each separate maneuver and the overall test in real time. When the participant didn’t provide three acceptable manoevers, the test was excluded from the analysis. The quality of the overall test was rated from A to E, where A is the best available grade. Grade D is defined as 'not reproducible', so if the participant did not reach at least grade C, the spirometry test was repeated until fatigue appeared. There were no missing values for FeNO since the NIOX VERO didn't provide a result if the measurement failed.

Comparison to previous work

The PASTA panel study used a repeated measures design to collect objective data on physical activity and air pollution in a free-living population living or working in three European cities. An overview of the added value of the PASTA panel study to previous research (summarized in Table 2, p. 31) is provided in Table 3.

Table 3 Contribution of the PASTA panel study to the research field regarding the subclinical responses to the combined effects of physical activity (PA) and air pollution (AP).

Previous work	The PASTA panel study
No direct assessment of PA	Habitual PA was measured continuously with the SenseWear armband.
Air pollution assessments rely on fixed monitoring sites or estimations at the home location	Black carbon concentrations are measured personally with a mobile sensor (microAeth).
Limited knowledge about exposure before the evaluation of subclinical markers	Detailed information about the real-life time-activity pattern during one week before the outcome assessments.
Conducted in only one city	Multicenter study with data collection in three European cities.
12-42 participants	122 participants
No repeated measurements	Repeated measurements resulting in enhanced statistical power: - Time-activity pattern: 3 assessments - Subclinical markers: 6-9 assessments Measurements in different seasons also enabled the approximation of long-term behaviour.
Focus on one subclinical marker	A set of subclinical markers is defined based on a literature review and assessed in an integrated fashion to provide an overview.
Broad range of study designs resulting in the lack of robust conclusions on the interactive behaviour between PA and AP	Continuous assessment of all predictors resulting in a transparent study design without prior restriction on the possible directions of the interaction effect.

Ethics

The PASTA panel study was approved by the Ethics Committee of the University hospital in Antwerp (UZA) on the 12th of January 2015, the Clinical Research Ethical Committee of the Parc de Salut Mar in Barcelona on the 24th of December 2014, and the Imperial College Research Ethics Committee in London on the 20th of April, 2015. All participants gave written informed consent prior to participation and received a small financial compensation at study completion (€150 for the completion of three measurement weeks).

Chapter 2 – Physical activity and sedentary behaviour in daily life: a comparative analysis of the Global Physical Activity Questionnaire (GPAQ) & the SenseWear armband

This chapter is based on:

Laeremans M, Dons E, Avila-Palencia I, Carrasco-Turigas G, Orjuela JP, Anaya E, Brand C, Cole-Hunter T, de Nazelle A, Götschi T, Kahlmeier S, Nieuwenhuijsen M, Standaert A, De Boever P, Int Panis L, 2017. Physical activity and sedentary behaviour in daily life: A comparative analysis of the Global Physical Activity Questionnaire (GPAQ) and the SenseWear armband. PLoS ONE 12(5).

Abstract

Reduction of sedentary time and an increase in physical activity offer potential to improve public health. However, quantifying physical activity behaviour under real world conditions is a major challenge and no standard of good practice is available. Our aim was to compare the results of physical activity and sedentary behaviour obtained with a self-reported instrument (Global Physical Activity Questionnaire (GPAQ)) and a wearable sensor (SenseWear) in a repeated measures study design.

Healthy adults (41 in Antwerp, 41 in Barcelona and 40 in London) wore the SenseWear armband for seven consecutive days and completed the GPAQ on the final day. This was repeated three times. We used the Wilcoxon signed rank sum test, Spearman correlation coefficients, mixed effects regression models and Bland-Altman plots to study agreement between both methods. Mixed models were used to assess the effect of personal characteristics on the absolute and relative difference between estimates obtained with the GPAQ and SenseWear.

Moderate to vigorous energy expenditure and duration derived from the GPAQ were significantly lower ($p<0.05$) compared to the SenseWear, yet these variables showed significant correlations ranging from 0.45 to 0.64. Estimates of vigorous-intensity physical activity in particular showed high similarity ($r>0.59$). Results for sedentary behaviour did not differ, yet were poorly correlated ($r<0.25$). The differences between all variables were reproducible across repeated measurements. In addition, we observed a relationship between these differences and BMI, body fat and physical activity domain.

Due to the lack of a standardized protocol, results from different studies measuring physical activity and sedentary behaviour are difficult to compare. Therefore, we suggested an easy-to-implement approach for future studies adding the GPAQ to the wearable of choice as a basis for comparisons.

Introduction

Physical inactivity is an important modifiable risk factor for premature mortality.[128] It has been shown that sedentary behaviour and lack of physical activity independently increase the risk for metabolic syndrome[129], type 2 diabetes[130], cardiovascular disease[131] and mortality[132]. Therefore, both reducing sedentary time and increasing physical activity levels offer beneficial health effects, caused by different molecular and physiological mechanisms.[133] An accurate measurement of the total and intensity-specific physical activity level and sedentary behaviour in real-world conditions is a crucial element in studying the association between physical activity, sedentary behaviour and health. Characterization of daily patterns of physical activity will enable researchers to distinguish structured exercise, sedentary behaviour and non-exercise physical activity. This is important in the context of surveillance, intervention studies and the accuracy of dose-response relationships for physical activity.

Quantification of physical activity and sedentary behaviour under real-world conditions is an important challenge in epidemiological research.[21,46] Due to the poor quality of measurements and lack of internationally comparable data, the International Physical Activity Questionnaire (IPAQ) and the Global Physical Activity Questionnaire (GPAQ) were developed.[1,23,34] Both questionnaires capture information on physical activity duration and intensity, but the burden of filling out the longer version of the IPAQ is too high for routine surveillance. On the other hand, and contrary to the GPAQ, the short IPAQ version does not cover multiple physical activity domains such as work, transport and leisure time.[23,34] The GPAQ was developed by the World Health Organization (WHO) in 2002 as part of the WHO STEPwise approach to surveillance (STEPS).[23,35] Although it performs well compared to other surveys, self-reported tools to measure physical activity levels are prone to sources of error such as recall bias and social desirability.[23–25] Sex, age, body measures, ethnicity and type of activity have been shown to influence reporting behaviour.[24,46,134]

During the last decade, wearable monitors have become a key element in physical activity research.[22] These devices register physiological responses (e.g. heart rate) and/or mechanical bodily movements (accelerometry).[38] Such monitors have evolved from mechanical pedometers that count steps to electronic multi-sensor devices like the SenseWear armband.[36,38] The SenseWear uses pattern recognition algorithms of various activities to provide valid estimates of physical activity energy expenditure.[38,40,41] The gold standard to accurately assess free-living energy expenditure is doubly labelled water (DLW), yet this method does not allow to differentiate between light-, moderate- and vigorous-intensity physical activity or to identify time-activity patterns.[36] In addition, measuring energy expenditure using DLW is expensive and labour-intensive. St-Onge et al.[42], Johannsen et al.[40], Mackey et al.[43] and Brazeau et al.[44] studied the difference between DLW and SenseWear results and reported a mean difference ranging from an overestimation of 25 kcal/day to an underestimation of 117 kcal/day by the SenseWear armband. They concluded that the SenseWear delivers accurate results for the measurement of total energy expenditure. Despite the advances in the field of objective monitoring, assessment of physical activity volume still lacks standards of good practice.[22]

In this study, we used two methods to estimate physical activity and sedentary behaviour under real-world conditions: the SenseWear armband, one of the most advanced wearable devices, and the GPAQ, a questionnaire developed to systematically collect data on physical activity levels. Both methods use a different data collection approach, yet are widely used to study the effects of activity behaviour on health. Therefore, our objectives are:

1) To determine the level of agreement between measurements of physical activity and sedentary behaviour collected with the SenseWear armband and the GPAQ.
2) To assess whether the differences between SenseWear and GPAQ estimates within a person change across repeated measures.
3) To identify personal characteristics that affect the difference between both methods.

Materials and Methods

Study design and participants

This study was part of the FP7 PASTA project (Physical Activity through Sustainable Transport Approaches), which has been described previously.[4,5] Briefly, data on physical activity and travel behaviour was collected through an online survey in over 12,500 volunteers in seven European cities (Antwerp, Barcelona, London, Örebro, Rome, Vienna, Zurich). From this sample, 122 eligible and willing respondents were selected in three cities (Antwerp: 41 participants, Barcelona: 41 participants, London: 40 participants). They took part in an experiment allowing us to collect objective physical activity sensor data and to compare this to the subjectively reported levels of physical activity. Eligible participants were healthy, non-smoking, 18 to 65 year olds with a self-reported BMI below 30. Participants wore the SenseWear armband (model MF-SW, BodyMedia, USA) for seven consecutive days, only removing the sensor when there was contact with water (bathing, showering, etc.). At the end of the measurement period participants filled out the GPAQ via the online PASTA platform. Each participant repeated this procedure three times: in the mid-season, in the summer and in the winter. The repeated measurements are referred to as session 1 (mid-season), session 2 (summer) and session 3 (winter). During the participant's last visit to the research centre, weight and percentage body fat were measured with a body composition monitor (model BF511, Omron, Japan). The study was approved by the Ethics Committee of the University hospital in Antwerp (UZA), the Comité Ético de Investigación Clíníca Parc de Salut MAR in Barcelona and the Imperial College Research Ethics Committee in London. All participants gave written informed consent prior to participation.

Measures of physical activity

The GPAQ is developed and validated by the WHO to systematically monitor global physical activity levels.[1,23] It is included in the PASTA online questionnaire to collect information on the duration and frequency of moderate- and vigorous-intensity physical activity during work, transportation and leisure time (the

GPAQ is provided in the appendix: Figure S 4, p. 150). Participants were asked only to report activities lasting 10 minutes or longer. The standard GPAQ was adjusted to ask for duration and frequency of walking, cycling and e-biking separately. Moderate/vigorous intensity activities are described as activities that require moderate/hard physical effort and cause small/large increases in breathing or heart rate. Average sitting time per day is reported as a proxy for sedentary behaviour. Participants filled out the GPAQ in their preferred language. Moderate-intensity activities, vigorous-intensity activities, walking, cycling and e-bike trips were assigned a value for their metabolic equivalent of task (MET) of 4, 8[135], 4, 6.8[136] and 5[137,138] respectively. Cleaning of GPAQ data and calculation of moderate to vigorous METminutes per week, minutes per day and sedentary minutes per day was performed according to the WHO GPAQ analysis guidelines.[135]

The SenseWear armband is a multi-sensor body monitor that measures heat flux, galvanic skin response, skin temperature and 3-axis accelerometry. It is worn on the tricep muscle of the left arm. Age, sex, body weight and height of the participants are provided manually to the SenseWear professional software (version 8.0). The SenseWear calculates energy expenditure and METs on a one-minute basis using proprietary algorithms based on pattern recognition.[38] For each measurement week, energy expenditure and minutes during intensity-specific activities were calculated in R version 3.3.1: moderate- to vigorous-intensity physical activity (MVPA), moderate-intensity physical activity, vigorous-intensity physical activity and sedentary behaviour. Bouts of at least 10 consecutive minutes with an intensity ≥ 3 METs were identified to match the data collection and analysis of the GPAQ.[135] If the intensity was ≥ 6 METs during at least half of the bout's duration, it was labelled 'vigorous-intensity'. If not, the bout was labelled 'moderate-intensity'. Energy expenditure and minutes during moderate-intensity physical activity, vigorous-intensity physical activity and MVPA were calculated as the sum of respective METs and minutes during the identified bouts. Minutes of sedentary behaviour per day are calculated as the sum of minutes with METs ≤ 1.5 minus time spent sleeping.[135,139–141]

Analysis

Medians and interquartile ranges (IQR) were reported because the calculated variables were not normally distributed. Physical activity measurements collected with GPAQ and SenseWear during each session were compared using the Wilcoxon signed rank sum test and Spearman correlation. To assess the overall level of agreement, Spearman correlation coefficients adjusted for repeated observations were calculated according to Bland & Altman.[142] These overall correlation coefficients could not be tested for significance. For the interpretation of the Spearman correlation coefficients, we used the scale defined by Landis & Koch.[143]

To study individual differences, Bland-Altman plots were constructed using 95% limits of agreement adjusted for repeated measures according to Bland & Altman.[144] Absolute differences showed an increase in variability when the magnitude of the physical activity variables increased. Therefore, the percentage difference Bland-Altman plots were used. However, in the GPAQ, 0 minutes of moderate- and vigorous-intensity physical activity were reported 43 and 50 times, respectively. Furthermore, 0 minutes of vigorous-intensity physical activity were measured by the SenseWear 25 times. These are influential observations and cause deviations from the normal distribution of the differences. Consequently, the mean difference and 95% limits of agreement were calculated both with and without these observations. When the limits of agreement exceeded 200%, the largest possible percentage difference, the limit was drawn at a percentage difference of 200. In addition, for vigorous-intensity physical activity, 0 minutes were both reported in the GPAQ and measured by the SenseWear 40 times. This results in an undefined percentage difference. In order to include these observations in the calculation of the mean difference and 95% limits of agreement, we used 0.001 instead.

Mixed effect regression models were used to study: (1) whether the difference between GPAQ and SenseWear measurements changed per session ($\Delta(t)$ model; Table 4), and; (2) the effect of participant characteristics on the difference between both methods (attribute models; Table 4). For the latter objective, we used separate models to study the effect of sex, age, BMI, percentage body fat and physical activity domain as a descriptive analysis. We assumed that BMI and percentage body fat were stable during the observation period. Consequently, inclusion of all measurement weeks increased our statistical power. For each measurement week, the dominant physical activity domain (work, transport or leisure-time) was determined using the GPAQ data. This was based on the assumption that the participants' recall bias depended on the physical activity domain in which they were most active. All mixed models included random participant effects nested in city effects.

Table 4 Mixed effect regression models used to analyse (1) the change in difference between GPAQ and SenseWear measurements; (2) the effect of personal attributes on the difference between both methods.

Δ(t) model	$\Delta_{ijk} = \beta_0 + a_i + b_{j\,(i)} + \beta_1 session\ 2_{ijk} + \beta_2 session\ 3_{ijk} + \varepsilon_{ijk}$
Attribute models	$\Delta_{ijk} = \beta_0 + a_i + b_{j\,(i)} + \beta_1 attribute_{ijk} + \varepsilon_{ijk}$
Assumptions	$a_i \sim N(0, \sigma^2_{cities}); b_{ij} \sim N(0, \sigma^2_{participants}); \varepsilon_{ijk} \sim N(0, \sigma^2_{residuals})$

Where Δ_{ijk} is the difference between the estimates of both methods for the k^{th} measurement of individual j in city i.

Results

A total of 122 healthy adults took part in the study between February 2015 and March 2016 (45% males, 89% with higher education degrees, 94% Caucasian, age: 35±10 years, BMI: 24±3 kg/m²; Appendix: Table S 1, p. 146). 119 participants completed three 7-day measurement periods (1 participant participated during 2 periods; 2 participants completed only 1 period). Out of 361 GPAQ and SenseWear estimates per variable respectively 11 (reported time was invalid) and 3 (failed read-out and incorrect wearing) were missing. Consequently, 16 participants had less than 3 pairs of data (13 had 2 pairs; 3 had 1 pair). The SenseWear armband was worn 96±4 % of the time (average and SD of the average wearing time per individual).

Table 5 shows the results from both measurement methods aggregated over three sessions per individual. We recruited an active sample: only 13% (GPAQ) and 7% (SenseWear) of METminutes/week were below 600 i.e. the WHO recommendation on physical activity.[139] This corresponds to 5% (GPAQ) and 2% (SenseWear) of our participants having a physical activity level below the recommendation during all sessions.

Table 5 Median and IQR of the physical activity measures aggregated over three sessions per participant for both measurement methods (GPAQ and SenseWear). Number of participants included in the analysis is 122.

	MVPA EE (METmin/wk)	**Moderate EE** (METmin/wk)	**Vigorous EE** (METmin/wk)	**MVPA** (min/day)	**Moderate PA** (min/day)	**Vigorous PA** (min/day)	**Sedentary behaviour** (min/day)
GPAQ (median (IQR))	2029 (1112-3237)	720 (310-1268)	1057 (321-2169)	53 (33-78)	26 (11-45)	22 (6-40)	535 (420-635)
SenseWear (median (IQR))	2569 (1688-4280)	1575 (1133-2256)	807 (207-2154)	71 (49-111)	47 (34-67)	17 (4-44)	550 (481-653)
% Difference [a] (median (IQR))	%						
	39 (0-75)	77 (30-144)	7 (-56-69)	34 (0-79)	62 (9-136)	7 (-53-71)	8 (-12-30)

GPAQ = global physical activity questionnaire, MVPA = moderate to vigorous physical activity, EE = energy expenditure, PA = physical activity.

[a] The percentage difference was calculated by subtracting GPAQ from SenseWear results divided by their average and reported as the median and IQR of the average difference per participant.

Estimates for moderate to vigorous and moderate energy expenditure obtained by the GPAQ were significantly lower compared to the SenseWear (Figure 5). However, no statistically significant differences between both methods were observed for vigorous energy expenditure alone. Measures of overall MVPA and vigorous energy expenditure from the GPAQ and SenseWear were significantly correlated. Moderate energy expenditure only showed a significant correlation on session 2. Moderate, MVPA and vigorous energy expenditure had an overall Spearman correlation (r_{rm}) of fair (0.33), moderate (0.54) and high (0.64) strength, respectively. The differences between both methods were reproducible as they did not change over time (i.e. no significant overall effect of session in the Δ(t) model; $p_{\Delta(t)} > 0.05$). The same trends were observed for minutes per day of MVPA, moderate- and vigorous-intensity physical activity (Appendix: Figure S 1, p. 147).

Sedentary minutes measured by the GPAQ and SenseWear were significantly different on session 1 only (Figure 5). Sedentary behaviour was poorly correlated between both methods. Hence, for each amount of sedentary minutes measured by the SenseWear, we observed a large range of estimates reported in the GPAQ (Appendix: Figure S 2, p. 148). Similarly to the results for physical activity variables, the difference in sedentary behaviour between both methods did not change significantly across sessions.

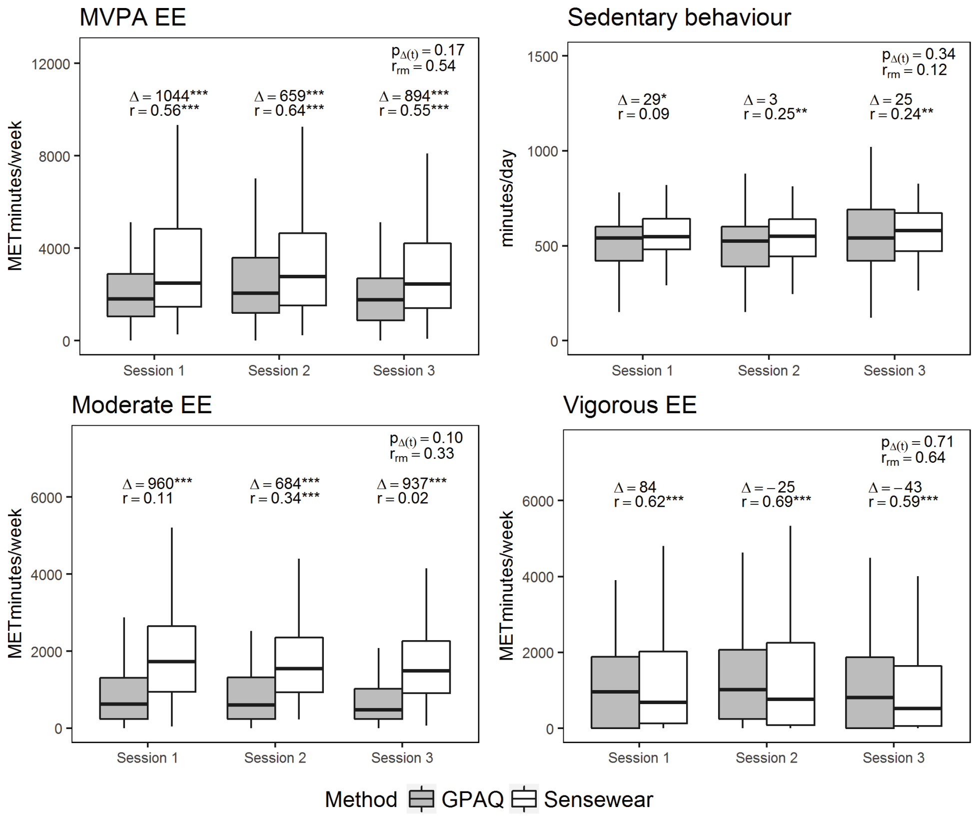


Figure 5 Boxplots of MVPA energy expenditure (EE), sedentary behaviour, moderate EE and vigorous EE per method and session. For each session, the difference Δ and Spearman correlation coefficient r is specified. Δ was calculated as the mean difference between both methods and was tested for significance using the Wilcoxon signed rank sum test. r_{rm} is the overall Spearman correlation adjusted for repeated measures (rm); $p_{\Delta(t)}$ = the p-value of the effect of session in the Δ(t) model which indicates if the difference between GPAQ and SenseWear measurements changes per repeated measurement. Statistical significance is expressed as *p<0.05, **p<0.01, and *** p<0.001

Bland-Altman plots using the percentage difference further illustrate that lower levels of moderate to vigorous and moderate energy expenditure were obtained with the GPAQ, while the mean difference for vigorous energy expenditure was close to 0 (Figure 6). On the other hand, the 95% limits of agreement were wide. For moderate and vigorous energy expenditure, 3 out of 4 limits reached the maximum percentage difference of 200. This was due to the inclusion of influential observations where either the GPAQ or SenseWear estimate was 0. Excluding these observations still resulted in limits where the magnitude of the differences exceeded the size of the mean difference. Analysis of MVPA, moderate- and vigorous-intensity physical activity minutes per day showed similar results (Appendix: Figure S 3, p. 149).

The Bland-Altman plot for sedentary behaviour indicates a mean difference close to 0 and narrower 95% limits of agreement (Figure 6). However, a relationship between the differences and the mean was observed. When the amount of sedentary behaviour increased, the amount of sedentary time reported in the GPAQ exceeded the estimate of the SenseWear.

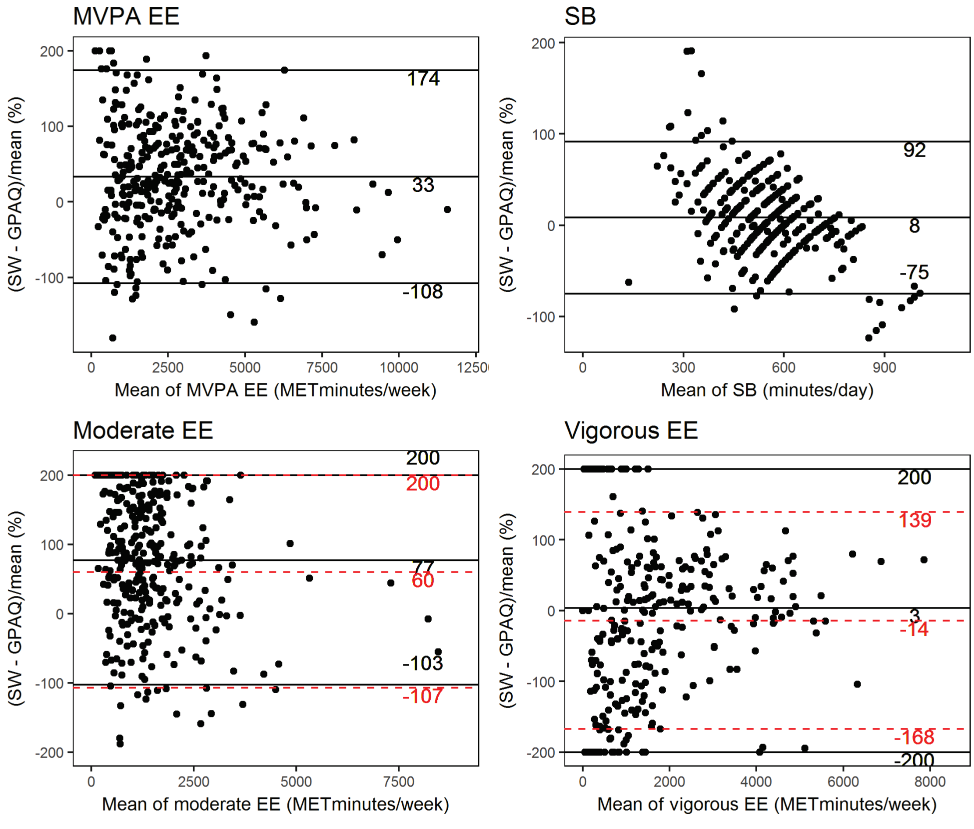


Figure 6 Bland-Altman plots comparing MVPA, moderate and vigorous energy expenditure (METminutes/week) and sedentary behaviour (minutes/day) measured by the SenseWear armband (SW) and the GPAQ. All percentage differences on the Y-axis are calculated by subtracting GPAQ from SenseWear results divided by their average. Moderate- and vigorous-intensity activities included influential observation. The red, dashed lines represent the mean difference and 95% limits of agreement excluding these observations. EE = energy expenditure. SB = Sedentary behaviour.

The discrepancy between SenseWear and GPAQ estimates was not affected by sex or age (Table 6). Per unit increase in BMI and percentage body fat, the absolute difference decreased significantly for MVPA and vigorous-intensity physical activity. For vigorous-intensity physical activity, similar, non-significant trends were observed for the percentage differences. Furthermore, participants mainly active during work compared to those mainly active during leisure time had significantly lower differences between SenseWear and GPAQ estimates for MVPA and moderate-intensity physical activity. For vigorous-intensity activities and MVPA, the percentage differences increased significantly for participants mainly active during transport compared to leisure time. Regarding the differences between both methods in sedentary behaviour, we observed the opposite trends for BMI and body fat compared to the estimates of activity. For physical activity domain, both the absolute and percentage difference increased for sedentary behaviour in participants mainly active during work.

Table 6 β_1-coefficients of the attribute models to indicate the effect of sex, age, BMI, body fat and physical activity domain on the absolute and percentage difference (Δ) between measured (SenseWear) and reported (GPAQ) METminutes/week, minutes physical activity (PA)/day and sedentary minutes/day. The difference between both methods is used as the dependent variable. Absolute differences were calculated as SenseWear minus GPAQ results. Percentage differences are calculated by dividing the absolute difference by the average of the measurements from both methods. Separate models were fitted for each characteristic. Each model included random participant effects clustered per city.

β_1 ± SE		Sex[a]	Age (years)	BMI (kg/m²)	Body fat (%)	PA domain[b] Transport	Work
Δ MVPA EE	METmin/week	431.7±344.7	-6.12±17.59	-151.57±55.49**	-57.04±20.84**	153.9±277.5	-1370.4±521.8**
	%	6.12±10.7	-0.47±0.54	-2.64±1.73	-0.43±0.66	21.24±8.47*	-38.14±15.91*
Δ Mod EE	METmin/week	27.78±233.52	-9.75±11.8	-51.50±38.32	-13.57±14.47	-53.13±200.13	-1136.84±368.99**
	%	-5.9±13.96	-0.99±0.70	-2.35±2.29	-0.32±0.87	1.68±10.87	-78.25±20.28***
Δ Vig EE	METmin/week	400.84±223.6	3.37±11.5	-99.25±36.31**	-43.62±13.47**	320.83±218.18	20.41±396.06
	%	30.32±16.41	-1.01±0.84	-5.15±2.68	-1.91±1.01	40.76±15.68**	33.27±28.58
Δ MVPA	minutes/day	4.73±8.91	-0.24±0.45	-2.96±1.45*	-0.93±0.55	1.42±7.36	-48.36±13.69***
	%	0.31±10.92	-0.51±0.55	-1.96±1.78	-0.13±0.67	14.45±8.38	-52.29±15.77**
Δ Mod PA	minutes/day	-2.36±7.45	-0.29±0.38	-0.97±1.23	-0.10±0.46	-1.54±6.45	-47.82±11.79***
	%	-7.15±14.43	-1.01±0.73	-1.6±2.38	-0.09±0.9	2.04±11.20	-83.03±20.88***
Δ Vig PA	minutes/day	6.99±4.20	0.04±0.22	-1.96±0.68**	-0.83±0.25**	4.71±4.21	2.1±7.58
	%	28.32±16.34	-1.05±0.83	-5.25±2.66	-1.84±1	38.44±15.66*	31.53±28.52
Δ SB	minutes/day	-50.9±37.35	1.05±1.91	12.46±6.03*	5.03±2.26*	-15.19± 25.64	123.45±49.18*
	%	-9.32±6.75	0.11±0.34	1.77±1.1	0.82±0.41 *	-7.06±4.47	32.55±8.57***

[a] women are the reference category; [b] leisure time physical activity is the reference category; PA = physical activity, Mod = moderate, Vig = vigorous, SB = sedentary behaviour. EE = energy expenditure.
Statistical significance is expressed as *p<0.05, **p<0.01, and *** p<0.001

Discussion

This is a comparative analysis of physical activity and sedentary levels measured by the GPAQ and the SenseWear armband. Compared to previous studies, we collected a large dataset using a repeated measures design on 122 participants in three different European cities.[39,47,48,52] The estimates obtained with the GPAQ were significantly lower for MVPA and moderate-intensity physical activity; no difference was observed for vigorous-intensity activities and sedentary behaviour. Significant positive correlations were found for MVPA and vigorous-intensity physical activity and the overall Spearman correlation was of respective moderate and high strength. Non-significant poor to fair correlations were observed for sedentary behaviour and moderate-intensity physical activity.

Cleland et al.[47] compared the GPAQ to the Actigraph and did not find a significant difference for MVPA time, while sedentary behaviour did differ between both methods. Though, similar to our results, moderate[47] and fair[145] correlations were reported for MVPA time, and poor agreement for sedentary behaviour.[47] The low correlation for sedentary behaviour could be explained by the use of a single item to measure sedentary time in the GPAQ. Hence, including multiple, domain-specific items to assess sedentary behaviour is needed as recent papers stress the importance of sedentary behaviour for public health.[146]

Other studies compared the SenseWear armband to an interviewer-administered 24-hour physical activity recall (PAR)[25] and the Flemish Physical Activity Computerized Questionnaire (FPACQ)[46]. The time between the activity and recall is only 24 hours when using PAR, compared to seven days for the GPAQ, which results in lower recall bias.[24,147] Similar to our results, both studies reported moderate to high correlations for total energy expenditure and MVPA time.

Our participants reported more vigorous-intensity physical activity compared to moderate-intensity physical activity. The tendency towards overestimating vigorous activity and underreporting moderate activity has already been shown.[45,46,48,52,148,149] Possibly, participants perceive or recall the exertion different than the objective physical output. This makes it difficult to distinguish between moderate and vigorous intensity when reporting physical activity levels.[34,45] However, when reporting physical activity, respondents think about vigorous or organized activities such as sports, while they forget about moderate, routine activities (e.g. household chores or active transportation) or incidental daily movements.[46] This might explain the better agreement between the GPAQ and the Sensewear for vigorous energy expenditure and time. Besides, it illustrates the importance of reporting domain specific activities.

Accordingly, we found a significant effect of physical activity domain on the difference between the SenseWear and GPAQ estimates. When looking at percentage differences, the amount of physical activity engaged in is filtered out. Consequently, participants mainly active during transport compared to leisure-time had a similar activity level, yet the difference between both methods increased for MVPA energy expenditure and vigorous-intensity activities. Hence, besides underreporting moderate, routine activities, we hypothesise that vigorous, routine activities such as cycling are underreported as well. In addition, the difference was significantly smaller for MVPA and moderate-intensity activities in participants mainly active during work compared to leisure time. Other studies comparing physical activity questionnaires to more objective measurement techniques also found higher correspondence for reported physical activity during work compared to leisure time or transport.[150,151] In our study with participants with a BMI below 30, we observed an effect of BMI and body fat on the difference between estimates of the SenseWear and GPAQ. We found that the absolute differences for MVPA energy expenditure and time decreased per unit increase in BMI and percentage body fat, similar to Welk et al.[25] In addition, we observed the opposite effect for sedentary behaviour. However, when the percentage differences were tested, the effect did not remain for activity measures due to the association between physical activity level as such and BMI or percentage body fat. When studying the effects of BMI and body fat on moderate- and vigorous- intensity physical activity separately, we observed a (non-significant) decrease in the difference for vigorous-intensity physical activity for both the absolute and percentage differences. Therefore, we hypothesise that heavier participants report more organized physical activity and less sedentary behaviour due to social desirability. On the other hand, METs, as measured by the SenseWear, do not take the perceived rate of exertion into account.[21] Hence, an activity experienced as vigorous by an overweight person could be classified as moderate by the SenseWear. Finally, contrary to previous work, we did not find an effect of sex and age on MVPA energy expenditure and time.[25,46]

Bland-Altman plots showed a wide range of individual differences between GPAQ and SenseWear measurements for all variables, which agrees with previous work.[34,46,47] This indicates that the GPAQ was unable to assess differences on an individual level, yet it was not developed for this purpose.[1]

A major strength of our study is the use of repeated measures. We observed a reproducible difference between the estimates of both methods. To our knowledge only Cleland et al.[47] assessed the validity of GPAQ in measuring changes in physical activity level and sedentary behaviour. The authors suggested that the GPAQ provides reliable results to assess change in MVPA time while it didn't for sedentary behaviour. The results from our study and those of Cleland et al.[47] show the GPAQ to be an easy-to-use and inexpensive instrument to monitor levels of change in physical activity on a population level.

In contrast to other studies, we obtained a significantly higher level of overall and moderate-intensity physical activity with the SenseWear compared to the self-reported instrument.[46,52] We suggest the following explanations for this observation:

1) Because our participants were highly active, we hypothesise that they were unable to recall all moderate activities that were part of their daily habits. Only 2 to 5% did not meet the physical activity recommendation of 600 METminutes/week, while the inactivity level of the global population is 31%.[1] Similarly to our study, Welk et al. used the SenseWear armband (software version 8.0) and recruited a highly active sample (MVPA time: 131 minutes/day).[25] They also obtained higher values of total energy expenditure with the SenseWear compared to the used self-reported instrument. However, on average, total energy expenditure was 10% higher when using the Sensewear. This is lower than our observation of 33%.

2) Another possible explanation is based on the variation in performance of different wearable sensors. Both Actigraph and SenseWear are widely used by researchers to measure physical activity and sedentary behaviour.[39] Both methods overestimate time in MVPA. This might explain why Cleland et al. reported a non-significant overestimation of MVPA time per day with the Actigraph (56 minutes/day) compared to the GPAQ (30 minutes/day).[47] In addition, Actigraph has been shown to underestimate energy expenditure during MVPA while SenseWear overestimates/underestimates energy expenditure during moderate-/vigorous-intensity physical activity, respectively.[41,49] Moreover, contrary to Actigraph and other hip-worn accelerometers, the SenseWear armband uses activity pattern recognition to estimate energy expenditure. This results in a more accurate measure of upper body movements or cycling, while hip-worn accelerometers don't register these movements.[38,41,46–48] This could lead to higher results obtained with the SenseWear compared to the Actigraph and other hip-worn accelerometers.
3) Our last hypothesis is based on the low wearing time of most accelerometers.[46,48] In our study, the SenseWear was worn during waking and sleeping hours and there were no battery issues. This resulted in a wearing time of 96%. Consequently, we were able to track movement during periods of time when other activity monitors are not worn (before and during the night, early mornings etc.).

A review by Prince et al. found that there is no consensus about the trends in level of agreement between self-reported instruments and wearables.[45] Results of comparisons between self-reported and measured physical activity differ depending on the characteristics of the wearable, the questionnaire and the sample.[45,152] When comparing wearables and self-reported tools, the intensity assigned to reported activities never equals the intensity measured by the wearable. This is an important limitation. According to the WHO GPAQ analysis guide, we assigned 4 and 8 METs to moderate- and vigorous- physical activity respectively. The SenseWear measured on average 4.62/6.72 METs for moderate/vigorous physical activity bouts of at least 10 minutes. This impacts the comparability of MVPA, moderate and vigorous energy expenditure, yet does not affect the comparability of durations. Furthermore, a lot of wearable sensors use proprietary algorithms to calculate energy expenditure[22,153]; this limits the possibilities to compare results of different studies. To illustrate, the SenseWear armband uses proprietary algorithms based on activity pattern recognition to calculate energy expenditure and METs. On the other hand, the Actigraph reports proprietary counts which cause confusion for the conversion into estimates of physical activity and the interpretation of results.[40] Clearly, in order to enable a systematic evaluation of physical activity levels and sedentary behaviour, it is advised to limit the use of such proprietary algorithms.

The quest for a standardized protocol to measure physical activity and sedentary behaviour is still ongoing and the use of one standard wearable in all studies is challenging.[45] To enable both characterisation of daily movement patterns and comparison of physical activity data across studies, we suggest to use both the GPAQ, a standardized and generally accepted questionnaire, and a wearable meeting the specific needs of the study. A questionnaire is practical, budget-friendly and has a low participant burden. Systematic use of the GPAQ, a WHO validated questionnaire, in addition to a wearable offers a basis for the comparison of different studies measuring physical activity and sedentary behaviour. Such an approach could help assess and disentangle the different factors causing variation between physical activity estimates of different methods e.g. recall bias due to sample characteristics or study design and performance of specific wearables.

Conclusion

Physical activity estimates obtained with the GPAQ were significantly lower compared to those obtained using the SenseWear armband. MVPA energy expenditure and time were moderately correlated between both methods and sedentary behaviour was poorly correlated. In contrast to moderate-intensity physical activity, estimates of both methods highly agreed for vigorous-intensity physical activity.

We observed reproducible differences between the estimates of both methods. Thus, the GPAQ is an acceptable tool to measure levels of change in the population's activity behaviour. We also found associations between the difference in results and BMI, body fat and physical activity domain. The strength of these associations depended on the intensity of the activity which emphasizes the importance of analyzing sedentary, moderate- and vigorous-intensity physical activity separately.

Chapter 3 – Black carbon reduces the beneficial effect of physical activity on lung function

This chapter is based on:

Laeremans M, Dons E, Avila-Palencia I, Carrasco-Turigas G,Orjuela-Mendoza J, Anaya-Boig E, Cole-Hunter T, de Nazelle A, Nieuwenhuijsen M, Standaert A, Van Poppel M, De Boever P, Int Panis L, 2018. Black carbon reduces the beneficial effect of physical activity on lung function. Medicine & Sports in Science & Exercise 50(9).

Abstract

When physical activity is promoted in urban outdoor settings (e.g. walking and cycling), individuals are also exposed to air pollution. It has been reported that short-term lung function increases as a response to physical activity, but this beneficial effect is hampered when elevated air pollution concentrations are observed. Our study assessed the long-term impact of air pollution on the pulmonary health benefit of physical activity.

Wearable sensors were used to monitor physical activity levels (SenseWear) and exposure to BC (microAeth) of 115 healthy adults during one week in three European cities (Antwerp, Barcelona, London). The experiment was repeated in three different seasons to approximate long-term behaviour. Spirometry tests were performed at the beginning and end of each measurement week. All results were averaged on a participant level as a proxy for long-term lung function. Mixed effect regression models were used to analyze the long-term impact of physical activity, BC and their interaction on lung function parameters FEV_1, FVC, FEV_1/FVC, FEF_{25-75} and PEF. Interaction plots were used to interpret the significant interaction effects.

Negative interaction effects of physical activity and BC exposure on FEV_1 ($p=0.07$), FEV_1/FVC ($p=0.03$) and FEF_{25-75} ($p=0.03$) were observed. For BC concentrations up to approximately 1 µg/m³, an additional METhour per week resulted in a trend towards lung function increases (FEV_1, FEV_1/FVC and FEF_{25-75} increased 5.6 mL, 0.1% and 14.5 mL/s, respectively).

We found that lung function improved with physical activity at low BC levels. This beneficial effect decreased in higher air pollution concentrations. Our results suggest a greater need to reduce air pollution exposures during physical activity.

Introduction

Today's global physical inactivity pandemic is responsible for approximately five million premature deaths annually.[2] However, when physical activity in urban outdoor settings (e.g. walking and cycling) is promoted, air quality may need to be taken into account.

Long-term air pollution exposure contributes to increased prevalence of cardiovascular and respiratory diseases.[7] Globally, many people live in urbanized regions, which are hotspots of air pollution due to poor urban and transport planning.[70] Consequently, walking, cycling and other forms of both incidental and intentional physical activity in urban environments often involve exposure to high concentrations of air pollution.

Higher ventilation rates during physical activity result in an increased pollutant dose and deeper penetration of particles into the lungs.[10,94] Nevertheless, various health impact assessments have been carried out and reported that the overall benefits of physical activity outweigh the risks of air pollution.[12,13,154] Also, the Danish Diet, Cancer and Health Cohort did not observe a modification effect of air pollution on the relationship between physical activity and all-cause mortality.[14] However, short-term respiratory responses to physical activity have been shown to alter with increasing air pollution concentrations.[15,90] A recent study in older adults, with and without pre-existing COPD or ischaemic heart disease, found that the beneficial effects of walking on lung function were lost after a two-hour walk in a polluted environment.[90] In addition, lung function responses to physical activity in healthy participants also differed when air pollution was taken into account.[15,17] Evidence on the chronic impact of air pollution on the health benefit of physical activity for specific physiological systems remains absent.[94] Therefore, the main aims of the current study were: (1) to assess the chronic effects of physical activity and air pollution on lung function and (2) to assess whether the pulmonary responses to physical activity and air pollution interact in a sample of healthy adults across three European cities.

Methods

Study design and participants

The framework of this study was the PASTA project (Physical Activity through Sustainable Transport Approaches). In this project, data on physical activity and travel behaviour among over 12,500 volunteers living or working in seven European cities (Antwerp, Barcelona, London, Örebro, Rome, Vienna, Zurich) was collected through an online survey.[4,5] From this sample, 122 participants were selected to participate in a real-life monitoring study in three cities (Antwerp: 41 participants, Barcelona: 41 participants, London: 40 participants). High-resolution data on physical activity and BC exposure was collected during seven consecutive days using wearable sensors. Lung function was assessed with a spirometry test at the beginning and end of the measurement week. Each participant repeated the measurement week three times: in the mid-season (spring or autumn), in the summer and in the winter, between February 2015 and March 2016. Eligible participants were non-smoking, 18-65 year olds with a BMI below 30 and no self-reported cardiovascular, respiratory or neurological condition. The study was approved by the Ethics Committee of the University hospital in Antwerp (UZA), the Clinical Research Ethical Committee of the Parc de Salut Mar in Barcelona and the Imperial College Research Ethics Committee in London. All participants gave written informed consent prior to participation.

Physical activity assessment

Physical activity was objectively measured with the SenseWear armband (model MF-SW, BodyMedia, USA). This multi-sensor body monitor measures heat flux, galvanic skin response, skin temperature and 3-axis accelerometry. The sensor was worn on the triceps muscle of the left arm and was only removed when there was contact with water (bathing, showering, etc.); wearing time was on average 96±4% of the total time. Age, sex, body weight and height of the participants were entered in the SenseWear professional software (version 8.0). Energy expenditure and METs were calculated on a one-minute basis using proprietary algorithms based on pattern recognition.[38] For each measurement week, the amount of METhours was calculated in R version 3.3.1. Only bouts of at least 10 consecutive minutes with an intensity ≥ 3 METs were considered. This is in accordance with the WHO recommendations on physical activity for health.[139] To clarify, an activity that requires 3 METs is walking the dog and doing this for one hour results in 3 METhours (according to the compendium of physical activities[141]).

Black carbon exposure assessment

Personal exposure to BC was assessed with the microAeth (model AE51, Aethlabs, USA). Ambient air was drawn over a Teflon-coated borosilicate glass fibre filter at a flow rate of 100 mL/min, resulting in BC accumulation on the filter. Light attenuation at a wavelength of 880 nm was measured and converted into a BC concentration (ng/m^3). The device was set to estimate the average BC concentration every five minutes. Participants always carried the device with them and replaced the filter every two days to prevent saturation. For indoor activities, participants left the microAeth in the room where they spent most of their time. A short tube was attached to the inlet of the microAeth which enabled participants to carry the device in a bag. Raw BC data were smoothened with the ONA algorithm (Optimized Noise-Reduction Algorithm; developed by the Environmental Protection Agency), and data tagged with an error code that could potentially impact concentration levels were excluded.

Lung function measurement

Spirometry (EasyOne, ndd Medical Technologies) was performed according to the European Respiratory Society and the American Thoracic Society guidelines.[125,155] Lung function parameters selected for this study are forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), the Tiffeneau index (FEV_1/FVC), peak expiratory flow (PEF) and forced expiratory flow at 25% to 75% of the FVC (FEF_{25-75}). Percentage predicted values of FEV_1 and FVC were calculated based on the Global Lung Function 2012 equations.

Analysis

For each measurement week, the participants' physical activity level was calculated as total METhours, and their BC exposure was averaged. We calculated the mean of the three measurements per participant as a proxy for long-term physical activity behaviour and long-term personal BC exposure. This is justified as we objectively measured physical activity and BC concentrations during a whole week in three different seasons. Consequently, differences due to seasonal changes were accounted for. Three participants did not complete all three measurement weeks and were excluded from the analysis. Also, only non-smokers (n=100) and former smokers (n=19) were recruited. Pack years of former smokers were low with an average of 2.7±2.9. This corresponds to 20 cigarettes per day during 2.7 years. Four of those former smokers were excluded from the analysis since they quit smoking less than 5 years ago. This resulted in a total of 115 eligible participants out of 122. Long-term lung function parameters were approximated by calculating the average FEV_1, FVC, FEV_1/FVC, FEF_{25-75} and PEF per participant.

The study population was described by sex, age, ethnicity, height, BMI, and education level. For categorical variables the amount (n) and percentage was reported. Arithmetic mean and standard deviation (SD) were used for continuous variables and pulmonary outcomes. In addition, the overall and city-specific median and interquartile range (IQR) of physical activity and BC concentrations were calculated.

The effects of long-term physical activity, air pollution and their interaction were tested using mixed effect regression models where city was introduced as a random variable. First, we tested the effects of physical activity and BC on lung function separately. Then the effects of physical activity and BC were estimated together in one single model. Finally, models including the interaction term were tested. All models included sex, age and height based on the European Respiratory Society and the American Thoracic Society guidelines.[155] To account for socio-economic differences, education level was also added as a covariate.

The significance level was set at $p<0.05$. When trends towards significant interaction effects were observed ($p<0.1$), regression estimates of physical activity were plotted with their 95% confidence interval as a function of BC concentrations in an interaction plot.

R software version 3.3.1 was used for data processing and analysis. Random intercept models were tested with the lme4 and lmerTest R-packages for mixed effect regression analysis. All marginal residuals were normally distributed. Interaction plots were made using the R-package Interplot.

Results

A total of 115 healthy adults were included in the analysis. Almost half of the study population (44%) were males; 94% were Caucasian and 90% had a higher education degree (Table 7). They were on average 36.6±10 years old, 1.7±0.1 m tall with a BMI of 23.7±3 kg/m². Long-term FEV_1 ranged from 2.28 to 5.13 L with an average of 3.53±0.70 L (mean %predicted FEV_1: 93.9±10.6%); FVC ranged from 2.63 to 6.78 L with an average of 4.44±0.92 L (mean %predicted FVC: 96.8±10.7%). The median, long-term FEV_1/FVC ratio was 80.7%. Hence, we recruited a sample of participants with normal lung function.[155]

Table 7 Characteristics of the study population and pulmonary outcomes aggregated over all sessions per individual (n=115). Categorical characteristics are reported as n (%); continuous variables are reported as mean±SD. The percentqge predicted values of FEV_1 and FVC were calculated based on the 2012 Global Lung Function equations.

Personal characteristics				
	Overall	**Antwerp**	**Barcelona**	**London**
Men	51 (44%)	21 (54%)	16 (42%)	14 (37%)
Age (years)	36.6±10	37.2±11	34.9±9	34.7±10
Ethnicity (Caucasian)	108 (94%)	39 (100%)	37 (97%)	32 (84%)
Height (m)	1.7±0.1	1.8±0.1	1.7±0.1	1.7±0.1
BMI (kg/m²)	23.7±3	23.8±3	23.3±3	23.9±4
Higher education	104 (90%)	37 (95%)	34 (89%)	33 (87%)
Pulmonary outcomes (mean±SD)				
	Overall	**Antwerp**	**Barcelona**	**London**
FEV_1	3.53±0.69 L 93.9±10.6 %pred.	3.66±0.64 L 92.6±9.6 %pred	3.48±0.69 L 95±12 %pred	3.45±0.74 94.1±10 %pred
FVC	4.44±0.92 L 96.8±10.7 %pred.	4.73±0.88 L 97.2±9.6 %pred	4.29±0.93 L 96.2±12 %pred	4.30±0.9 L 96.9±10 %pred
FEV_1/FVC	80±6.3 %	77.7±5.7 %	81.8±7.3 %	80.4±5.2 %
PEF	8.58±1.87 L/s	8.61±1.84 L/s	8.70±1.86 L/s	8.43±1.95 L/s
FEF_{25-75}	3.41±0.95 L/s	3.24±0.86 L/s	3.66±1 L/s	3.33±0.95 L/s

Table 8 shows the participants' physical activity level and BC exposure aggregated over all three measurement weeks per individual. In general, we recruited a physically active sample: the median amount of METhours per week was 42, which exceeds the WHO recommendation of 10 METhours. The least active population was recruited in Barcelona, while a similar physical activity level was observed for participants in Antwerp and London. On the other hand, BC concentrations were highest in Barcelona. Overall, median BC exposures were 1.4 µg/m³.

Table 8 Median and IQR of the average physical activity level (PA) and BC concentrations per participant (n=115).

	Overall	**Antwerp**	**Barcelona**	**London**
PA level (METhours/week)	42 (28-74)	56 (36-89)	32 (17-59)	51 (29-82)
BC (µg/m³)	1.4 (1.1-1.7)	1.3 (1.1-1.6)	1.7 (1.5-1.9)	1.3 (1.0-1.5)

Mixed effect regression analysis was performed using models with and without the interaction term of physical activity and BC exposure (Table 9). There were no significant effects of physical activity and BC on the lung function parameters in models excluding the interaction term. This changed when the interaction term was included. These models showed significant, negative interaction effects of physical activity and BC exposure on FEV_1 ($p=0.07$), the FEV_1/FVC ratio ($p=0.03$) and FEF_{25-75} ($p=0.03$).

Table 9 Estimated effects of physical activity (METhour/week), BC (µg/m³) and their interaction (PAxBC) on lung function parameters: FEV_1, FVC, FEV_1/FVC, PEF and FEF_{25-75}. Estimates were obtained using mixed effect regression analysis (n=115). The first column specifies the main effects that were included in the associated models. All models included sex, age, height and education levels as covariates. City was introduced as a random effect. PA = Physical activity; BC = Black carbon

Outcome	PA (METhour/week)		BC (µg/m³)		PAxBC	
FEV_1 (mL)	**β (95% CI)**	**p**	**β (95% CI)**	**p**	**β (95% CI)**	**p**
PA	-1.01 (-3.14;1.12)	0.35				
BC			-74.30 (-248.21;96.62)	0.40		
PA+BC	-0.95 (-3.09;1.19)	0.39	-68.87 (-243.40;105.66)	0.44		
PA+BC+PAxBC	5.61 (-1.65;12.87)	0.13	189.90 (-133.77;513.57)	0.25	-4.73 (-9.74;0.28)	0.07
FVC (mL)						
PA	0.10 (-2.40;2.60)	0.94				
BC			19.59 (-184.81;223.99)	0.85		
PA+BC	0.08 (-2.44;2.61)	0.95	19.11 (-186.74;224.95)	0.86		
PA+BC+PAxBC	2.01 (-6.67;10.70)	0.65	95.22 (-292.22;482.65)	0.63	-1.39 (-7.39;4.60)	0.65
FEV_1/FVC (%)						
PA	-0.03 (-0.06;0.004)	0.09				
BC			-2.57 (-5.10;-0.04)	0.05		
PA+BC	-0.02 (-0.05;0.01)	0.23	-2.18 (-4.73;0.36)	0.10		
PA+BC+PAxBC	0.09 (-0.009;0.19)	0.08	2.21 (-2.30;6.72)	0.34	-0.08 (-0.15;-0.01)	0.03
PEF (mL/s)						
PA	-3.90 (-9.66;1.86)	0.19				
BC			-208.10 (-698.29;282.08)	0.41		
PA+BC	-3.54 (-9.41;2.34)	0.24	-144.61 (-641.56;352.34)	0.57		
PA+BC+PAxBC	5.60 (-13.61;24.82)	0.57	225.03 (-663.26;1131.32)	0.62	-6.64 (-19.90;6.63)	0.33
FEF_{25-75} (mL/s)						
PA	-2.37 (-6.78;2.04)	0.30				
BC			-336.44 (-718.72;45.83)	0.10		
PA+BC	-1.60 (-6.04;2.85)	0.49	-306.46 (-695.13;82.20)	0.14		
PA+BC+PAxBC	14.54 (-0.20;29.27))	0.06	344.86 (-337.35;1027.07)	0.33	-11.70(-21.89;-1.51)	0.03

The effect of physical activity increased to borderline significant levels when the interaction term was included. According to these models, FEV_1, FEV_1/FVC and FEF_{25-75} would gain 5.6 mL ($p=0.13$), 0.1% ($p=0.08$) and 14.5 mL/s ($p=0.06$) per additional weekly METhour. However, the interaction term also shows trends towards significance which means that the value and significance of the main physical activity effect should be interpreted for specific BC concentrations. None of the models could detect a significant effect of physical activity and BC on FVC and PEF.

The effects of physical activity on FEV_1, FEV_1/FVC and FEF_{25-75} were plotted as a function of BC concentration. Figure 7 visualizes how increasing BC concentrations modify the effect of physical activity on FEV_1, FEV_1/FVC and FEF_{25-75}. The dashed vertical line represents the BC concentrations where the 95% confidence interval of the estimated effect of physical activity on FEV_1, FEV_1/FVC and FEF_{25-75} only included negative values. Consequently, based on our results, FEV_1, FEV_1/FVC and FEF_{25-75} improves with physical activity at lower BC concentrations. At high BC levels, additional physical activity may reduce these lung function parameters.

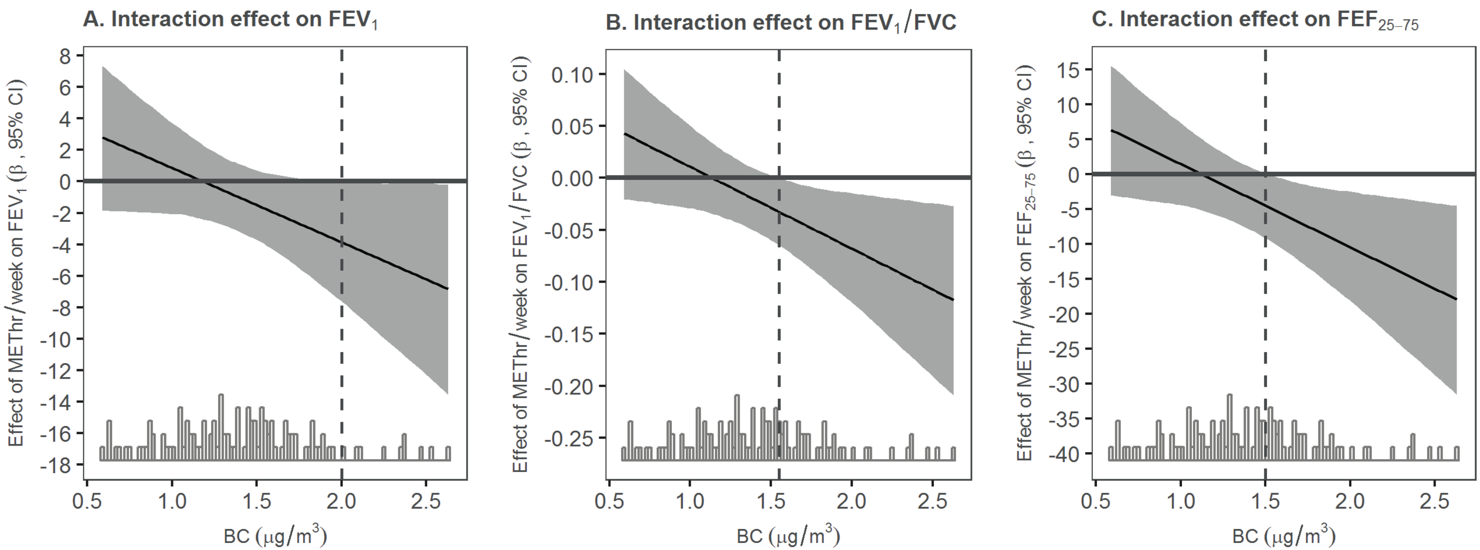


Figure 7 Regression estimates of physical activity effects (95% confidence interval; METhours per week) on FEV_1 (A), FEV_1/FVC (B) and FEF_{25-75} (C) as a function of BC. Barplots on the x-axis represent the number of participants exposed to the corresponding BC concentration. The dashed vertical line represents the BC concentration where the 95% confidence interval of the effect of physical activity only includes negative values. This means that the lung function parameters decrease with additional physical activity at BC concentrations above the highlighted levels.

Discussion

This is the first study to examine the combined long-term effects of air pollution and physical activity on lung function using personal measurements obtained with wearable devices. We found that physical activity is associated with an improved pulmonary function at low BC concentrations. This respiratory benefit decreased when BC concentrations increased.

BC is a fraction of particulate matter with most particles having an aerodynamic diameter smaller than 1 µm. Such small particles are believed to be more harmful than particles of larger sizes.[59] This makes BC a valuable marker to study physiological effects of air pollution.[60] Overall, we measured a median BC concentration of 1.4 µg/m³, with the highest concentrations in Barcelona (1.7 µg/m³). These concentrations were similar to those measured in the ESCAPE project, which covered a broad range of areas in Europe (PM2.5 absorbance was converted to BC based on the formulas in Dons[32] and Gan[156] et al.). The ESCAPE cohort found a negative association between long-term NO_2 and PM_{10} exposure and lung function in adults.[157] However, the relationship was not significant for $PM_{2.5}$absorbance (a marker for BC) which is consistent with our results.

At low BC concentrations up to approximately 1 µg/m³, the effect of an additional METhour per week on FEV_1, FEV_1/FVC and FEF_{25-75} was an increase of 5.6 mL, 0.1% and 14.5 mL/s respectively. This benefit decreased with increasing BC concentrations. Our findings are in line with observations from a study by Cheng et al. (2003), executed in Texas. In this study, self-reported exercise patterns of healthy adults between 25 and 55 years old were associated with spirometry results.[158] These participants were categorized according to their physical activity level where the highly active participants were approximately 16 METhour per week more active than those with a low physical activity level (based on the formulas in Ainsworth et al.[141]). Highly active men had a 130 mL higher FEV_1; for women, the difference was 80 mL. This corresponded to a 8 mL and 5 mL FEV_1 increase per METhour for men and women respectively with an annual BC concentration 0.7 µg/m³ in Texas, similar to our results.[159] Other studies have also observed correlations between physical activity related variables and FEV_1 or FVC.[160,161]

Little research has been done on the chronic impact of air pollution on the respiratory benefits of physical activity. A review by Giles et al. (2014) suggested that chronic exposure during exercise has important consequences for respiratory function.[162] Hence, a higher prevalence of exercise-induced bronchoconstriction, asthma and lower lung function has been observed in athletes who train in environments with high particulate matter emissions.[94] Moreover, reductions in respiratory mortality associated with cycling were greater for participants with low to moderate compared to high air pollution exposure in the Danish Diet, Cancer and Health Cohort.[14]

It is well documented that inhalation of air pollutants causes oxidative damage and inflammation in the respiratory system resulting in airway obstruction and a reduced FEV_1.[69,75,94] Exercising in polluted air also results in inhalation of a higher pollutant dose which may enhance its adverse respiratory effects.[10,69,94] Contrary, regular physical activity improves endurance and strength of the respiratory muscles resulting in an increased FEV_1.[75] However, compared to the cardiovascular system, respiratory adaptations to physical activity are rather small because the lungs are already equipped to deal with the demands of high intensity exercise. Consequently, exercise in elevated air pollution concentrations may lead to a level of obstruction potentially outweighing the beneficial effect of physical activity on FEV_1 and FEF_{25-75}. Therefore, chronic exposure to high air pollution doses during physical activity may result in a decreased FEV_1,FEV_1/FVC and FEF_{25-75}. Since FEF_{25-75} specifically informs us about small airway function, this may indicate that physical activity and BC interact at this level.[163,164]

Contrary, various HIAs reported that the overall physical activity benefits outweigh the negative physiological effects of air pollution.[13] However, the modification effects on physiological systems are potentially masked in previous studies as they did not investigate such subclinical reactions. Our results suggest that there may be a greater need to reduce air pollution exposures during physical activity than was previously thought.

Our sample consisted of healthy, highly educated adults with an average FEV_1/FVC ratio of 80.1±6.4%. The level of physical activity measured in our sample meets the WHO recommendation of 600 METminutes to maintain health. Overall, the IQR of measured METhours falls within light to moderate physical activity levels.[165] Our findings aren't entirely representative of the general population as we recruited mostly active and highly educated volunteers of Caucasian ethnicity. We covered, however, multiple European cities which is unique in this field of research. We also recruited a larger sample compared to previous studies where air pollution exposure was assessed on a personal level.[17,100] Moreover, measuring air pollution exposure and physical activity with wearable sensors reduces the risk of exposure misclassification. This is of particular importance when studying traffic-related pollutants such as BC of which the concentrations rapidly change in space and time.[94] Accurately assessing both physical activity and air pollution exposure remains a major challenge in epidemiological research. In addition, we were able to use our personal measurements as a proxy for long-term behaviour as all participants collected exposure data in three different seasons.

It has been reported that the SenseWear underestimates energy expenditure during high intensity activities which is a limitation of our study design.[50] However, especially MET values above 10 METs are underestimated which our participants only achieved during 0.2% of their time awake.[50] In addition, the most recent version of the SenseWear provides the best estimation compared to previous SenseWear versions and the Actigraph, the most widely used wearable physical activity tracker in research. Another limitation is that only a limited number of participants was exposed to the highest BC concentrations where we found a negative relationship between physical activity and respiratory health. Consequently, we were unable to robustly characterize the association at these BC concentrations. Also, the addition of an interaction term to our models resulted in wider confidence intervals. This may be due to the limited amount of data available for each combination of physical activity and BC levels. While our results are of potential interest to public health, the study needs to be replicated in a larger study sample to confirm them. Finally, high average physical activity levels of our participants may have affected the results. Potentially, the respiratory benefit of physical activity in low BC concentrations is larger in a less active sample. In addition, the interaction effect may be smaller since the baseline amount of inhaled particles during physical activity would be lower, also lowering the chances to provide a respiratory hazard.

Due to the potentially high impact on public health, we recommend future research to further characterize the modification effect of air pollution on the relationship between physical activity and long-term respiratory health. We suggest to (1) complement spirometry with other measures providing information on different parts of the respiratory system (e.g. frequency of self-reported respiratory symptoms of both the upper and lower tract), and (2) to investigate the combined effects of air pollution and physical activity in vulnerable sub-populations such as children, athletes and pulmonary patients.

Conclusion

This is the first study executed in multiple European cities simultaneously that integrated effects of air pollution and physical activity to assess their combined impact on pulmonary function. We found a long-term beneficial effect of long-term physical activity on respiratory health at low BC concentrations. The beneficial effect decreased with increasing yearly, average BC concentrations in a healthy adult population. This illustrates the need for an integrated approach to better understand the health impact of strategies and policy measures that try to tackle physical inactivity and pollution.

Chapter 4 – Short-term effects of physical activity, air pollution and their interaction on the cardiovascular and respiratory system

This chapter is based on:

Laeremans M, Dons E, Avila-Palencia I, Carrasco-Turigas G,Orjuela-Mendoza J, Anaya-Boig E, Cole-Hunter T, de Nazelle A, Nieuwenhuijsen M, Standaert A, Van Poppel M, De Boever P, Int Panis L, 2018. Short-term effects of physical activity, air pollution and their interaction on the cardiovascular and respiratory system. Environment International 117.

Abstract

Physical activity in urban environments may lead to increased inhalation of air pollutants. As physical activity and air pollution have respectively beneficial and detrimental effects on the cardiorespiratory system, the responses to these exposures can interact. Therefore, we assessed the short-term effects of physical activity, air pollution and their interaction on a set of subclinical cardiovascular and respiratory outcomes in a panel of healthy adults: HRV, retinal vessel diameters, lung function and FeNO.

One hundred twenty two participants measured their physical activity level and exposure to BC, a marker of air pollution exposure, with wearable sensors during an unscripted week in three different seasons. The study was part of the PASTA project in three European cities (Antwerp: 41 participants, Barcelona: 41 participants, London: 40 participants). At the end of each measurement week, the health outcomes were evaluated. Responses to physical activity, BC and their interaction were assessed with mixed effect regression models. Separate models were used to account for a 2-hour and 24-hour time window.

During the 2-hour time window, HRV and lung function changed statistically significantly in response to physical activity (METhours) and logarithmic BC (%change). Changes in HRV marked an increased sympathetic tone with both physical activity (LF/HF: +7% per METhour; $p<0.01$) and BC (HF: -0.19% per BC %increase; $p<0.05$). In addition, physical activity provoked bronchodilation which was illustrated by a significant increase in lung function (FEV_1: +15.63 mL per METhour; $p<0.05$). While a BC %increase was associated with a significant lung function decrease (PEF: -1 mL; $p<0.05$), the interaction indicated a potential protective effect of physical activity ($p<0.05$). We did not observe a response of the retinal vessel diameters. Most subclinical outcomes did not change in the 24-hour time window (except for a few minor changes in LF/HF, FeNO and PEF).

Our results on the separate and combined effects of short-term physical activity and air pollution exposure on subclinical markers of the cardiorespiratory system are relevant for public health. We provide insights on the physiological responses of multiple, complementary markers. This may move further research towards elucidating potential pathways to disease and the long-term clinical impact of the observed physiological changes.

Introduction

Particulate matter air pollution provokes over four million annual deaths worldwide and contributes to the onset and development of cardiovascular and respiratory conditions.[7–9] Epidemiological studies frequently study non-invasive outcomes such as HRV, retinal vessel diameters, lung function and FeNO to assess subclinical responses to air pollution that may be on the pathway of disease development.[100,113,117,118,166]

Increased sympathetic tone, indicated by decreased HRV, and responses of the retinal vessel diameters are recognized as early markers of cardiovascular conditions.[167] In healthy individuals, the sympathetic and parasympathetic branch form the autonomic nervous system and are in dynamic balance.[81] Sympathetic domination provokes reductions in HRV which induces arrhythmias potentially leading to sudden death.[8,86] In addition, microvascular narrowing as quantified through retinal image analysis is related to elevated blood pressure potentially leading to hypertension and cardiovascular diseases.[107,108] Airway inflammation, as estimated with FeNO, and lung function are used to diagnose respiratory conditions.[95,125,155] High FeNO concentrations are associated with eosinophilic airway inflammation and impaired lung function indicates pulmonary diseases such as asthma or COPD. Elevated air pollution exposure has been associated with increased FeNO, reduced HRV, narrowed retinal arteriolar lumen and impaired lung function.[8,112,117,121] In contrast, better cardiovascular fitness is related to increased HRV[74], wider retinal arterioles[168], and improved respiratory function.[158]

An adequate amount of physical activity prevents premature mortality and increases quality of life, yet 31% of the world’s adult population does not reach the WHO recommended level of 600 METminutes per week.[1] Active mobility (e.g. walking and cycling) has been introduced as an innovative and accessible measure to promote physical activity.[4,5] However, when physical activity takes place in urban outdoor settings, individuals may be exposed to elevated air pollution concentrations and higher ventilation rates during physical activity could increase the inhaled pollutant dose.[4,10,70] Physical activity and air pollution exposure activate biological pathways that provoke subclinical changes in the cardiovascular and respiratory system.[8,76,169] Hence, the responses may interact. A recent study in 135 older adults, with and without pre-existing COPD or ischaemic heart disease, assessed acute cardiorespiratory responses after a 2-hour walk in high versus low air pollution concentrations.[90] They found that the beneficial effects of walking on arterial stiffness and lung function were lost in a polluted environment. This illustrates that air pollution may attenuate the cardiorespiratory benefits of physical activity in vulnerable populations. Such results need to be complemented with studies of physiological responses in healthy volunteers. An experimental study in 29 healthy participants found that physical activity may protect against the short-term blood pressure increase associated with air pollution.[16,96] Lung function improved with physical activity, while reductions were observed in association with air pollution.[17] The adverse effects of air pollution on respiratory markers were negated by physical activity in a comparable study in Barcelona.[15]

Considering the fact that many people are physically active in an urban environment, more evidence is needed to disentangle the short-term physiological responses to physical activity and air pollution. Assessing subclinical changes in healthy individuals may provide relevant information on the development of cardiovascular and respiratory diseases. The aim of the current study was to evaluate the short-term subclinical, cardiorespiratory effects of real-life physical activity, air pollution and their interaction in a sample of healthy adults.

Methods

Study design and participants

This study was part of the FP7 PASTA project (Physical Activity through Sustainable Transport Approaches) in which data on physical activity and travel behaviour was collected in seven European cities (Antwerp, Barcelona, London, Örebro, Rome, Vienna and Zürich).[4,5] Data was collected through online, longitudinal surveys that were completed by over 12,500 volunteers across a period of up to two years. From this sample, 122 participants took part in a real-world monitoring study between February 2015 and March 2016 in three cities: Antwerp (41 participants), Barcelona (41 participants) and London (40 participants). The participants collected high-resolution data on physical activity level and air pollution exposure with wearable sensors during seven consecutive days while performing their habitual activities. A battery of non-invasive measurements were used: HRV, retinal vessel diameters, FeNO, and lung function. These outcomes were measured at a research center in each of the three cities at the end of each measurement week. Each participant repeated the measurement week three times: in the mid-season (autumn or spring), in the summer and in the winter. Eligible participants were non-smoking, 18-65 years old with a self-reported BMI below 30 and no self-reported cardiovascular, respiratory or neurological condition. The study was approved by the ethics committee of each research center involved and all participants gave written informed consent prior to participation.

Physical activity assessment

Physical activity was measured with the SenseWear armband (model MF-SW, BodyMedia, USA). This multi-sensor body monitor measures heat flux, galvanic skin response, skin temperature and 3-axis accelerometry on a one-minute basis. Participants wore the armband on the triceps muscle of the left arm and only removed it during contact with water (bathing, showering, etc.). Wearing time was 96±4% (mean±SD) of total participation time. Age, sex, body weight and height were provided to the SenseWear professional software (version 8.0) that calculates energy expenditure and METs using proprietary algorithms based on pattern recognition.[38] The total amount of METhours was calculated in R version 3.3.1 for each time window. Only bouts of at least 10 consecutive minutes with an intensity ≥ 3 METs were considered for the METhour calculations, in accordance with the WHO recommendations on physical activity for health.[139] According to the updated compendium of physical activities by Ainsworth et al. (2000), 3 METs is the amount of energy required to e.g. walk the dog.[141] Doing this for one hour results in 3 METhours.

Black carbon exposure assessment

Personal air pollution exposure was assessed by measuring BC. A major element of diesel exhaust emissions and therefore abundantly present in urban areas.[60] Since BC consists of particles that are mostly smaller than one micrometer, they have a high pulmonary penetration capacity. Therefore, such small particles are believed to be more harmful than larger ones, which makes BC a valuable marker to study the physiological effects of air pollution.[59,60]

Exposure to BC was measured on a personal level using the microAeth (model AE51, Aethlabs, USA). Air was drawn over a Teflon-coated borosilicate glass fibre filter at a flow rate of 100 mL/min, resulting in BC accumulation on the filter. The microAeth detects the changing optical absorption of light transmitted through the filter at wavelength of 880 nm. The microAeth logs the average BC concentration on a five-minute basis. Participants were instructed to always carry the device with them and replace the filter every two days to prevent saturation. For indoor activities, participants kept the microAeth in the room where they spent most of their time. A short tube was attached to the inlet of the microAeth so participants could carry the device in their bag while it measured BC in ambient air. Raw BC data were smoothened with the Optimized Noise-reduction Algorithm (ONA), developed by the Environmental Protection Agency. Data with an error code for filter saturation or flow out of range were excluded.

Assessment of subclinical cardiorespiratory outcomes

Subclinical markers were measured in the following order: (1) HRV, (2) retinal vessel diameters, (3) FeNO, and (4) lung function. HRV at rest was assessed in a seated position using the Zephyr BioHarness (Medtronic, USA). Time-domain (standard deviation of normal-to-normal intervals (SDNN) and root mean square of successive differences in adjacent NN intervals (rMSSD)) and frequency-domain (high frequency (HF; 0.15–0.40 Hz) power, low frequency (LF; 0.05–0.15 Hz) power and the ratio of LF to HF (LF/HF)) measures of HRV were determined with the R package RHRV. SDNN estimates overall HRV while rMSSD and HF have been linked to parasympathetic activity specifically.[102] LF/HF informs about the degree of domination by the sympathetic system. Decreases in SDNN, rMSSD and HF and increases in LF/HF are associated with increased sympathetic tone which may result in negative health outcomes such as arrhythmias.[8,86] Data collection started after the participants wore the Zephyr for 10 minutes, also in a seated position. All HRV measures were calculated and averaged over four consecutive 5-minute intervals.

During each visit to the research center, the retina of the right eye was photographed twice with the Canon CR-2 digital non-mydriatic retinal camera (Hospithera, Belgium).[170] Retinal vessel diameters were analyzed with the IFLEXIS software (VITO, Belgium) according to previously reported protocols.[170] The resulting central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) were averaged per participant, per visit.

FeNO was measured according to the manufacturer's protocol: NO-free air was inhaled through the NIOX VERO device (Circassia Pharmaceuticals Inc., USA) and exhaled with a constant flow of 50 mL/s. Participants performed two measurements per visit which were averaged to obtain the final FeNO value per visit. Spirometry tests (EasyOne, ndd Medical Technologies, USA) to measure lung function were executed according to the European Respiratory Society and the American Thoracic Society guidelines.[125,155] The EasyOne device automatically assessed the quality of both the overall test and each separate maneuver. The device rates the quality of each maneuver from A to E, where A is the best available grade. If the participant did not reach at least grade C, the test was repeated. All technicians were trained together at the study center in Antwerp in February 2015 before the start of the data collection. The lung function parameters for this study were forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), the Tiffeneau index (FEV_1/FVC) and peak expiratory flow (PEF). During the last visit to the research center, BMI was assessed with a body composition monitor (model BF511, Omron, Japan).

All measurements were performed during late afternoon at the campuses of VITO (Antwerp, Belgium), ISGlobal (Barcelona, Spain) or Imperial College London (London, UK).

Analysis

R software version 3.3.1 was used for data processing and analysis. Categorical variables were described as the amount (n) and percentage. Arithmetic mean and standard deviation were used for continuous variables. Physical activity, BC concentrations and subclinical outcomes were summarized as the overall and city-specific median and IQR. Variables were aggregated per individual before the calculation of the median and IQR. Mixed effect regression models were used to test for differences between cities; 'city' was the only fixed effect included in these models and random participant effects were used to correct for repeated measures.

Short-term physiological effects of physical activity, BC and their interaction were also assessed with mixed effect regression models over a 2-hour and 24-hour time window before the health outcomes assessments (R-packages: lme4 and lmerTest[171,172]). For each time window, physical activity was calculated as the total amount of METhours and BC as the average exposure concentration during the respective time period. The Kenward-Roger's approximation was used for the calculation of p-values.[172] We tested both unadjusted and adjusted models with physical activity and BC only (PA model, PA+C model, BC model, BC+C model; C=confounders), physical activity and BC in the same model (PA+BC model, PA+BC+C model) and models with physical activity, BC and their interaction (PAxBC model, PAxBC+C model). HRV measures and FeNO were log-transformed to comply with the normality assumption of the residuals.

BC was log-transformed as well to reduce the impact of influencing measurements. Since log-transformation of variables complicates the interpretation of effect sizes, we clarified this in Table 10. Throughout the results, we will refer to β as the model estimate and specify its conversion to ease the interpretation of effect sizes. The distribution of physical activity measures was also skewed, yet physical activity could not be log-tranformed due to the amount of observations with 0 METhours. Therefore, a sensitivity analysis was performed where physical activity was categorized to check the robustness of the results (two categories: (1) no physical activity (0 METhours); (2) physical activity (> 0 METhours)). Physical activity and BC were measured on a 1- and 5-minute basis respectively and measurements where half of the data points were missing from the respective time windows were excluded from the analysis. Adjusted models included sex, age, BMI or height, season, education level, and physical activity and BC during the whole measurement week as confounders. Confounders were identified based on a directed acyclic graph (DAG) with the R-package 'dagitty' (Appendix: Figure S 5, p. 153). Height was used as a confounder in models with lung function outcomes[155], all other models included BMI. Education level was used as a proxy for socio-economic status. Physical activity and BC during the whole week were included to account for pre-exposure and were calculated as the total amount of METhours and the average BC concentration respectively. Random variables were introduced to account for the effects of city and repeated measures. All marginal residuals were normally distributed and the significance level was set at 0.05. The variance inflation factors (VIF) of all variables in all models was smaller than three which reflects low correlation between predictors.

Table 10 Interpretation of effect sizes when the outcome and/or predictor are log-transformed. y = outcome, x = predictor, β = model estimate, log = natural logarithm.

Model	Interpretation
$\boldsymbol{y} = \boldsymbol{\beta}.\mathbf{x}$	A unit-increase in x, gives a β unit-change in y
$\boldsymbol{log}(\boldsymbol{y}) = \boldsymbol{\beta}.\mathbf{x}$	A unit-increase in x, gives a β.100 %change in y
$\boldsymbol{y} = \boldsymbol{\beta}.\boldsymbol{log}(\mathbf{x})$	A %increase in x, gives a β/100 unit-change in y
$\boldsymbol{log}(\boldsymbol{y}) = \boldsymbol{\beta}.\boldsymbol{log}(\mathbf{x})$	A %increase in x, gives a β %increase in y

Results

From the total of 122 healthy adults who participated in the study, 119 completed all three measurement weeks (one participant completed two measurement weeks and two participants only one). Participants were on average 35±10 (mean±SD) years old, 1.7±0.1 m tall and had a BMI of 23.7±3 kg/m² (Table 11). The majority was highly educated (89% greater than secondary education) and almost half of them were males (45%). The characteristics of the study sample were comparable in all three cities.

Table 11 Overall and city-specific characteristics of the study population (n=122). Categorical characteristics are reported as n (%); continuous variables are reported as mean±SD

Personal characteristics	Overall	Antwerp (n=41)	Barcelona (n=41)	London (n=40)
Men	55 (45%)	23 (56%)	16 (39%)	16 (40%)
Age (years)	35±10	37±11	34±9	35±10
Height (m)	1.7±0.1	1.8±0.1	1.7±0.1	1.7±0.1
BMI (kg/m²)	23.7±3	23.9±2.9	23.4±2.8	24.0±3.4
Higher education	109 (89%)	37 (90%)	37 (90%)	35 (88%)

Missing values: BMI=3

We recruited individuals that were observed to be physically active as the median weekly physical activity level assessed with the SenseWear armband was 42 METhours, which exceeds the WHO recommendation of 10 METhours. We observed a small intra-individual difference in weekly physical activity levels (intraclass correlation coefficient =0.74), which illustrates the measurement of habitual physical activity levels. However, the range of physical activity during the 24-hour time window was wide with statistically significant differences between cities (Table 12). The most active participants were recruited in London and Antwerp and the lowest physical activity level was observed in Barcelona. Differences between cities were similar during the 2-hour time windows.

The median BC exposure during the 24-hour time window differed between cities with the highest level in Barcelona (1.7 µg/m³) and similar levels in Antwerp and London (1.2 and 1.4 µg/m³) (Table 12). Differences between cities changed during the 2-hour time window, with the highest median BC concentrations observed in London.

The median and IQR of all subclinical outcomes are also reported in Table 12. The average FEV_1/FVC ratio observed was above 70%, which corresponds to normal lung function.[155] In addition, 75% of our study population had a FeNO concentration below 27 ppb, indicating that eosinophilic airway inflammation was not likely in our participants.[95] HRV markers, CRAE and CRVE lack established clinical reference values. Nunan et al. (2010) reviewed markers of HRV in over 20,000 healthy individuals and the reported ranges capture all our results, except for the 25th HF power percentile. The majority of the outcomes reached similar levels in all cities. FVC and FEV_1/FVC differed significantly between cities due to differences in the number of males and the age and height distribution of the city-specific samples.

Table 12 Overall, sex- and city-specific median and IQR of the physical activity level, BC concentrations and biomarker results aggregated per participant (n=122).

	Overall (n=122)	Males (n=55)	Females (n=67)	ANT (n=41)	BCN (n=41)	LDN (n=40)	p
Exposures							
24-hour exposure window							
PA (METhr)	5.9 (2.9-9.5)	6.7 (3.9-11.8)	5.4 (2.7-8.7)	6.4 (3.9-11.8)	3.7 (1.8-6.9)	7.2 (3.0-9.8)	*
BC (μg/m³)	1.4 (1.1-1.8)	1.3 (1.0-1.8)	1.4 (1.3-1.8)	1.2 (1.0-1.5)	1.7 (1.3-2.0)	1.4 (1.1-1.6)	***
2-hour exposure window							
PA (METhr)	0.7 (0.1-1.8)	0.8 (0.3-2.0)	0.6 (0-1.2)	1.5 (0.6-2.2)	0.4 (0-1.0)	0.6 (0.2-1.1)	*
BC (μg/m³)	1.8 (1.3-3.0)	1.8 (1.2-2.6)	1.9 (1.3-3.3)	1.5 (1.2-2.0)	1.6 (1.0-2.4)	3.5 (1.9-4.2)	***
Biomarkers							
HRV							
SDNN (ms)	71 (53-81)	75 (53-86)	65 (51-79)	72 (57-85)	74 (53-82)	66 (52-77)	
rMSSD (ms)	45 (28-58)	47 (28-58)	45 (28-59)	44 (30-51)	50 (29-66)	39 (26-59)	
HF (ms²)	138 (61-278)	120 (49-278)	142 (77-273)	149 (77-242)	201 (70-354)	96 (42-283)	
LF/HF	2.8 (1.9-4.6)	3.4 (2.3-5.7)	2.5 (1.7-3.9)	3.3 (2.1-4.8)	2.3 (1.6-3.5)	3.0 (2.0-6.6)	
Retinal microvasculature							
CRAE (μm)	161 (153-171)	159 (150-169)	162 (155-171)	161 (156-172)	164 (159-172)	156 (150-163)	
CRVE (μm)	236 (223-248)	233 (221-243)	239 (227-250)	233 (219-245)	243 (223-256)	237 (227-245)	
Lung inflammation							
FeNO (ppb)	20 (14-27)	24 (17-33)	18 (11-24)	21 (14-29)	18 (11-26)	23 (18-28)	
Lung function							
FEV_1 (L)	3.5 (3.0-4.0)	4.1 (3.8-4.5)	3.0 (2.8-3.3)	3.7 (3.3-4.2)	3.3 (3.0-3.9)	3.4 (3.0-4.1)	
FVC (L)	4.3 (3.7-5.1)	5.3 (4.8-5.6)	3.8 (3.4-4.2)	4.9 (4.1-5.5)	4.1 (3.6-4.7)	4.3 (3.7-5.2)	*
FEV_1/FVC (%)	80 (75-85)	79 (74-83)	82 (77-87)	77 (73-83)	83 (78-87)	80 (76-85)	**
PEF (L)	8.3 (7.3-10.0)	10.2 (9.3-11.4)	7.3 (6.7-8.0)	8.5 (7.2-10.0)	8.2 (7.4-9.8)	8.0 (7.1-9.9)	

Missing values: BC, 24h = 13; PA, 24h = 11; BC, 2h = 18; PA, 2h = 9; SDNN & rMSSD = 78; HF & LF/HF = 87; CRAE & CRVE = 9; FEV1, FVC, FEV1/FVC & PEF = 5; FeNO = 0; Mixed effect models were used to test for significant differences between cities correcting for sex, age and BMI or height (in case of lung function markers). HRV markers, FeNO and BC were logtransformed; physical activity (PA) was not transformed as the data contained 0-values. p<0.05 = * p<0.01 = **; p<0.001 = ***.

Results of the regression analysis are shown in Figure 8 and Figure 9. Effect estimates are provided in the appendix (Table S 2, p. 155 and Table S 3, p. 157) and β_{PA}, β_{BC} and β_{PAxBC} will be used to refer to the effects of physical activity, BC and their interaction respectively.

HRV markers showed a statistically significant increase in the log-transformed LF/HF with physical activity in the 2-hour time window (all models that did not include the interaction term; β_{PA}=0.07 (PA+BC+C model)) (Figure 8). In addition, log-transformed SDNN (β_{BC}=-0.06 (BC model)), rMSSD (β_{BC}=-0.11 (BC model)) and HF (β_{BC}=-0.19 (BC and PA+BC model)) statistically significantly decreased with logarithmic BC in the unadjusted models. The adjusted models showed similar trends, but these effects were not significant. The statistically significant changes were not present in the 24-hour time window, except for a modest change in LF/HF in the PAxBC model (β_{PA}=0.01). No short-term subclinical changes were observed for the retinal vessel diameters. Physical activity and BC also did not show interaction effects on the cardiovascular markers (HRV and retinal vessel diameters).

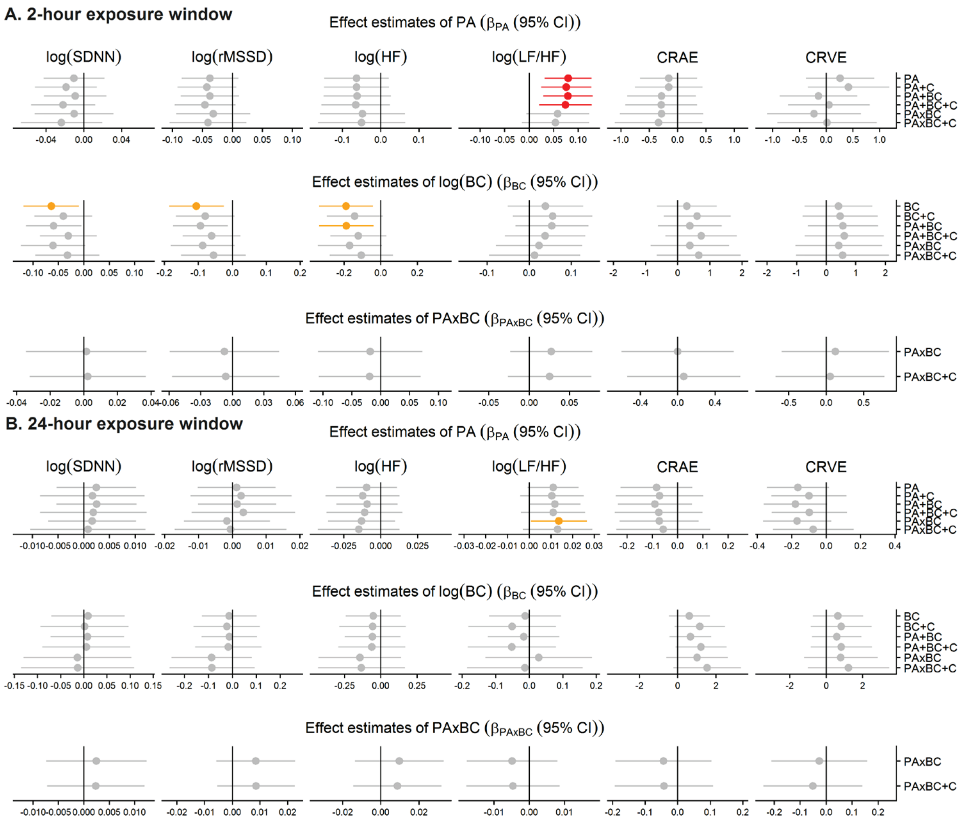


Figure 8 Effect estimates of physical activity (PA), logarithmic BC and their interaction (PAxBC) on the cardiovascular outcomes of (A) the 2-hour exposure window and (B) the 24-hour exposure window (based on the mixed effect regression analysis with continuous variables). β_{PA}, β_{BC} and β_{PAxBC} refer to the estimates of physical activity (total METhours), BC (average BC concentration in µg/m³, log-transformed) and their interaction respectively. The model where the respective estimate was observed is specified on the right. Orange-colored estimates have a p-value <0.05; red-colored estimates have a p-value <0.01. C = confounders (sex, age, BMI , season, education level, physical activity during the whole week (total METhours) and BC during the whole week (average concentration in µg/m³)).

The respiratory outcomes were also associated with physical activity and BC during the 2-hour time window preceding the outcome measurements. In the physical activity model, physical activity was statistically significantly associated with an increase in FEV_1 (β_{PA}=15.6) and FEV_1/FVC (β_{PA}=0.2) (Figure 9). The other models showed similar, non-significant trends. On the other hand, PEF significantly decreased per %increase BC in the BC (β_{BC}=-134.2), BC+C (β_{BC}=-102.19) and PA+BC (β_{BC}=-129.22) models. The statistically significant association between BC and PEF was also present for the 24-hour time window

(β_{BC}=-128.1 (BC model), β_{BC}=-124.06 (PA+BC model), β_{BC}=-209.84 (PAxBC model)). Logarithmic BC was also associated with decreases in FeNO (β_{BC}=-0.09) in the 24-hour time window. Significant, positive interaction effects between physical activity and logarithmic BC were observed for FeNO (β_{PAxBC}=0.04), FEV_1 (β_{PAxBC}=15.45) and FVC (β_{PAxBC}=22.07) during the 2-hour time window in both the adjusted and unadjusted models. The interaction effects were not present in the 24-hour time window (except for a modest positive interaction effect on FEV_1/FVC (β_{PAxBC}=0.05)).

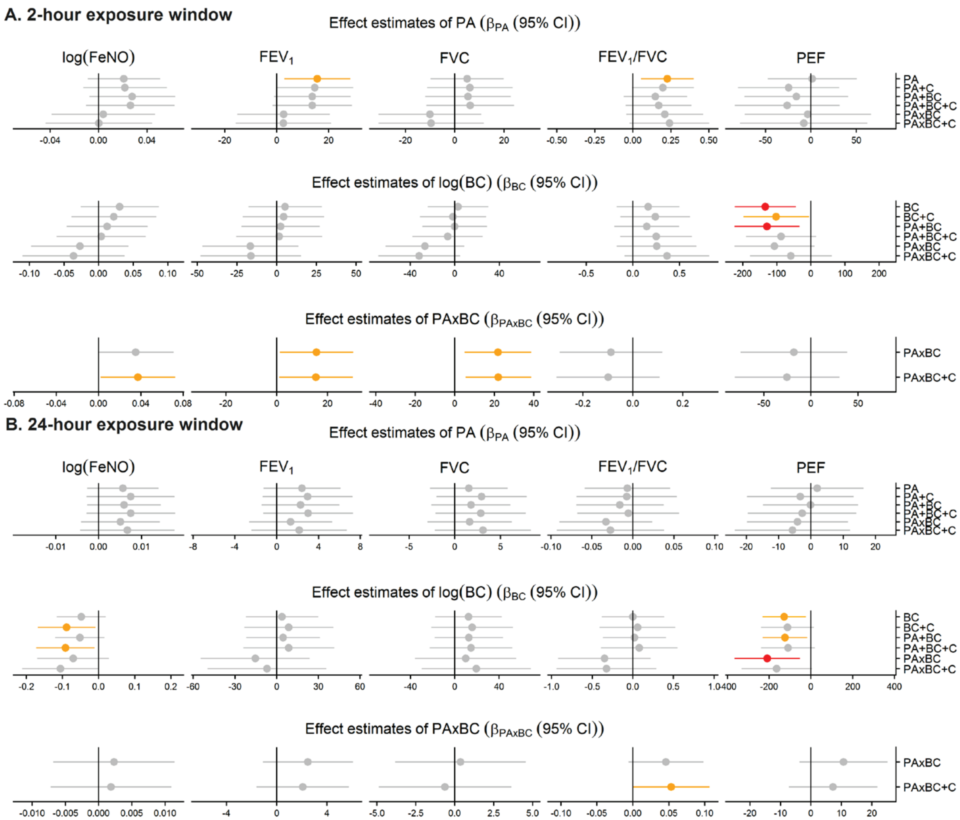


Figure 9 Effect estimates of physical activity (PA), logarithmic BC and their interaction (PAxBC) on the respiratory outcomes of (A) the 2-hour exposure window and (B) the 24-hour exposure window (based on the mixed effect regression analysis with continuous variables). β_{PA}, β_{BC} and β_{PAxBC} refer to the estimates of physical activity (total METhours), BC (average BC concentration in µg/m³, log-transformed) and their interaction respectively. The model where the respective estimate was observed is specified on the right. Orange-colored estimates have a p-value <0.05; red-colored estimates have a p-value <0.01. C = confounders (sex, age, BMI or height in case of lung function biomarkers, season, education level, physical activity during the whole week (total METhours) and BC during the whole week (average concentration in µg/m³)).

During the 2-hour and 24-hour time windows, 48% and 6% of the physical activity measurements resulted in zero METhours respectively. Therefore, we assessed the robustness of the results by repeating the regression analysis with physical activity as a categorical variable (no physical activity (0 METhours) versus physical activity (> 0 METhours)) which resulted in a similar pattern (Appendix: Figure S 6, p. 159). A minor difference was the statistically significant FVC increase with physical activity during the 24-hour time window. At the same time, FEV_1 remained unchanged which provoked a significant decrease in FEV_1/FVC associated with physical activity.

We observed positive interaction effects between physical activity and BC on FeNO, FEV_1, FVC (2-hour time window) and FEV_1/FVC (24-hour time window) in the regression analysis with continuous variables. Due to the presence of an interaction, the main BC effects need to be interpreted at specified physical activity levels. Therefore, we calculated the effect estimates of logarithmic BC for each physical activity category separately (Figure 10). For FeNO, the difference between responses to BC disappeared when physical activity was introduced as a categorical variable. In the case of FEV_1 and FVC trends towards positive interaction effects remained since the p-values of the interaction effects were respectively 0.13 and 0.03. However, except for a borderline statistically significant decrease in FVC with BC in the absence of physical activity (β_{BC}=-34.91, Figure 10C), effects of logarithmic BC were not significantly different from zero for specific physical activity categories.

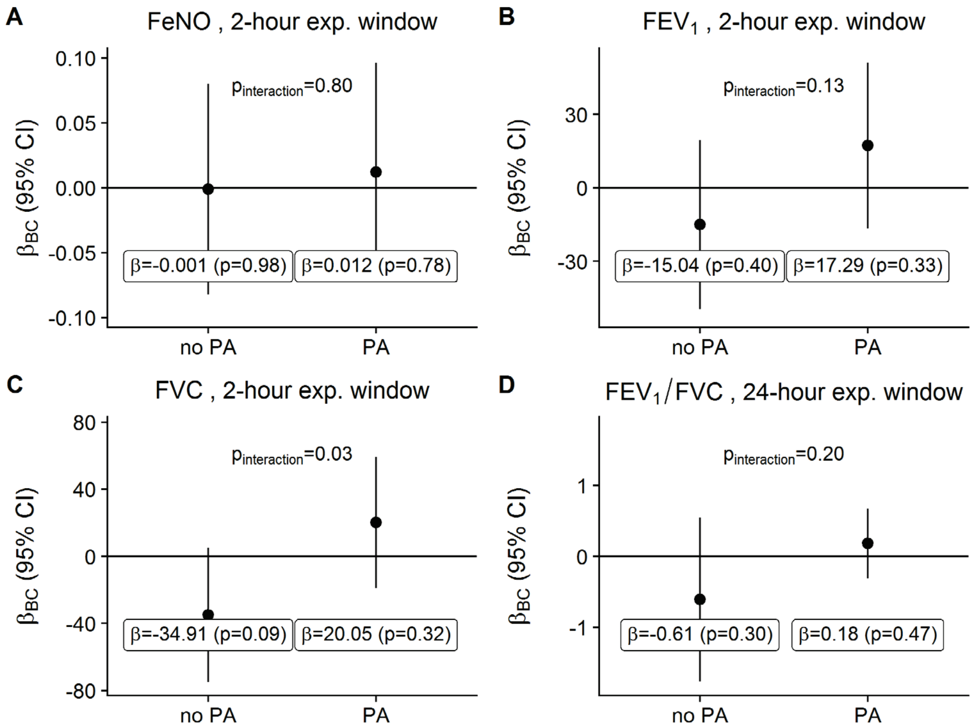


Figure 10 Effect estimates of logarithmic BC on (A) FeNO, (B) FEV_1, (C) FVC (2-hour time window) and (D) FEV_1/FVC (24-hour time window) per physical activity category (no physical activity vs physical activity). Results were obtained from the PAxBC+C regression models where $p_{interaction}$ represents the p-value of the interaction term. PA = physical activity.

Discussion

We found statistically significant, immediate HRV and respiratory responses to real-world physical activity and BC exposure. For the 2-hour time window, sympathetic tone dominated with both physical activity (increased LF/HF) and BC (decreased SDNN, rMSSD and HF). In addition, physical activity and BC had opposite, independent effects on respiratory markers. Physical activity was associated with an increase in FEV_1 and FEV_1/FVC and BC with a decrease in PEF. Moreover, we observed an interaction effect between physical activity and BC on FEV_1 and FVC, which may point to a protective effect of physical activity to counterbalance the BC effects on lung function. No effects were observed for the 24-hour time window apart from a few minor changes in LF/HF, FeNO and PEF.

The effects of both physical activity and BC on HRV mark increased sympathetic tone after the 2-hour time window. Sympathetic domination induces a rise in blood pressure and has been related to adverse health outcomes.[8,86] Previous studies also found associations between air pollution and HRV indicative of increased sympathetic activity.[96,100,118] As part of the TAPAS project in Barcelona, Cole-Hunter et al. (2016) assessed HRV responses to physical activity and BC in a 2-hour window. They observed that air pollution concentrations modified the relationship between HRV changes and physical activity.[96] On the high air pollution site, they found that the decrease in HRV was smaller when people were active compared to resting. Contrary, on the low air pollution site, larger HRV decreases with physical activity were observed compared to rest which resembles the HRV response to physical activity in our study. It should be noted that even the BC concentrations on the low traffic site of the TAPAS project exceeded our observations during the 2-hour time window. In addition, it has been shown that the sympathetic nervous system dominates during physical activity. During physical activity, the blood pressure increase associated with sympathetic domination provokes a defense mechanism against oxidative stress.[78,173] Consequently, physical activity may protect against the potential detrimental effects of air pollution on the vasculature. This mechanism is supported by the results of a previous study that observed that an increase in systolic blood pressure associated with air pollution was attenuated by physical activity.[16] However, our observations failed to confirm this hypothesis based on the measurements of retinal vessel diameters. Earlier work indicated that the retinal vessel diameters reflect an acute response to physical activity or BC.[112,113,115] During a smog episode, Louwies et al. (2013) observed a decreased CRAE associated with BC.[113] In addition, the Multi-Ethnic Study of Atherosclerosis (MESA) reported a CRAE decrease with exposure to short-term air pollution exposure and an increased CRVE in people with low physical activity levels.[112,115] The latter may indicate higher levels of systemic inflammation in people with low physical activity levels compared to highly active people. However, the MESA cohort included participants of older age (between 46 and 87 years old) who could be more susceptible to the adverse effects of air pollution and low physical activity.

In our study, BC was associated with decreases in FeNO in the 24-hour time window. This is in contrast to previous results where BC was associated with higher FeNO concentrations as a marker for increased airway inflammation.[117,118] However, we also found a small, yet statistically significant interaction effect of physical activity and BC on FeNO. This may reflect that higher ventilation rates during physical activity stimulate airway inflammation provoked by BC. Similarly, Bos et al. (2013) found that FeNO did not increase after a training program in a rural environment, yet an increase was observed after training in an urban environment.[93] In that study, higher BC concentrations were measured in the urban environment, so physical activity potentially enhanced the effect of BC. On the other hand, the interaction effect observed in our study was not replicated in the analysis with physical activity as a categorical variable. In clinical practice, FeNO is used to measure eosinic inflammation, while airway inflammation as a response to different triggers is more complex.[95] In addition, FeNO may also originate from other biological processes than airway inflammation in healthy adults. NO acts as a vasodilator during physical activity, so FeNO increases have been associated to physical activity in healthy individuals.[17] It follows that the observed positive interaction effect of physical activity and BC may be due to the different origins of FeNO during physical activity in polluted air (increased airway inflammation due to inhalation of BC and elevated systemic NO due to the vasodilating effect of physical activity).

We also observed an FEV_1 and FEV_1/FVC increase with physical activity after the 2-hour time window which is similar to recent results of the TAPAS project.[15,17] It is known that bronchodilation occurs with physical activity, potentially explaining these changes.[75] Besides, we found a statistically significant interaction effect of physical activity and BC on lung function markers FEV_1 and FVC. This may indicate a protective effect of physical activity on airway constriction provoked by inhalation of pollutants.[94] None of the effects of physical activity on the respiratory system were present for the 24-hour time window except for a small, positive interaction effect on FEV_1/FVC. Previous studies looking at the combined effects of physical activity and BC on lung function found either no interaction effects[17], or a protective effect of physical activity on PEF[15]. In our study, a decreased PEF was associated with BC, both in the 2- and 24 hour time window, but no interaction effect was observed for this marker. Zuurbier et al. (2011) found a similar decrease in PEF after two hours of air pollution exposure.[166]

Our sample consisted of healthy, highly educated adults with a physical activity level that meets the WHO recommendation of 10 METhours per week. Consequently, our findings cannot be extrapolated to the general population. We covered multiple European cities, which is unique in this field of research. In addition, our sample of over 100 individuals, who repeated the measurements in different seasons, was larger compared to previous studies in healthy adults designed to disentangle the short-term subclinical responses to physical activity and air pollution.[11,16,96,101] Contrary to previous experimental studies that assessed the interaction between physical activity and air pollution, we opted for a non-scripted panel study to measure real-world physical activity levels and air pollution exposure. This enabled us to assess the short-term effects of daily exposures and to correct for previous physical activity engagement and air pollution exposure. A limitation of this approach is the lack of available, a priori knowledge on the effect size of an interaction between continuous physical activity and BC. Therefore, we didn’t perform a formal sample size calculation which is a limitation of our study. However, we advise future research to use our observed effects sizes as a starting point. To minimize the risk of exposure misclassification, we measured air pollution exposure and physical activity on a personal level with wearable sensors. We opted for the microAeth to measure BC as a marker of air pollution. The microAeth is a reliable and validated mobile device for personal BC monitoring and such devices lack for other pollutants.[174] Although, the measurement of only one air pollutant is a limitation of our study, our goal was to assess the combined subclinical effects of physical activity and air pollution in an urban environment where BC, a marker of traffic-related air pollution, is highly relevant. Regarding the SenseWear armband, the estimation of its total energy expenditure has been validated against the doubly labelled water technique.[40,42–44] A recent study compared the SenseWear armband, its previous version and the Actigraph to indirect calorimetry and reported that all devices underestimate energy expenditure, especially at high intensities.[50] However, the most recent version of the SenseWear provided the best available estimate which justifies the use of this wearable sensor in our study. A final limitation of our study design is that participants didn't fill out an activity diary, so we couldn't categorize activities into different domains. Future studies may benefit from the use of such an activity diary to identify the combined effects of domain-specific physical activity and air pollution on subclinical markers.

Conclusion

Our study adds evidence on the separate and combined, short-term effects of physical activity and air pollution on subclinical cardiorespiratory markers. We found that both physical activity and BC immediately decreased HRV, but we failed to show effects on the microvasculature assessed with retinal image analysis. In addition, air pollution inhalation provoked lung function decreases while physical activity acted as an acute bronchodilator potentially providing a protective effect. In conclusion, we report short-term physiological changes in response to physical activity and air pollution in healthy individuals during everyday life in an urban environment. We advocate further research to elucidate how such short-term changes (1) behave over time and (2) translate into the long-term clinical effects that are associated with physical activity and air pollution. This may yield insight into the effects of modifiable environmental and behavioral risk factors on the development of cardiopulmonary diseases.

General discussion

Discussion

We aimed to estimate the long- and short term independent and combined cardiorespiratory effects of physical activity and air pollution in exposure-response relationships:

- **Exposures:** We focused on personal, real-life and continuous measurements to assess both physical activity and air pollution. This approach limits exposure misclassification.
- **Outcomes:** We integrated a set of non-invasive physiological outcome measurements related to cardiorespiratory health that have been shown relevant to investigate the responses to physical activity and air pollution.
- **Analysis:** We aimed at a transparent analysis where real-world exposures are implemented as continuous variables which prevents prior restriction on the possible directions of the interaction effect. Participants tracked their physical activity level and air pollution exposure during three weeks in three different seasons to approximate long-term behaviour.

Main Findings

Our study is the first to investigate the exposure-response relationship of real-world, personal physical activity and air pollution in an healthy adult population from multiple European cities. We found statistically significant acute and long-term cardiorespiratory responses to the combination of both factors that contribute to the current evidence base. An overview is provided in Figure 11 and Table 13 which respectively compare the levels of physical activity and air pollution and the results of our studies to those of the most relevant previous research.

We focused on the use of real-life, continuous measurements of both physical activity levels and air pollution concentrations. However, no standard of good practice exists to assess these variables. For air pollution, we based our measurement technique on previous work done in a VITO/IMOB PhD.[32] We measured personal BC concentrations as a proxy of exposure to air pollution. Regarding physical activity, we found that the GPAQ estimated significantly lower MVPA levels compared to the SenseWear armband. The difference depended on the intensity as it disappeared for vigorous-intensity activities. We also found that the differences between the estimates of both techniques were reproducible across repeated measurements. An easy-to-implement approach for future studies was suggested, adding the GPAQ to the wearable of choice as a basis for comparisons.

In Figure 11, physical activity levels of different studies, and measured with different techniques, are converted to METhours as a basis for comparisons. If our suggestion would be applied, this overview could also be provided based on estimates from a standardized technique over all studies: the GPAQ. The comparison of air pollution estimates is based on the range of concentrations measured in the ESCAPE project since this project measured number of pollutants consistently in different European cities (i.e. the ESCAPE scale).[157]

We found a beneficial effect of long-term physical activity on lung function at low BC concentrations. The beneficial effect decreased with increasing yearly, average BC concentrations (markers: FEV_1, FEV_1/FVC and FEF_{25-75}). HRV, the retinal microvasculature and FeNO didn't show a statistically significant, subchronic response to physical activity and/or BC.

Regarding short-term responses, we found statistically significant effects of real-world physical activity and BC on HRV and respiratory markers (Table 13). During the 2-hour time window, sympathetic tone dominated with both physical activity (increased LF/HF) and BC (decreased SDNN, rMSSD and HF). Physical activity acted as an acute bronchodilator (increased FEV_1 and FEV_1/FVC), while BC was associated with lung function decreases (decreased PEF). An interaction term was also observed that pointed towards a protective effect of physical activity to counterbalance the negative impact of BC on lung function (markers: FEV_1 and FVC). Few effects were observed in the 24-hour time window (minor changes in LF/HF, FeNO and PEF) and no effects were observed on the retinal microvasculature.

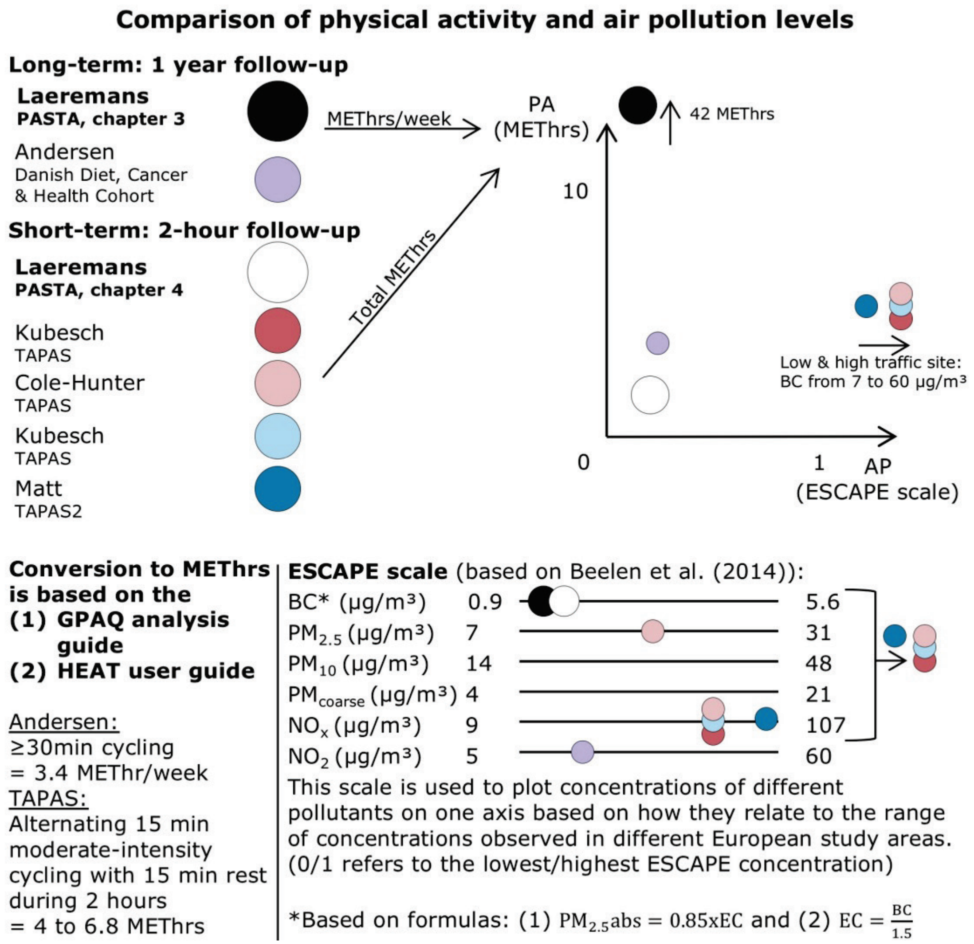


Figure 11 Comparison of the levels of physical activity and air pollution in our studies (black[175], white[176]) to those in the most relevant previous research (purple[14], red[16], pink[96], light blue[17], dark blue[15]). To compare exposures beyond studies, levels of physical activity (PA) are converted to METhours and air pollution (AP) concentrations are related to results of the ESCAPE project[177] where a number of pollutants was measured consistently in different European cities. Studies from the TAPAS project included a high and low traffic site with higher and lower air pollution concentrations respectively. For $PM_{2.5}$ and NO_x, some TAPAS studies reported air pollution concentrations on the low traffic site that are within the range measured in ESCAPE[177]. All other air pollution concentrations reported in TAPAS studies are larger than the maximum measured in ESCAPE[177]. This is represented by the corresponding coloured dots after the bracket including all pollutants reported in TAPAS. Formulas to convert $PM_{2.5}$abs to BC are extracted from Dons (2013)[32] and Gan et al. (2011)[156]. Conversions to METhours are based on METvalues from the WHO GPAQ analysis guide[135] and the HEAT (Health Economic Assessment Tool) user guide[136].

Table 13 Comparison of our results to the most relevant previous studies that focused on the combined effects of physical activity (PA) and air pollution (AP) on outcomes of the cardiovascular and respiratory health. Figure 11 presents the levels of the exposure observed in the summarized studies.

Study	Exposures	Responses
Cardiovascular markers		
Laeremans[175] *	↑1 METhour ↑1% BC	↑8% LF/HF ↓0.06% SDNN, ↓0.11% rMSSD, ↓0.19% HF
Cole-Hunter[96] **	Low traffic: ↑10,000/cm³ UFP, ↑10 μg/m³ BC	SDNN↓: active > rest (UFP & BC: Δ 3.6 & 10% resp.) rMSSD↓: **active** > rest (UFP & BC: Δ 4.2 & 20% resp.)
	High traffic: ↑10,000/cm³ UFP, ↑10 μg/m³ $PM_{2,5}$/BC	SDNN↓: active < **rest** ($PM_{2,5}$ & BC: Δ 3.4 & 1.7% resp.) rMSSD↓: active < **rest** (UFP , $PM_{2,5}$ & BC: Δ 0.9, 3.2, & 2% resp.) LF↓: active < **rest** ($PM_{2,5}$ & BC: Δ 5.6 & 5% resp.) HF↓: active < **rest** (UFP , $PM_{2,5}$ & BC: Δ 1, 7.2, & 4.4% resp.)
Kubesch[16]	4 to 6.8 METhrs compared to rest ↑IQR BC, PMcoarse, PM_{10}, NOx & UFP 4 to 6.8 METhrs compared to rest, or change from low to high AP (ref.: low AP & rest)	↓2.4 mm/Hg systolic blood pressure ↑1.0-1.2 mm/Hg systolic blood pressure High AP & PA: -1.4 mm/Hg; Low AP & PA: -3.1 mm/Hg; High AP & rest: 0.38 mm/Hg
Respiratory markers		
Laeremans[176] *	↑1 METhour ↑1% BC ↑1 METhr or ↑1% BC (interaction)	↑15.6 mL FEV_1, ↑0.2 % FEV_1/FVC ↓1.02-1.34 mL/s PEF ↑15 mL FEV_1, ↑22 mL FVC, 0.04% FeNO
Kubesch[17]	4 to 6.8 METhrs compared to rest ↑IQR BC, $PM_{2.5}$, PM10, NOx & UFP	↑34 mL FEV_1, ↑ 0.9 ppb FeNO ↓0.5% FEV_1/FVC
Matt	4 to 6.8 METhrs compared to rest ↑1 μg/m³ PM_{coarse} ↑1%HR_{max} or ↑1 μg/m³ $PM_{2.5}$,PM_{10},PM_{coarse}	↑49 mL FEV_1, ↑0.6% FEV_1/FVC, ↑98 mL/s FEF_{25-75} ↓1.3 mL FEV1, ↓1.7 mL FVC ↑ 0.02-0.03 L/min = 0.3-0.5 mL/s PEF

The BC measures in our study and all HRV markers were log-transformed to fit the regression model. Refer to Table 10 (p. 103) for the interpretations of the effects. *Chapter 4; Few changes were observed in the retinal microvasculature and during the 24 hour follow up period which are not reported here. **Bold text represents that significant effects of air pollution on HRV were observed for this physical activity category.

Exposure assessment

We compared the results of the GPAQ, a WHO-validated questionnaire, to those of the SenseWear armband, one of the most advanced wearable devices to measure physical activity. Since the GPAQ provides estimates of weekly physical activity performed in bouts of >10 minutes, measurements of the SenseWear were aggregated to provide measurements over the same time window. Both MVPA duration and METminutes showed significant moderate to strong correlations similar to previous studies.[25,46,47,145] We also observed that estimates of MVPA derived from the GPAQ were significantly lower compared to the SenseWear. This is in contrast to previous studies where higher physical activity levels were obtained with the self-reported tool compared to the wearable sensor.[46,52] The following reasons may explain this observation:

1) We recruited a study sample of relatively active participants who may have been unable to recall all activities that were part of their daily pattern. The median amount of weekly physical activity was 42 METhours which exceeds the WHO recommendation of 10 METhours per week.
2) Specific wearables perform differently and the SenseWear provides more accurate measures of upper body movements as its measurement technique is based on pattern recognition.[38,41,46–48]
3) Finally, our participants wore the SenseWear 96±4% of time during the measurement week. This is considerably higher than the wearing time of wearables in previous studies.[46,48]

The difference between GPAQ and SenseWear depended on the intensity of physical activity. Measures of moderate-intensity activity were significantly different and did not correlate, while measures of vigorous-intensity activity did not differ and showed high similarity. This was also observed in previous studies as respondents mainly think about vigorous or organized activities during physical activity reporting (e.g. sports). At the same time, they forget about moderate, routine activities (e.g. household chores or active transportation) or incidental daily movements.[46] Also, a scatter plot of the GPAQ and SenseWear estimates for sedentary behaviour showed a cloud where the center corresponded to similar GPAQ and SenseWear values (Figure S 2, p. 148). Hence, results for sedentary behaviour did not differ, but were poorly correlated, similar to Cleland et al. (2014).[47] Furthermore, similar to previous studies[25,150,151], the difference between the GPAQ and SenseWear estimates depended on BMI, body fat and physical activity domain. However, there was no relationship with gender or age which is in contrast to previous studies.[25,46]

We also observed that the differences between SenseWear and GPAQ estimates did not change across repeated measures. To our knowledge, only one study assessed the reproducibility of the differences between results of a self-reported tool and a wearable sensor, the GPAQ and the Actigraph, and the results were similar to ours.[47] Hence, the differences between the GPAQ and both the Actigraph and SenseWear, the most widely used wearable sensors in physical activity research, are reproducible.

The assessment of daily physical activity patterns lacks a golden standard technique. Wearable devices are highly valuable because they provide a detailed look into an individual's activity level. However, different wearable devices are used across studies since the choice depends on e.g. cost, availability and perception of user burden. Therefore, based on the reproducibility of the differences between the GPAQ and wearables, we propose a good practice for physical activity assessment. We recommend to use both a wearable sensor and the GPAQ. The GPAQ, a WHO-validated questionnaire providing robust estimates of overall activity, offers a reference to compare physical activity levels across studies. The wearable provides objective estimates of daily time-activity patterns at the desired time-resolution which is crucial to answer a broad range of research questions (e.g. responses to physical activity during different exposure windows, analysis of time-activity patterns and volumes of domain- and intensity specific activities to inform promotion actions).

Good practices for the personal measurement of air pollution exposure have been established by a previous VITO/IMOB PhD.[32] Hence, we used the microAeth to measure BC concentrations as a marker of personal air pollution exposure. The overall, median long-term, 24-hour and 2-hour BC concentrations of respectively 1.4 μg/m³ (Table 8, p. 85), 1.4 μg/m³ and 1.8 μg/m³ (Table 12,

p. 106) were low compared to the TAPAS project and the range of those measured in the ESCAPE project as illustrated in Figure 11 (p. 122).

The microAeth is a reliable and validated mobile device for personal BC monitoring and such devices lack for other pollutants.[174] BC is a fraction of particulate matter with most particles having an aerodynamic diameter smaller than 1 μm. Such small particles are believed to be more harmful than particles of larger sizes.[59] According to the WHO there is sufficient evidence of an association of cardiopulmonary morbidity and mortality with exposure to BC.[178] This makes BC a valuable marker to study physiological effects of air pollution.[60]

We also collected data for BC, NO, NO_2, PM_{10}, and $PM_{2.5}$ from fixed monitoring stations to check the correlation between BC concentrations and other pollutants (Antwerp, Barcelona and London; one monitoring station per city). Over the study period (February 2015–March 2016), daily NO and NO_2 concentrations were highly correlated with BC ($r=0.93$ and $r=0.91$ respectively). Correlations between BC and PM_{10} ($r=0.40$) and $PM_{2.5}$ ($r=0.38$) were lower, and differed between cities. We decided not to use these data in sensitivity analyses as we would introduce error from using proxy exposure estimates compared to personal BC monitoring which is in contrast with our objectives. Moreover, we only had daily averages available, so we would not be able to assess responses in the 2-hour window, or the exact 24-hour exposure window.

Dons et al. (2017) compared various formulas to estimate inhaled air pollution dose based on data collected in the PASTA panel study.[10] These calculations may be important to investigate the adverse effects of air pollution on health. However, the calculated inhaled dose was not used for the analysis in this PhD since it is a linear combination of the physical activity estimates measured by the SenseWear and air pollution concentrations measured by the microAeth. Using this variable in the models would not allow to disentangle the responses to both variables. A more interesting approach for future research would be to identify the deposited dose i.e. the fraction of the inhaled dose that reaches the target tissue. This approach might help to estimate precise effect sizes of the physiological responses to a combination of physical activity and air pollution.

Though, accurate determination of the deposited dose at a high time resolution remains challenging and only a few panel studies have used this exposure measure. Moreover, the combination with physical activity also complicates the deposited dose calculations since breathing patterns change with exercise and are highly variable between individuals.[11] Deposition models have been developed to estimate the deposited fraction, or alternative approaches can be used to determine biomarkers of deposition over longer time spans (e.g. sampling lower airway macrophages and analyse them for BC content).[73,179]

Subclinical cardiovascular effects

We observed immediate sympathetic domination with both physical activity (increased LF/HF) and air pollution exposure (decreased SDNN, rMSSD and HF) independently (Table 13, p. 123). Short-term exposure to air pollution has been shown to increase sympathetic domination, assessed by markers of HRV.[123] This induces a rise in blood pressure and has been related to adverse health outcomes.[8,86] It has also been shown that the sympathetic nervous system dominates during physical activity. In this case, the associated blood pressure increase provokes a defense mechanism against oxidative stress to protect the blood vessels.[78,173] We didn't find larger HRV responses with a combination of physical activity and air pollution. This was observed by Cole-Hunter et al. (2016) where sympathetic domination associated with BC, UFP and $PM_{2.5}$ exposure was larger during exercise compared to rest in an environment identified as the low traffic site.[96] Although the authors referred to that site as 'low traffic', its BC concentrations still exceeded those measured during our study (1.7 µg/m³ vs 8.6 µg/m³; Figure 11, p. 122). Contrary, on the 'high traffic' site, sympathetic domination was stronger during rest compared to exercise. The authors hypothesise a protective effect of physical activity on the HRV decrease associated with air pollution, but the current evidence cannot confirm this theory.

We didn't find long-term, independent or combined effects of physical activity and BC on HRV (Figure S 7, p. 160). HRV is highly variable and a limited amount of evidence is available on the long-term HRV responses to physical activity and air pollution.[8] However, lower HRV indices have been reported in individuals at higher risk of or with established cardiovascular diseases.[8,81]

We did not find any long- or short-term effects of physical activity and air pollution on the retinal microvasculature as a marker of cardiovascular health (Table 13, p. 123 and Figure S 7, p. 160). Earlier work indicated that the retinal vessel diameters reflect an acute response to physical activity or BC.[112,113,115] During a smog episode, Louwies et al. (2013) observed a decreased CRAE associated with BC.[113] In addition, the MESA study reported a CRAE decrease with exposure to short-term air pollution and an increased CRVE in people with low physical activity levels.[112,115] The latter may indicate higher levels of systemic inflammation in low compared to highly active individuals. However, the MESA cohort included participants of older age (between 46 and 87 years old) who could also be more susceptible to the adverse effects of air pollution and low physical activity. No previous studies are available on the combined effects of physical activity and air pollution on the retinal microvasculature in healthy individuals.

The pathways underlying changes of the retinal microvasculature have not been elucidated yet, but they may involve responses of the endothelium.[113] Regarding cardiovascular effects in general, it is believed that physical activity has a protective effect on the vasculature while air pollution contributes to impaired endothelial function and vascular stiffening.[86] A recent study by Sinharay et al. (2017) in more vulnerable adults found that the beneficial effect of physical activity on the arteries disappeared after a walk in a polluted environment (central aortic pulse wave velocity and augmentation index were used to describe arterial stiffness).[90] This observation suggests that air pollution attenuates the physical activity benefit which points towards an impaired built-up of the beneficial effects on the cardiovascular system. A complementary vision supported by Kubesch et al. (2014) is that physical activity protects against the adverse effects of air pollution on the vasculature (marker: blood pressure).[16] However, the study design of Sinharay et al. (2017) did not allow to test this hypothesis. Zhang et al. (2017) contributed to the evidence base of the combined effect of long-term physical activity and air pollution on cardiovascular health by providing information on cellular markers.[19] They found that physical activity and air pollution were respectively associated with a decreased and increased white blood cell count as markers of systemic inflammation. No interaction effect was observed at relatively high air pollution concentrations. The 90th percentile of $PM_{2.5}$ exposure was used to test the interaction effect and. The cut-off point was 39.8 µg/m³ which is high compared to $PM_{2.5}$ concentrations observed in the ESCAPE study (Figure 11, p. 122).[177] Similarly, the Danish Diet, Cancer, and Health cohort observed lower cardiovascular mortality rates among physically active adults, but air pollution did also not modify the relationship.[14] In this study, air pollution concentrations were relatively low (Figure 11, p. 122).[180]

Subclinical respiratory effects

Regarding respiratory markers, we observed an interaction effect between both long- and short-term physical activity engagement and air pollution exposure on lung function. We found that long-term, weekly physical activity is associated with an improved pulmonary function at low BC concentrations. A 5.6 mL FEV_1, 0.1% FEV_1/FVC and 14.5 mL/s FEF_{25-75} increase was associated with an additional, weekly METhour at BC concentrations up to 1 µg/m³. This respiratory benefit decreased when BC concentrations increased, similar to previously reported findings.[92,94] This may explain why the benefits of physical activity on respiratory mortality were attenuated in high air pollution concentrations in the Danish Diet, Cancer, and Health cohort.[14] In addition, a higher prevalence of exercise-induced bronchoconstriction, asthma and lower lung function has been observed in athletes who train in environments with high particulate matter emissions.[94]

The public health impact of long-term, small effects on lung function markers has been demonstrated by Kunzli et al. (2000).[181] An *average* 3% FVC decrease (associated with a 10 µg/m³ PM_{10} concentration increase) resulted in a 47% higher prevalence of 'FVC<80% predicted'.[181] The latter cut-off point is used for the diagnosis of respiratory diseases and the clinical impact of such chronic effects on lung function have to be interpreted on a *population* level.[181,182] In our study, we found that the physical activity benefit on FEV_1 in low air pollution concentrations was similar to Cheng et al. (2003)[158] where an additional 15 METhours per week were associated with a 130 mL higher FEV_1. This corresponds to an increase of 3% in active individuals, assuming a FEV_1 population average of 4.3 L (ERS reference values for a 25 year old male of 175 cm tall, Quanjer et al. (2012)[155]). Taking into account the estimations of Kunzli et al. (2000)[181], this holds an important reduction in the prevalence of 'FEV_1<80% predicted' and respiratory diseases. According to our results, the public health benefit disappears in yearly average BC concentrations above 2 $\mu g/m^3$.

Regarding short-term effects, physical activity provoked bronchodilation illustrated by a significant increase in FEV_1 (15.6 mL) while a BC %increase was associated with a significant PEF decrease (-1 to -1.3 mL/s; Table 13, p. 123). Zuurbier et al. (2011) found a similar change in PEF after two hours of air pollution exposure.[166] In addition, Int Panis et al. (2017) reported short-term changes in lung function (FEV_1, FVC and PEF) associated with day to day changes in PM_{10}.[120] PEF also decreased with higher daily NO_2 concentrations. Kubesch et al. (2015)[17] and Matt et al. (2016)[15] observed decreases in FEV_1, FVC and FEV_1/FVC associated with air pollution, and found a FEV_1 increase with one additional METhour similar to our results. A 34 mL and 49 mL higher FEV_1 was associated with 4 to 6.8 METhours compared to rest (Table 13, p. 123), which corresponds to a 5 (34 mL divided by 6.8) to 12.3 mL (49 mL divided by 4) increase per additional METhour. They also looked at the combined effects of physical activity and air pollution on lung function and found either no interaction effects[17], or a protective effect of physical activity on PEF[15]. We also observed a statistically significant interaction between physical activity and BC on FEV_1 and FVC. This may indicate that physical activity counterbalances short-term airway constriction provoked by air pollution.

Estimated responses to physical activity and air pollution had an interaction effect on FeNO concentrations (Table 13, p. 123). This may reflect that higher ventilation rates during physical activity stimulate airway inflammation provoked by BC. We did not observe a subchronic effect of physical activity and BC on FeNO (Figure S 7, p. 160) and the short-term interaction effect disappeared in the analysis with physical activity as a categorical variable (Figure 10, p. 111). However, the short-term effects compare to the FeNO increase observed after a 12 week training program in an urban compared to a rural environment.[93] This study measured higher BC concentrations in the urban environment, so physical activity might have enhanced its effect.

Methodological considerations

Our sample consisted of healthy, highly educated adults with an average physical activity level that meets the WHO recommendation of 10 METhours per week.[183] We covered multiple European cities, enabling geographical extrapolation which is unique in this field of research. Since we recruited mostly active and highly educated volunteers of Caucasian ethnicity, our findings aren't entirely representative of the general population. We also focused on healthy adults, so we cannot extrapolate our findings to more sensitive population groups such as children, older adults or individuals with chronic conditions. Responses to (a combination of) physical activity and air pollution might differ or be more severe in these groups. Previous research already found more pronounced lung function decreases in participants with moderate compared to mild asthma after a 2 hour walk along Oxford street (high traffic site in London).[91]

To minimize the risk of exposure misclassification, we measured air pollution exposure and physical activity on a personal level, with wearable sensors. Our goal was to assess the combined subclinical effects of physical activity and air pollution in an urban environment where BC, a marker of traffic-related air pollution, is highly relevant. For personal BC monitoring, we used the microAeth. This is a reliable and validated mobile sensor and such devices lack for other pollutants.[174] To assess physical activity, we used the SenseWear armband for which the estimates of its total energy expenditure have been validated against the doubly labelled water technique.[40,42–44] A recent study compared the SenseWear armband, its previous version and the Actigraph to indirect calorimetry and reported that all devices underestimate energy expenditure, especially at high intensities.[50] However, the most recent version of the SenseWear provided the best available estimate which justifies the use of this wearable sensor in our study. In addition, especially MET values above 10 METs are underestimated by the SenseWear which our participants only achieved during 0.2% of their time awake.[50]

We opted for a non-scripted panel study to measure real-world physical activity levels and air pollution exposure where each participant repeated the measurements in three different seasons. The use of a repeated measures design has several advantages:

1) The power of our statistical analysis of the short-term effects increased (illustrated in Figure 12, p. 137).
2) Collection of personal, free-living data in three different seasons enabled us to calculate proxies for long-term behaviour. Hence, this approach complements large scale epidemiological studies that often use less precise exposures and focus on mortality or established morbidity instead of subclinical effects.
3) We found reproducible differences between the GPAQ and SenseWear across repeated measurements, adding to previous results on the Actigraph.[47] Hence, we proposed a good practice for the assessment of physical activity in future studies, based on both the GPAQ and a wearable sensor.

Our aim was to test whether the responses to physical activity and air pollution interact. The recommended study design to assess an interaction is a two-factor design which was used in the TAPAS project.[184] Each participant completed scripted activities in four different scenarios i.e. all combinations of two levels of each of the two factors: rest/physical activity and high/low air pollution levels.[15–17,96] This allows researchers to determine whether air pollution attenuated the benefits of physical activity and/or whether physical activity protected against the adverse effects of air pollution. This cannot be assessed when only two scenarios are used. In a number of previous studies, participants completed a scripted bicycle or walking tour in high and low air pollution, so they only took the modification effect of air pollution into account.[84,90,93,97,98] Another possibility to approach the two-factor design is to collect continuous measurements and divide the sample in subgroups based on their physical activity level and exposure to air pollution. In our analysis, we categorized physical activity in the

2-hour time window to check the robustness of the observed responses to continuous physical activity and air pollution. However, dichotomization reduces both the amount of information available in the data and the comparability of the modification effect between studies since thresholds often don't align (Figure 11, p. 122).[185] Such thresholds are mostly based on quantile information such as medians[14,20,92] or selected by design via locations with relatively high and low air pollution concentrations.[15–17,96] Since we focused on responses to real-world physical activity and air pollution, the use of a two-factor design where each participant completes four specified scenarios was complicated. Major strengths of the use of continuous variables for physical activity and BC are that (1) all collected information was used in the analysis and (2) it allows easy comparability between studies and provides a transparent analysis. The use of continuous variables enabled us to estimate interaction effects and we hypothesized a protective effect of physical activity or an attenuating effect of air pollution based on the effect estimates and the sign of the interaction.

Another strength of our analysis is that we used a DAG to identify confounders of the relationship between short-term cardiorespiratory responses, physical activity and air pollution (Figure S 5, p. 153). Such a graph visualizes the hypothesized causal framework on which the research question is based.[186,187] It has been recommended as a tool to formalize data analysis in epidemiological research since it allows easy communication on model building.[188] It also helps to identify bias or explain unexpected findings of the relationship of interest.

We integrated a battery of non-invasive outcome measurements to obtain an overall view on the cardiorespiratory responses to physical activity and air pollution: HRV, retinal vessel diameters, FeNO and lung function. Clinical threshold values are established for FeNO and lung function which enables interpretation regarding the likeliness of diseases.[95,155] In clinical practice, FeNO concentrations above 25 ppb mark increased probability of eosinic inflammation.[95] However, airway inflammation as a response to different triggers is more complex and FeNO may also originate from other biological processes than airway inflammation in healthy adults. NO acts as a vasodilator during physical activity, so FeNO has been observed to increase with physical activity in healthy individuals.[17] This complicates the interpretation of the contribution of FeNO to effects of physical activity in polluted air. Regarding cardiovascular markers, the interpretation of HRV is also more complicated than generally considered as it rapidly modulates in response to changing environments.[81,102] Still, responses involving sympathetic domination are highly relevant to evaluate health because they may require excessive energy demands. The retinal vessel diameters provide information on physiological effects of the microvasculature, but the pathways underlying these changes need to be further investigated. Based on these considerations, we recommend for future research to further characterize the relationship between physical activity, air pollution and cardiorespiratory responses. We suggest to complement the used set of markers with other cardiorespiratory outcomes to validate the selected outcome measures and to extract pathway information.

Finally, a limitation of our study is the lack of an a priori sample size calculation. The decision on the magnitude of the sample was based on the results and experiences from previous studies. We opted for a large sample size compared to the majority of previous panel studies focusing on physical activity and air pollution epidemiology where the number of participants was below 60 (Table 3, p. 51).

Sample size calculations often involve complicated mathematics and most approaches do not respond to all characteristics of panel studies in epidemiology.[189] Recently, Weichenthal et al. (2017) addressed this problem. The authors provide a user-friendly, online tool to calculate the sample size needed to observe the hypothesized effect size with a power of 80% in panel studies with a repeated measures design.[189] For random-intercept models, input on the residual variance of the responses, the number of measurements per subject and the within subject range of exposures (mean squared deviations) is required. Therefore, it is encouraged to report all variance components of fitted random-effect models in future research to improve evidence based assumptions for a priori sample size calculations.

We used the online tool to do an a posteriori sample size calculations for the observation of short-term, independent effects, based on our study characteristics (Figure 12). A set of markers were selected where significant effects were observed: (1) FEV_1 and LF/HF for the effects of physical activity, and (2) PEF and HF for the effects of BC. The following effect sizes were assumed based on a comparison of the estimates observed in previous research:

1) We assumed a 10 mL increase in FEV_1. Effect sizes of our study and TAPAS were compared previously and ranged from 5 to 15 ml (Paragraph: Subclinical respiratory effects).
2) The hypothesized effect size of physical activity in LF/HF was 0.08 and based on our study only because none of the previously discussed studies report (similar) effect estimates of independent physical activity on HRV of LF/HF.
3) We assumed a PEF decrease of 115 mL/s with logarithmic BC. This value is centered between our observations and is similar to the estimate of Zuurbier et al. (2011)[166]: PEF decreased 80 mL/s with a BC IQR increase of approximately 6 µg/m³, ranging from 6 to 12 ($PM_{2.5}$absorbance was measured and converted to BC with the formulas reported in Figure 11 on p. 122). Based on our effect estimate, PEF also decreased 80 mL with a similar change in BC concentrations: -115*log(12/6)=80 mL/s (with 'log' as the natural logarithm).
4) The assumed effect estimate of logarithmic BC on logarithmic HF was 0.2. Cole-Hunter et al. (2016)[96] observed a 23% HF decrease with a 10 µg/m³ BC increase on the low traffic site. The average BC concentration on this site was 8.6 µg/m³ with a standard deviation of 5.1. Based on these numbers, we assumed a 10 µg/m³ BC increase from 3.5 to 13.5 µg/m³ to compare the %change in HF in our study: $(13.5/3.5)^{-0.19}=0.77$. This is similar to a 23% decrease in HF with a 10 µg/m³ BC increase.

Figure 12 suggests that our sample size and study characteristics were adequate to observe the hypothesized effects on HRV and PEF. The distribution of mean squared deviations of the exposures in this study is shown in Figure 12C. They were generally low , but the range was large enough in combination with three repeated measures (Figure 12A and B, left). Regarding FEV_1, it appears that a larger sample size is required to observe a 10 mL increase with an additional

METhour. This might be due to a combination of high residual variation and a low range of within-person variability in exposures within 2 hours before the evaluation of cardiorespiratory markers. However, the magnitude of the physical activity effect on FEV_1 was similar in three different studies and our effect estimate was larger compared to the assumed size. It follows that the observed effects are robust and a valid contribution to the current evidence base.

While the described effects were observed in models without the interaction term, addition of a modification effect further complicates the calculation of the required sample size. To detect an interaction between two binary fixed effects in mixed effect regression models, it has been reported that the required sample size is fourfold due to increased variance.[190] Formal sample size and power calculations are highly valuable for the interpretation of effect sizes and interest in interactions between environmental and lifestyle exposures is increasing.[188] Therefore, further work is necessary to develop user-friendly tools to calculate the sample size for the detection of interactions between both continuous and categorical variables with high power.

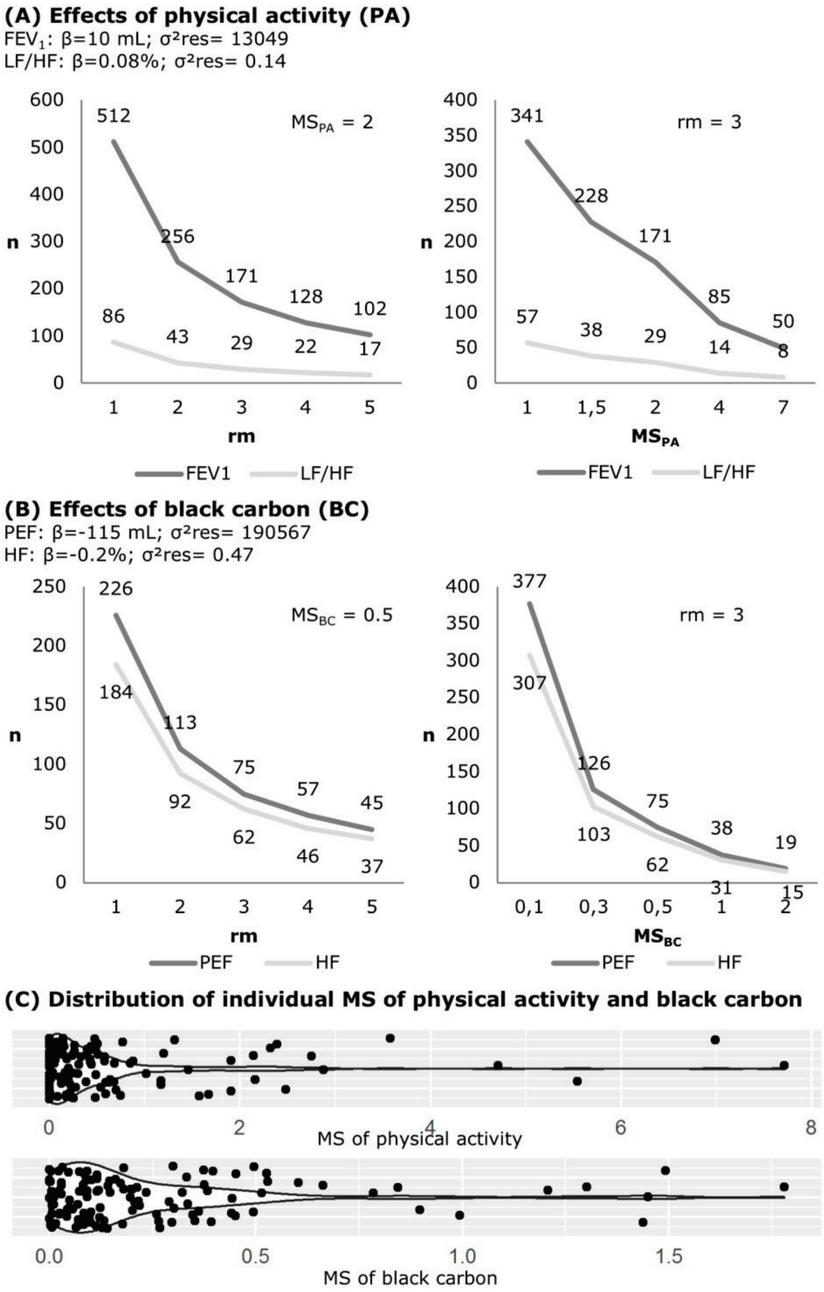


Figure 12 Sample size calculation based on Weichenthal et al. (2017)[189] to detect the hypothesized effect sizes (β) of independent physical activity (A) and BC (B) with a power of 80%. The effect size and residual variance (σ^2res) is kept constant (σ^2res is based on unadjusted models including only the exposure of interest and random participant effects; LF/HF, HF and BC was log-transformed). Sample sizes are calculated as a function of repeated measures (rm; left) and intrapersonal mean squares deviations (MS; right) in physical activity and black carbon (C).

Further research

Promotion of active mobility in urban areas is complex since health outcomes depend on many interactions.[70] Therefore, further research should expand on our research design and (1) track activities, air pollution concentrations and physiological responses at a high temporal and spatial resolution since large exposure variation exists within cities, and (2) validate the physiological information of previously used outcome measurements. Moreover, information should be collected on both healthy and more sensitive groups to improve urban health. Such studies are the next step towards smart cities collecting continuous data streams and crowdsourcing personal sensor measurements. This will revolutionise the concept of citizen science.

Knowledge on when and where the human body responds to a combination of environmental and lifestyle triggers holds valuable information on the health impact of specific locations and will aid to improve public health.[70] Propeller Health already developed smart inhalers and applications to help asthma patients manage their disease better by understanding their environment. On a population level, such databases may inform city administrations about locations with a high adverse health impact. New technologies enable large-scale follow-up of location, activity patterns and vital signals.[70,188] Smartphones have become widespread commodities that track locations and measure accelerometry. They enable large-scale, personal data collection since the smartphone is already at the individual's disposal. Complementary apps may provide additional information about air pollution exposure (e.g. aircheckr), physical activity and mobility patterns (e.g. Google Fit and Moves). Moreover, small and user-friendly sensors have become available that allow long-term, continuous and personal data collection about physical activity and vital signals (e.g. Fitbit and Byteflies).[70] The same goes for low-cost air quality monitoring devices.[191]

Less is known about the data quality of these new data collection approaches. Therefore, we recommend to also include the collection of validation data. Smart city sensor networks (i.e. sensors placed on various locations within the city) and remote sensing (e.g. satellites) will provide information on environmental exposures such as (within city variation in) air pollution.[188] These networks can be compared to monitoring of personal exposure to air pollution. Moreover, individuals can fill out the GPAQ on their smartphones as a comparison base for data on the overall physical activity level collected with different wearable approaches. Subsets of the population can also be recruited to provide validation data with (1) the microAeth for personal exposure to BC, and (2) a wearable sensor validated against doubly labelled water and indirect calorimetry such as the SenseWear armband.

We contributed to the evidence base on the independent and combined cardiorespiratory effects of physical activity and air pollution. These changes might be relevant as biomarkers of early physiological effects. We recommend to further characterize these cardiorespiratory responses of physical activity and air pollution to enhance the physiological interpretation. Regarding cardiovascular markers, we suggest to complement (1) HRV markers with electrodermal activity which also informs about sympathetic domination[192], and (2) retinal microvasculature measurements with other markers of the microvasculature (e.g. photoplethysmography to continuously monitor blood volume pulses) and endothelial function (e.g. flow mediated dilation). Additional markers of pulmonary inflammation such as induced sputum may also aid the interpretation of FeNO. Frequency of self-reported respiratory symptoms of both the upper and lower tract and continuous tracking of respiratory rate and volume will enhance the evidence base on respiratory responses such as lung function. Moreover, continuous time series data on vital signals such as heart rate, ventilation and blood pulses will (1) inform about the reversible nature of the short-term, physiological changes, and (2) enable long-term follow-up studies to elucidate the clinical impact in case of disease onset. In the PASTA panel study, we collected continuous heart rate and HRV data during waking hours, on two days of the measurement week (three weeks in one year). This may provide a first pilot dataset to address the reversible nature of changes in heart rate and HRV.

Implications

More than 80% of the European population is expected to live in urban areas by 2050.[193] It follows that city planning needs to be done across different sectors, including urban design, transport planning and health, to maintain and increase life quality of urban citizens. Despite high chances of elevated exposure to air pollution in cities, *it is vital that administrations keep promoting physical activity for health.* Physical inactivity has been linked to the prevalence of major non-communicable conditions such as coronary heart disease, type 2 diabetes and cancer, and accounts for more than five million premature deaths.[2] Decreasing inactivity by 10 to 25%, would save up to approximately one million lives. Higher urban active mobility levels also create co-benefits such as lower motorized traffic resulting in reduced air pollution concentrations and noise.[5,70] Active mobility promotion measures offer potential to increase overall physical activity levels and reach all groups of society. However, health outcomes of urban interventions often come with unforeseen effects since they depend on many interactions between the built environment, environmental factors, personal behaviour and health.[70] *The question that remains is when, where and how promotion measures need to be implemented.*

Various HIAs reported that the overall health benefits of physical activity outweigh the risks of air pollution exposure on a population level.[12,13] Tainio et al. (2016)[194] estimated how long we can cycle per day until the risks of air pollution surpass the physical activity benefits for all-cause mortality. This was done across a wide range of urban background $PM_{2.5}$ concentrations. For the average European city (14 µg/m³, WHO), people can cycle all day before the risks of air pollution exposure would exceed the benefits of daily cycling. Even in the world's most polluted city (Delhi, India, 153 µg/m³), people can cycle for 45 minutes per day until it becomes more harmful than beneficial. These models focus on mortality and established clinical conditions. In order to prevent such conditions, more evidence on the biomarkers of early effects is needed.

Current evidence on the short-term cardiorespiratory responses to a combination of physical activity and air pollution suggests that:

1) In healthy individuals, physical activity is related with improved lung function, and it attenuates bronchoconstriction, associated with exposure to air pollution. In more vulnerable individuals the pulmonary benefit of physical activity disappeared in a polluted environment.
2) A previous study observed a decreased blood pressure associated with physical activity which also attenuated the blood pressure increase related to air pollution exposure in healthy individuals. Similar to the respiratory benefit, more vulnerable individuals didn't experience a cardiovascular benefit after a walk in a polluted environment.

It appears that the short-term cardiorespiratory responses to physical activity in polluted environments are less beneficial in vulnerable compared to healthy populations, but clear harmful effects have not been reported.

Looking at subchronic and long-term effects of physical activity in elevated air pollution concentrations, evidence suggests caution for respiratory health. However, to prevent disease progression, physical activity is an important part of treatment plans. Therefore, patients need to be informed about their environment and be provided with location- and time-specific recommendations on physical activity. Within cities, environmental exposures such as air pollution, noise and green space vary substantially in space and time. We proposed a set of new methods and tools to aid further research and determine location-specific responses to air pollution, taking the individual's behaviour into account. This will identify physiological responses to the complex mixture of urban factors in order to inform policies and create healthy cities.

Meanwhile, the European Lung Foundation and the European Respiratory Society developed tips to gain Healthy Lungs For Life by taking air quality into account during exercise. They recommend to (1) be proactive and check the weather forecast since rain and wind comes with cleaner air, (2) avoid exercising during rush hours, and (3) take location and route into account. This involves the use of green space, to keep a two meter distance from the road and no cycling behind motorized traffic. Small roads between (tall) buildings should also be avoided since this is where air pollution accumulates. Most importantly, they advocate to be active and walk and cycle as a means transport so we don't add to the high air pollution concentrations and reach our daily physical activity goals.

Appendix

Supplemental material: Chapter 2

Table S 1 Characteristics of volunteers enrolled in (1) the PASTA online survey in all cities (2) the PASTA online survey in Antwerp (ANT), Barcelona (BCN) and London (LDN) only (3) the study using wearables (all participants and each city separately). Physical activity variables of the online survey sample are derived from the GPAQ asking about general behaviour. Physical activity variables of the subset enrolled in the study using wearables are derived from the GPAQ asking about activities during their measurement week.

	PASTA online survey	PASTA online survey ANT,BCN, LDN	Subset used for analysis ANT, BCN, LDN	Subset used for analysis ANT	Subset used for analysis BCN	Subset used for analysis LDN
n	10693*	4618*	122	41	41	40
Males %	46	43	45	56	39	40
Higher education %	78	86	89	90	90	88
Age years (mean±sd)	40±13	39±13	35±10	37±11	34±9	35±10
Reported BMI kg/m² (mean±sd)	24±4	24±4	23±3	23±3	23±3	22±3
MVPA EE METmin/week (median (IQR))	2820 (1560-4857)	2720 (1468-4717)	2029 (1112-3237)	1972 (1251-3271)	1600 (1000-2232)	2523 (1408-3334)
Moderate EE METmin/week (median (IQR))	960 (360-2280)	960 (240-2160)	720 (310-1268)	360 (120-850)	760 (413-1213)	870 (560-1580)
Vigorous EE METmin/week (median (IQR))	1440 (320-2880)	1410 (272-2796)	1057 (321-2169)	1632 (752-2285)	408 (107-1387)	1184 (147-2330)
MVPA min/day (median (IQR))	73 (40-129)	69 (39-122)	53 (33-78)	46 (31-78)	43 (30-66)	64 (40-90)
Moderate MVPA min/day (median (IQR))	34 (13-81)	34 (9-77)	26 (11-45)	13 (4-30)	27 (15-43)	31 (20-56)
Vigorous MVPA min/day (median (IQR))	26 (6-54)	26 (6-51)	22 (6-40)	30 (15-43)	9 (2-28)	23 (3-45)
SB min/day (median (IQR))	480 (330-600)	480 (330-600)	535 (420-635)	460 (390-580)	590 (480-720)	520 (415-600)

MVPA = moderate to vigorous physical activity, EE = energy expenditure, SB = sedentary behaviour; *cleaned data

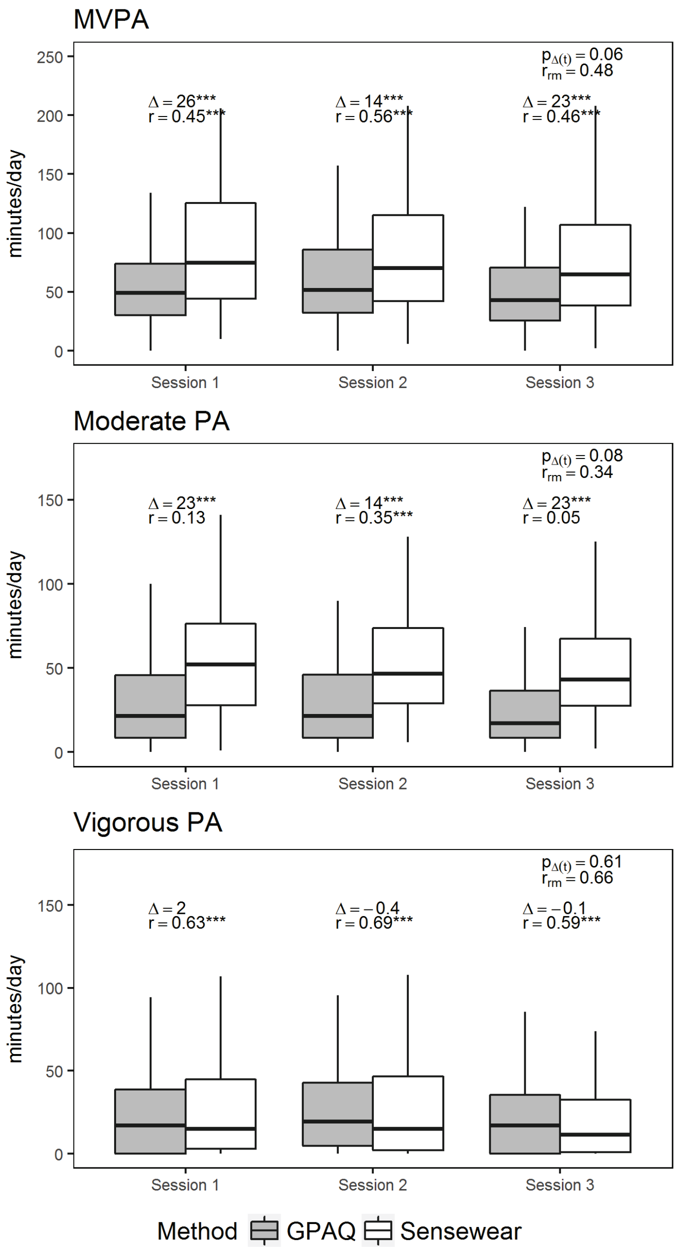


Figure S 1 Boxplots of MVPA time, moderate- and vigorous physical activity (PA) time per measurement method and session. Δ= the mean difference between both methods per session (tested for significance using the Wilcoxon signed rank sum test); r = the Spearman correlation coefficient per session; r_{rm} = the overall Spearman correlation adjusted for repeated measures (rm); $p_{\Delta(t)}$ = the p-value of the effect of session in the Δ(t) model which indicates if the difference between GPAQ and SenseWear measurements changes over time or sessions. Statistical significance is expressed as *p<0.05, **p<0.01, and *** p<0.001

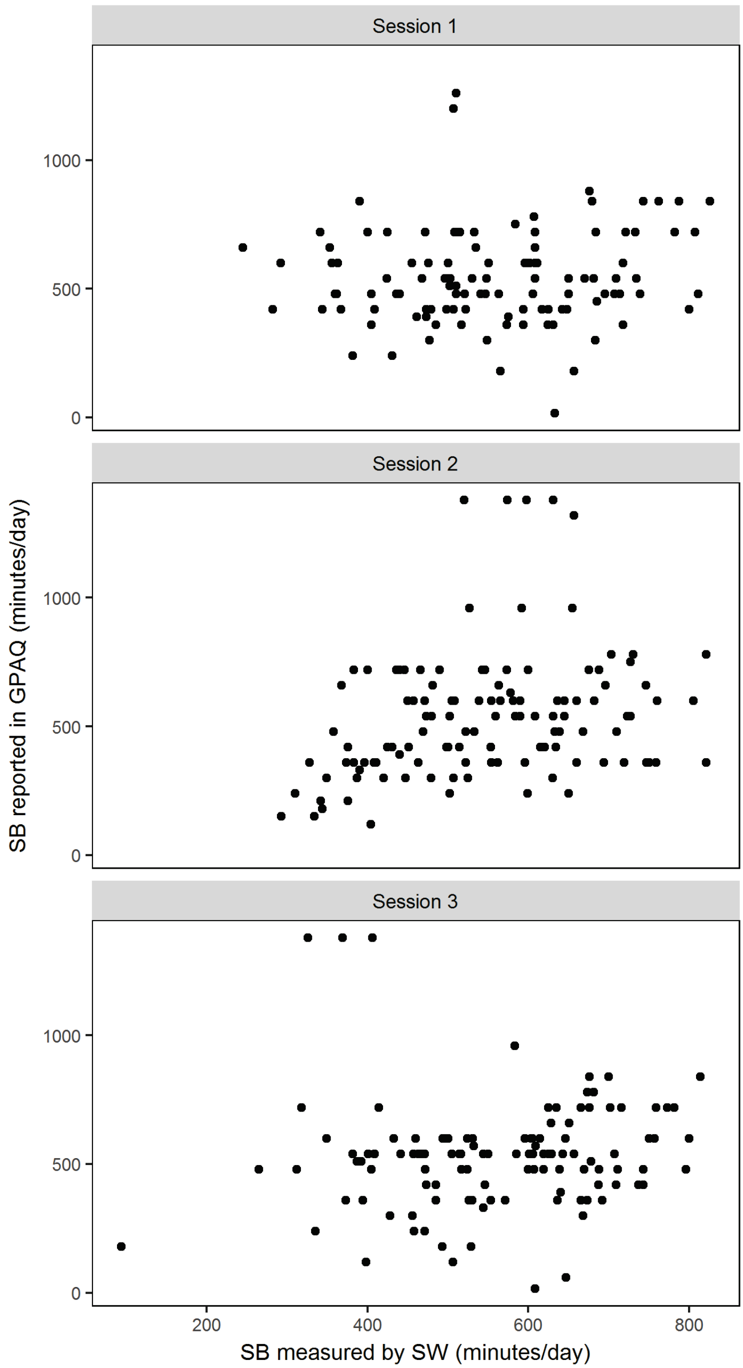


Figure S 2 Sedentary minutes measured by the GPAQ in function of sedentary behaviour (SB) measured by the SenseWear. The Spearman correlation coefficients for session 1, session 2 and session 3 are respectively 0.09, 0.25 and 0.24 (overall r_{rm} = 0.12). SW = SenseWear

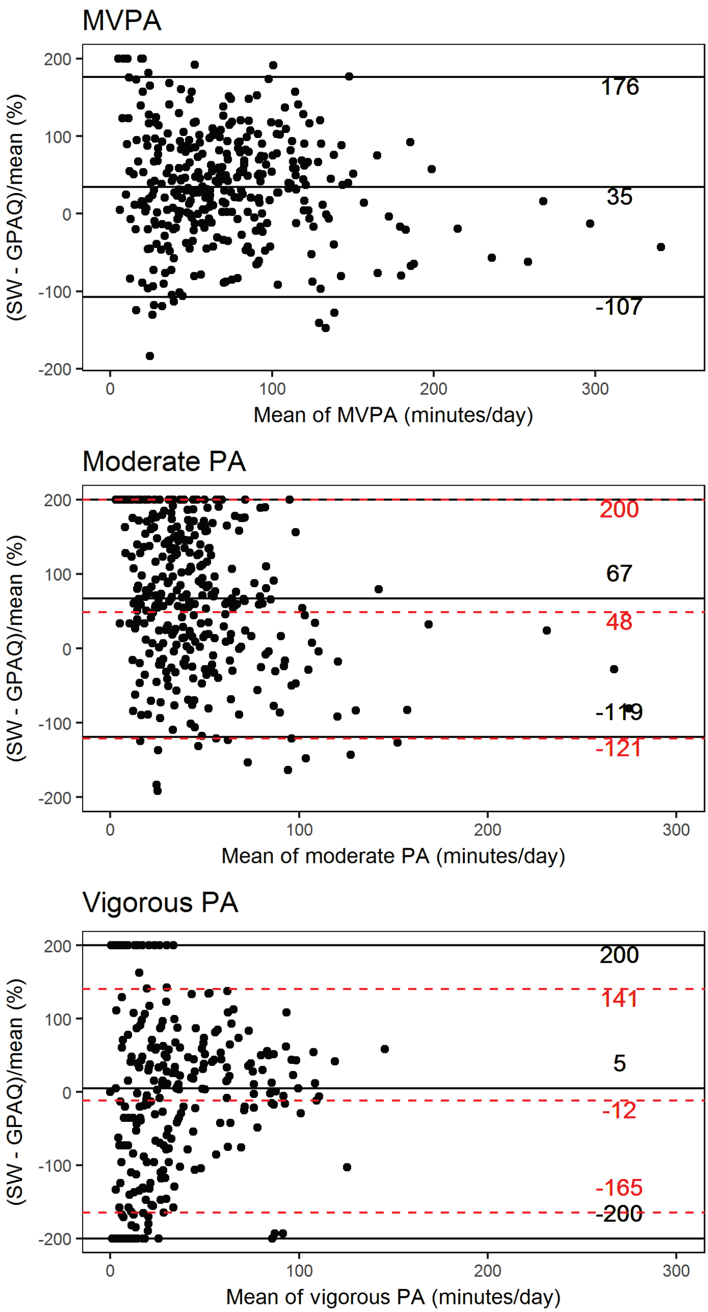


Figure S 3 Bland-Altman plots comparing MVPA, moderate and vigorous time (minutes/week) measured by the SenseWear armband (SW) and the GPAQ. All percentage differences on the Y-axis are calculated by subtracting GPAQ from SenseWear results divided by their average. Moderate and vigorous intensity activities included influential observation. The red, dashed lines represent the mean difference and 95% limits of agreement excluding these observations. PA = physical activity.

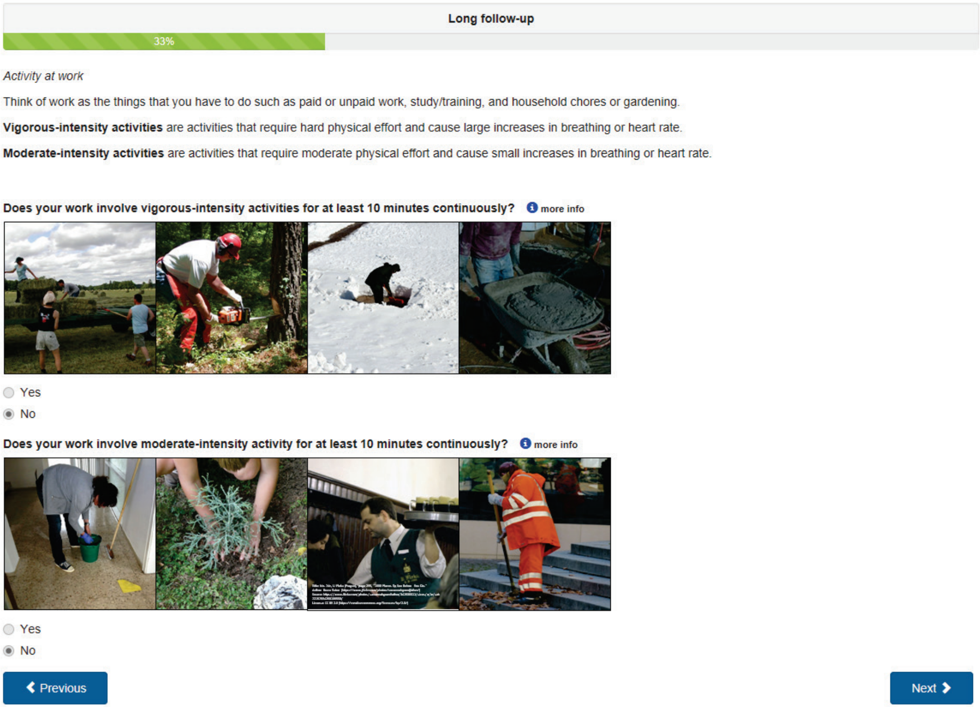
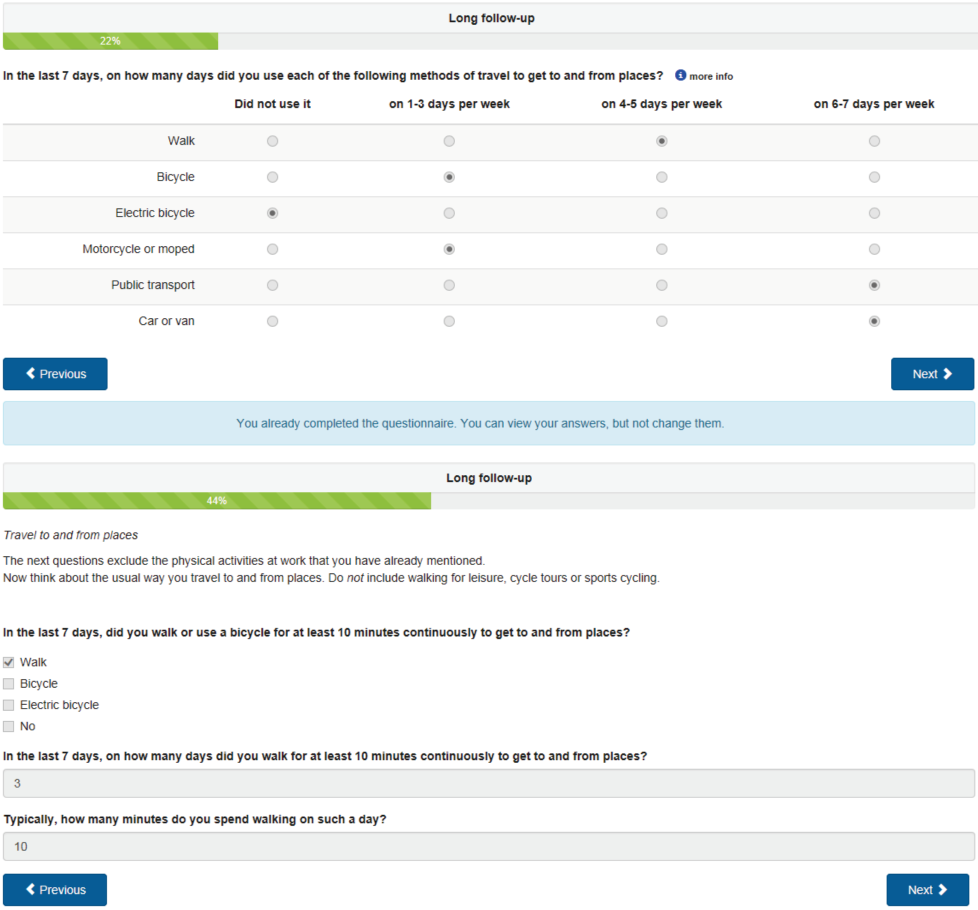


Figure S 4 The Global Physical Activity Questionnaire (GPAQ) used in the PASTA project to collect self-reported physical activity levels during the previous week. The GPAQ was adjusted to capture information on walking, cycling and e-biking trips separately. The results were compared to the SenseWear armband.



Long follow-up

22%

In the last 7 days, on how many days did you use each of the following methods of travel to get to and from places? more info

	Did not use it	on 1-3 days per week	on 4-5 days per week	on 6-7 days per week
Walk	○	○	●	○
Bicycle	○	●	○	○
Electric bicycle	●	○	○	○
Motorcycle or moped	○	●	○	○
Public transport	○	○	○	●
Car or van	○	○	○	●

Previous

Next

You already completed the questionnaire. You can view your answers, but not change them.

Long follow-up

44%

Travel to and from places

The next questions exclude the physical activities at work that you have already mentioned.
Now think about the usual way you travel to and from places. Do *not* include walking for leisure, cycle tours or sports cycling.

In the last 7 days, did you walk or use a bicycle for at least 10 minutes continuously to get to and from places?

- ☑ Walk
- ☐ Bicycle
- ☐ Electric bicycle
- ☐ No

In the last 7 days, on how many days did you walk for at least 10 minutes continuously to get to and from places?

3

Typically, how many minutes do you spend walking on such a day?

10

Previous

Next

Figure S 4 The Global Physical Activity Questionnaire (continued)

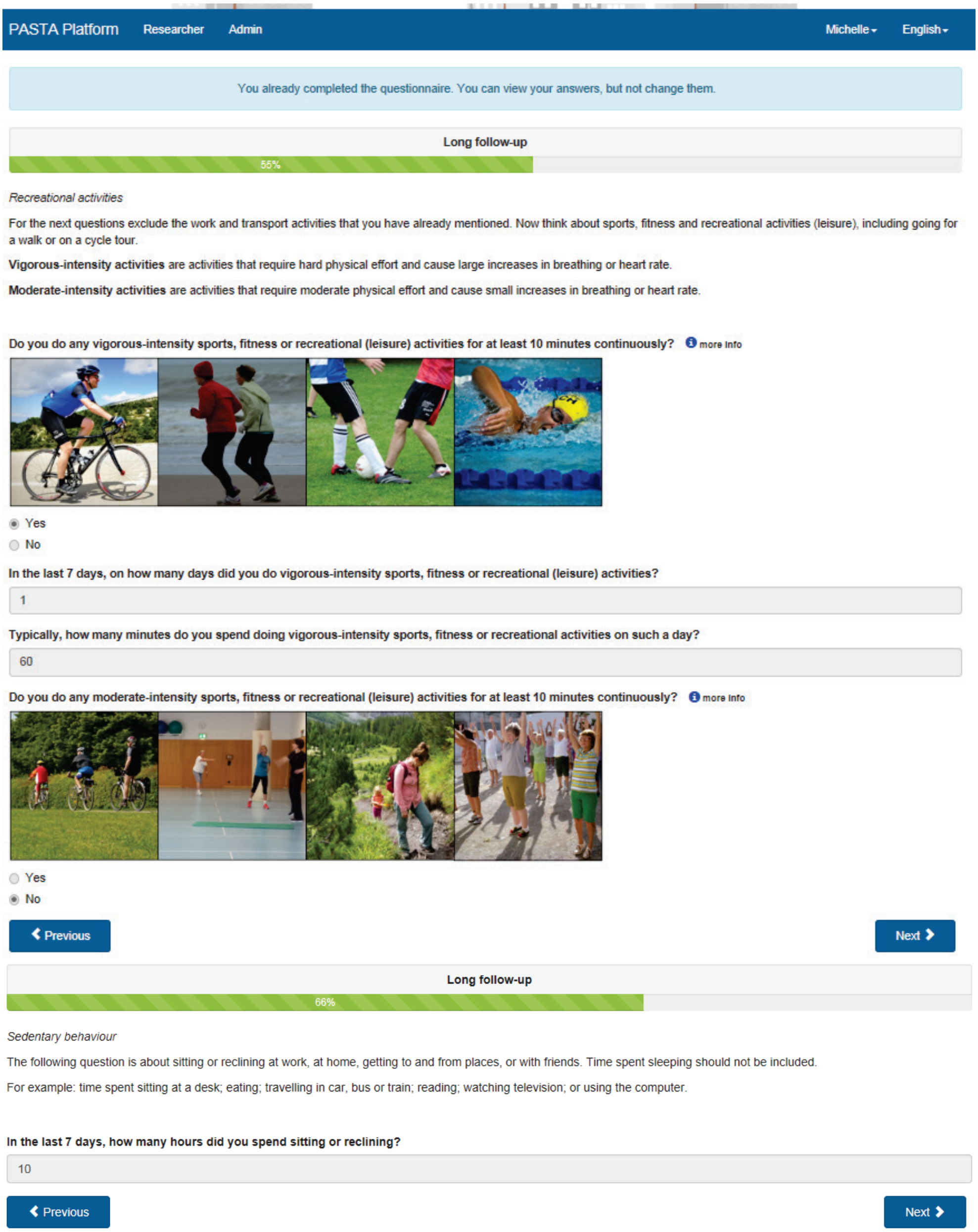


Figure S 4 The Global Physical Activity Questionnaire (continued)

Supplemental material: Chapter 4

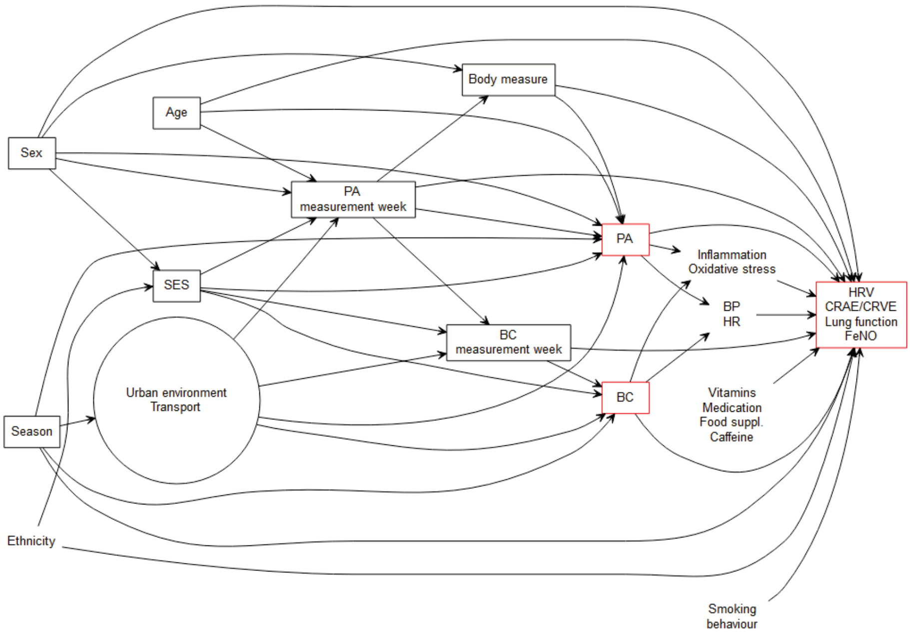


Figure S 5 DAG (directed acyclic graph) to identify confounding variables in the relationship between physical activity (PA), black carbon (BC) and the biomarkers (red boxes). The urban environment is included as a common cause of physical activity level and BC exposure. Boxed variables represent confounders that were identified to use in the minimal adjustment set; unboxed variables are confounders that were not identified for the minimal adjustment set. Education level is used as a proxy for socio-economic status (SES); Body measure = BMI or height, depending on the outcome; BP = blood pressure; HR = heart rate

Table S 2 Estimated effects of physical activity (METhours), BC (µg/m³, log-transformed) and their interaction on the subclinical outcomes in the 2-hour exposure window. Estimates were obtained using mixed effect regression analysis (n=122). The first column indicates the predictor and the model in which the respective estimates were obtained. Bold estimates have a p-value <0.05; bold, underlined estimates have a p-value <0.01. C = confounders (sex, age, BMI or height in case of lung function biomarkers, season, education level, physical activity during the whole week (total METhours) and BC during the whole week (average concentration in µg/m³)).

2-hour time window	**Outcomes (β (SE))**										
	HRV				Retinal microvasculature		FeNO	Lung function			
Predictor	SDNN (ms, log)	rMSSD (ms, log)	HF (ms², log)	LF/HF (log)	CRAE (µm)	CRVE (µm)	FeNO (ppb, log)	FEV1 (mL)	FVC (mL)	FEV1/ FVC	PEF (mL/s)
physical activity (METhours)											
PA	-0.01 (0.02)	-0.04 (0.02)	-0.06 (0.04)	<u>**0.08****</u> <u>**(0.02)**</u>	-0.16 (0.26)	0.26 (0.33)	0.02 (0.02)	**15.63*** **(6.46)**	5.02 (7.57)	**0.23*** **(0.09)**	1.53 (25.03)
PA+C	-0.02 (0.02)	-0.04 (0.02)	-0.06 (0.04)	<u>**0.08****</u> <u>**(0.03)**</u>	-0.16 (0.30)	0.42 (0.39)	0.02 (0.02)	14.63 (7.55)	6.14 (8.83)	0.20 (0.10)	-24.60 (28.31)
PA+BC	-0.01 (0.02)	-0.04 (0.02)	-0.06 (0.04)	<u>**0.08****</u> <u>**(0.03)**</u>	-0.29 (0.31)	-0.14 (0.37)	0.03 (0.02)	13.65 (7.53)	5.31 (8.82)	0.15 (0.11)	-16.13 (29.15)
PA+BC+C	-0.02 (0.02)	-0.05 (0.03)	-0.07 (0.05)	<u>**0.07****</u> <u>**(0.03)**</u>	-0.29 (0.32)	0.05 (0.39)	0.03 (0.02)	13.66 (7.80)	6.28 (9.06)	0.17 (0.11)	-26.09 (29.46)
PAxBC	-0.01 (0.02)	-0.03 (0.03)	-0.05 (0.06)	0.06 (0.03)	-0.29 (0.37)	-0.23 (0.45)	$3x10^{-3}$ (0.02)	2.63 (9.08)	-10.07 (10.61)	0.21 (0.13)	-3.46 (35.45)
PAxBC+C	-0.02 (0.02)	-0.04 (0.03)	-0.05 (0.06)	0.05 (0.03)	-0.34 (0.39)	0.02 (0.47)	$2x10^{-4}$ (0.02)	2.57 (9.40)	-9.55 (10.85)	0.24 (0.13)	-7.91 (35.81)

BC (µg/m³, log)											
BC	**-0.06***	**-0.11***	**-0.19***	0.04	0.28	0.41	0.03	5.30	2.84	0.16	**-134.17****
	(0.03)	**(0.04)**	**(0.08)**	(0.05)	(0.48)	(0.59)	(0.03)	(11.81)	(13.81)	(0.17)	**(45.49)**
BC+C	-0.04	-0.08	-0.14	0.06	0.61	0.47	0.02	4.30	-1.60	0.24	**-102.19***
	(0.03)	(0.04)	(0.08)	(0.05)	(0.53)	(0.65)	(0.03)	(13.06)	(15.12)	(0.19)	**(49.35)**
PA+BC	-0.06	-0.09	**-0.19***	0.05	0.38	0.57	0.01	2.49	-0.03	0.15	**-129.22****
	(0.03)	(0.04)	**(0.08)**	(0.04)	(0.51)	(0.61)	(0.03)	(12.58)	(14.76)	(0.18)	**(48.61)**
PA+BC+C	-0.03	-0.06	-0.12	0.04	0.73	0.61	4x10^{-3}	1.53	-6.37	0.25	-87.42
	(0.03)	(0.04)	(0.08)	(0.05)	(0.57)	(0.69)	(0.03)	(13.83)	(16.07)	(0.20)	(52.50)
PAxBC	-0.06	-0.09	-0.17	0.02	0.38	0.42	-0.03	-16.62	-26.81	0.25	-107.30
	(0.03)	(0.05)	(0.09)	(0.05)	(0.62)	(0.75)	(0.04)	(15.41)	(18.01)	(0.22)	(59.87)
PAxBC+C	-0.03	-0.06	-0.11	0.01	0.65	0.56	-0.04	-16.33	-31.88	0.37	-58.91
	(0.03)	(0.05)	(0.09)	(0.06)	(0.67)	(0.80)	(0.04)	(16.19)	(18.72)	(0.23)	(61.34)
PAxBC (log(BC))											
PAxBC	1x10^{-3}	-7x10^{-3}	-0.02	0.03	-7x10^{-4}	0.12	0.04	**15.67***	**21.88***	-0.09	-18.16
	(0.02)	(0.03)	(0.05)	(0.03)	(0.31)	(0.36)	(0.02)	**(7.37)**	**(8.60)**	(0.11)	(28.88)
PAxBC+C	2x10^{-3}	-6x10^{-3}	-0.02	0.03	0.07	0.05	**0.04***	**15.45***	**22.07***	-0.10	-25.42
	(0.02)	(0.03)	(0.04)	(0.03)	(0.31)	(0.37)	**(0.02)**	**(7.39)**	**(8.53)**	(0.11)	(28.34)

Table S 3 Estimated effects of physical activity (METhours), BC (µg/m³, log-transformed) and their interaction on the subclinical outcomes in the 24-hour exposure window. Estimates were obtained using mixed effect regression analysis (n=122). The first column indicates the predictor and the model in which the respective estimates were obtained. Bold estimates have a p-value <0.05; Bold, underlined estimates have a p-value <0.01. C = confounders (sex, age, BMI or height in case of lung function biomarkers, season, education level, physical activity during the whole week (total METhours) and BC during the whole week (average concentration in µg/m³))

24-hour time window	Outcomes (β (SE))										
	HRV				Retinal microvasculature		FeNO	Lung function			
Predictor	SDNN (ms, log)	rMSSD (ms, log)	HF (ms², log)	LF/HF (log)	CRAE (µm)	CRVE (µm)	FeNO (ppb, log)	FEV1 (mL)	FVC (mL)	FEV1/FVC	PEF (L)
physical activity (METhours)											
PA	2x10^{-3} (4x10^{-3})	1x10^{-3} (6x10^{-3})	-0.01 (0.01)	0.01 (6x10^{-3})	-0.08 (0.07)	-0.16 (0.09)	6x10^{-3} (4x10^{-3})	2.43 (1.88)	1.55 (2.17)	-7x10^{-3} (0.03)	1.92 (7.33)
PA+C	2x10^{-3} (5x10^{-3})	3x10^{-3} (8x10^{-3})	-0.01 (0.01)	0.01 (7x10^{-3})	-0.07 (0.09)	-0.1 (0.11)	7x10^{-3} (5x10^{-3})	2.96 (2.20)	2.93 (2.52)	-7x10^{-3} (0.03)	-3.3 (8.43)
PA+BC	2x10^{-3} (4x10^{-3})	1x10^{-3} (6x10^{-3})	-9x10^{-3} (0.01)	0.01 (6x10^{-3})	-0.09 (0.07)	-0.18 (0.09)	6x10^{-3} (4x10^{-3})	2.30 (1.90)	1.77 (2.21)	-0.02 (0.03)	-0.15 (7.51)
PA+BC+C	2x10^{-3} (5x10^{-3})	3x10^{-3} (8x10^{-3})	-0.01 (0.01)	0.01 (8x10^{-3})	-0.08 (0.09)	-0.10 (0.11)	8x10^{-3} (5x10^{-3})	3.01 (2.19)	2.83 (2.52)	-5x10^{-3} (0.03)	-2.75 (8.59)
PAxBC	2x10^{-3} (4x10^{-3})	-2x10^{-3} (6x10^{-3})	-0.01 (0.01)	**0.01*** **(7x10^{-3})**	-0.07 (0.08)	-0.17 (0.10)	5x10^{-3} (5x10^{-3})	1.34 (2.02)	1.63 (2.36)	-0.03 (0.03)	-4.18 (7.99)
PAxBC+C	8x10^{-4} (6x10^{-3})	-6x10^{-4} (8x10^{-3})	-0.01 (0.01)	0.01 (8x10^{-3})	-0.06 (0.09)	-0.08 (0.12)	6x10^{-3} (6x10^{-3})	2.15 (2.32)	3.09 (2.68)	-0.03 (0.03)	-5.73 (9.11)

BC (µg/m³, log)											
BC	9x10^{-3}	-0.01	-0.05	-0.01	0.61	0.65	-0.05	3.87	12.45	2x10^{-3}	**-128.13***
	(0.04)	(0.06)	(0.10)	(0.05)	(0.55)	(0.70)	(0.03)	(13.27)	(15.38)	(0.20)	**(53.15)**
BC+C	1x10^{-3}	-0.02	-0.06	-0.05	1.15	0.82	**-0.09***	8.67	15.65	0.06	-111.95
	(0.05)	(0.07)	(0.12)	(0.07)	(0.67)	(0.85)	**(0.04)**	(16.37)	(18.79)	(0.24)	(64.46)
PA+BC	8x10^{-3}	-0.01	-0.06	-0.02	0.66	0.58	-0.05	4.57	12.75	0.02	**-124.06***
	(0.04)	(0.06)	(0.10)	(0.05)	(0.56)	(0.69)	(0.03)	(13.58)	(15.72)	(0.20)	**(54.34)**
PA+BC+C	5x10^{-3}	-0.02	-0.06	-0.05	1.22	0.83	**-0.09***	8.69	14.69	0.08	-109.66
	(0.05)	(0.07)	(0.12)	(0.07)	(0.68)	(0.86)	**(0.04)**	(16.64)	(19.08)	(0.24)	(65.76)
PAxBC	-0.01	-0.09	-0.15	0.03	1.01	0.80	-0.07	-15.46	9.85	-0.35	**-209.84****
	(0.06)	(0.09)	(0.15)	(0.08)	(0.83)	(1.03)	(0.05)	(20.07)	(23.35)	(0.29)	**(80.20)**
PAxBC+C	-0.01	-0.08	-0.13	-0.01	1.55	1.22	-0.11	-6.98	19.43	-0.32	-164.27
	(0.06)	(0.09)	(0.15)	(0.09)	(0.90)	(1.14)	(0.05)	(21.84)	(25.17)	(0.31)	(86.08)
PAxBC (log(BC))											
PAxBC	2x10^{-3}	9x10^{-3}	0.01	-5x10^{-3}	-0.04	-0.03	2x10^{-3}	2.49	0.36	0.05	10.66
	(5x10^{-3})	(7x10^{-3})	(0.01)	(7x10^{-3})	(0.08)	(0.09)	(5x10^{-3})	(1.84)	(2.14)	(0.03)	(7.34)
PAxBC+C	2x10^{-3}	9x10^{-3}	9x10^{-3}	-5x10^{-3}	-0.04	-0.05	2x10^{-3}	2.07	-0.63	**0.05***	7.26
	(5x10^{-3})	(7x10^{-3})	(0.01)	(7x10^{-3})	(0.08)	(0.10)	(5x10^{-3})	(1.88)	(2.17)	**(0.03)**	(7.40)

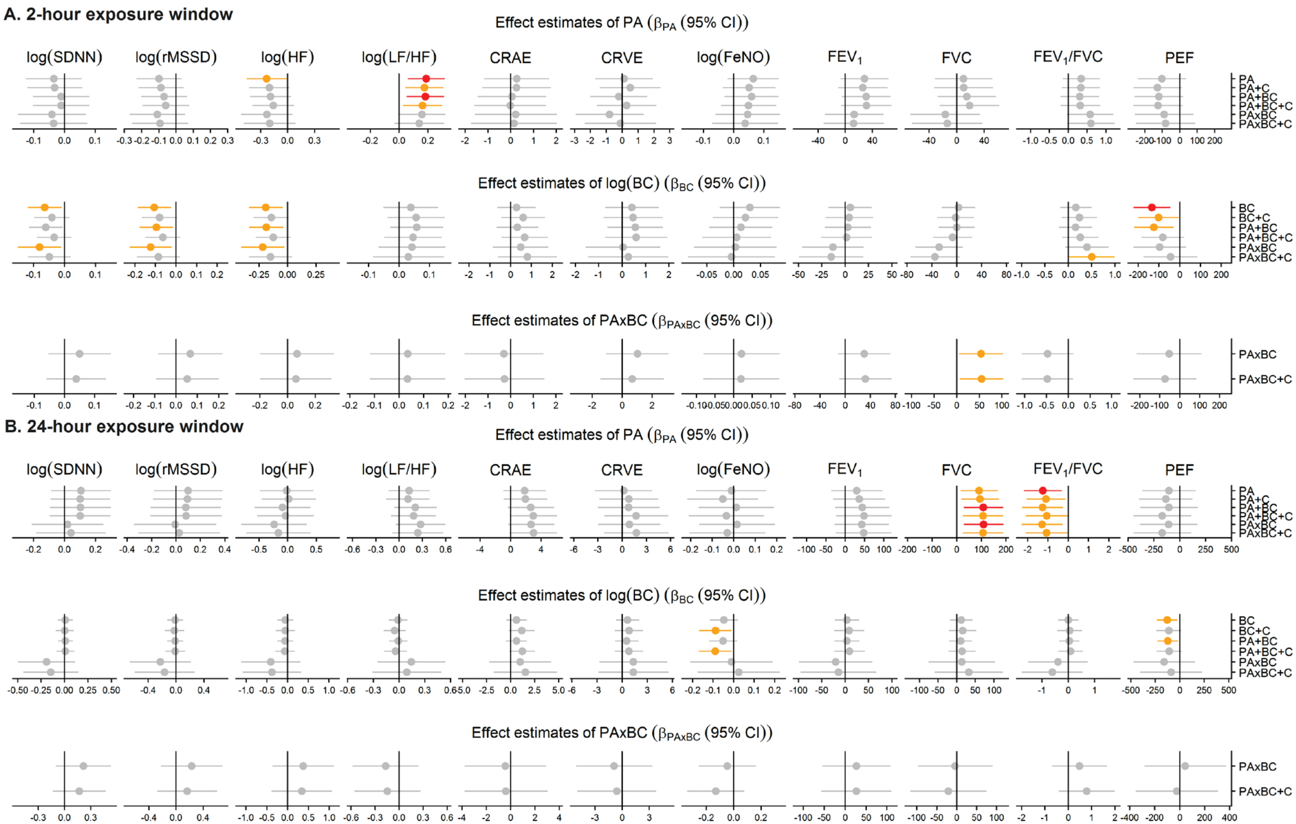


Figure S 6 Effect estimates of physical activity, logarithmic BC and their interaction (PAxBC) on the respiratory outcomes of (A) the 2-hour exposure window and (B) the 24-hour exposure window (based on the mixed effect regression analysis with physical activity as a categorical variable). β_{PA}, β_{BC} and β_{PAxBC} refer to the estimates of physical activity (reference category = 'no PA'), BC (average BC concentration in µg/m³, log-transformed) and their interaction respectively. The model where the respective estimate was observed is specified on the right. Orange-colored estimates have a p-value <0.05; red-colored estimates have a p-value <0.01. C = confounders (sex, age, BMI or height in case of lung function biomarkers, season, education level, physical activity during the whole week (total METhours) and BC during the whole week (average concentration in µg/m³)).

Supplemental material: General discussion

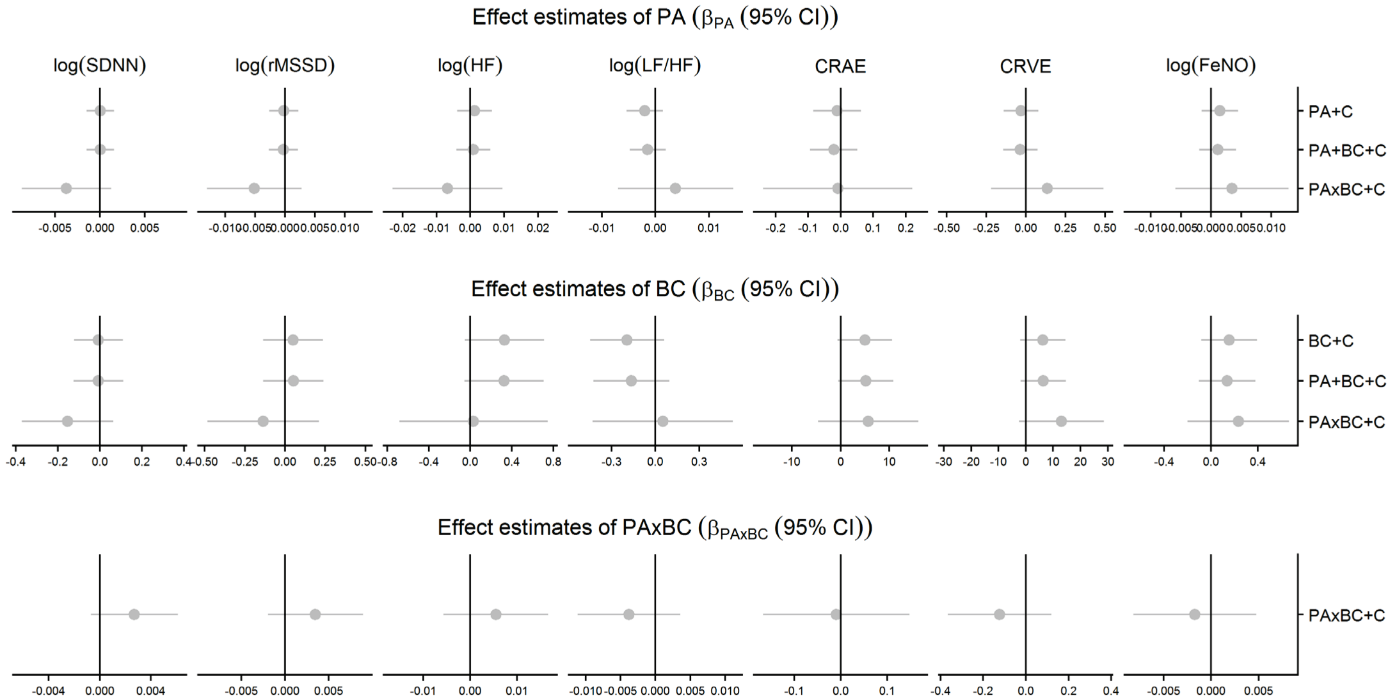


Figure S 7 Subchronic effects of physical activity (PA) and BC on HRV, the retinal vessel diameters (CRAE and CRVE) and FeNO (all p>0.1). Models resemble those described in the analysis of chapter 3. The PA+C and BC+C models include respectively physical activity and BC as exposure of interest. The PA+BC+C model includes both physical activity and black carbon and the PAxBC+C model also includes the interaction term. All models included random city effects and sex, age, BMI and level of education as confounders (C). Individuals who quit smoking less than 5 years ago are excluded (n=115).

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