HEALTH EFFECTS OF TRAFFIC-RELATED AIR POLLUTION AND PHYSICAL ACTIVITY

A real-world exposure experimental study

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“You never change things by fighting the existing reality. To change something, build a new model that makes the existing model obsolete.”

— R. Buckminster Fuller

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ABSTRACT

Background

Exposure to traffic-related air pollution (TRAP) has been associated with adverse health outcomes. Physical activity (PA) in polluted air may increase pollutant uptake and hence affect health. The main aim of this thesis is to determine the short-term health effects of TRAP in healthy participants and any possible modifying effect of PA.

Methods

A crossover real-world exposure study which compared in 28 healthy participants lung, systemic, and blood pressure responses to four different exposure scenarios: two hours of exposure in a high and low TRAP environment, each at rest and in combination with intermittent moderate PA, that comprised alternating 15-minute rest and cycling intervals. The data was analysed by using mixed effect models for repeated measures.

Results

High levels of TRAP induced airway and systemic inflammatory responses, as well as increased blood pressure. Physical activity improved lung function and attenuated the blood pressure increase. Physical activity in high levels of TRAP also induced lung and systemic inflammation, and decreased the circulating levels of the brain-derived neurotrophic factor (BDNF).

Conclusions

The short-term exposure to TRAP is associated with adverse health effects. Physical activity has beneficial effects on health, even when performed in high levels of TRAP. The clinical meaning of the decreases in BDNF as a response to the intermitted physical activity in the experimental setting and the more habitual physical activity in daily life remains unclear. This study also suggests that the health effects of traffic-related air pollution and physical activity are predominantly independent in high levels of TRAP.

RESUM

Antecedents

L'exposició a la contaminació de l'aire relacionada amb el tràfic (CART) s'ha associat amb resultats adversos per a la salut. L'activitat física (AF) realitzada en ambients amb aire contaminat pot augmentar l'absorció de contaminants i amb això produir efectes en la salut. L'objectiu principal d'aquesta tesi va ser determinar els efectes en la salut de l'exposició a la CART a curt termini en participants sans així com qualsevol possible efecte modificador de l'AF.

Mètodes

Estudi en disseny del creuament amb exposició del "món real" comparant en 28 participants sans les respostes pulmonars, sistèmics i de la pressió arterial en quatre escenaris diferents d'exposició: 2 hores d'exposició en un entorn d'alta i baixa CART, cadascun en repòs i en combinació amb AF moderada i intermitent, que consta de quatre intervals de 15 minuts de descans i ciclisme. Les dades es van analitzar utilitzant models d'efectes mixtes per a mesures repetides.

Resultats

Els nivells alts de CART van induïr una resposta inflamatòria sistèmica i de la via aèria, i un augment de la pressió arterial. L'AF va millorar la funció pulmonar i va atenuar l'augment de la pressió arterial. L'AF en alts nivells de CART també va induïr una inflamació pulmonar i sistèmica y una disminució dels nivells circulants del factor neurotròfic derivat del cervell (FNDC)[1].

Conclusions

L'exposició a curt termini a CART s'associa amb efectes adversos per a la salut. L'AF té efectes beneficiosos sobre la salut, encara quan es realitza en alts nivells de CART. El significat clínic de les disminucions en el FNDC com a resposta a l'AF intermitent en l'entorn experimental i l'AF més habitual de la vida quotidiana segueix essent poc clara. Aquest estudi també suggereix que els efectes sobre la salut de la CART i l'AF són predominantment independents dels nivells alts de CART.

[1] també conegut com "BDNF", de l'anglès "Brain-derived Neurotrophic Factor".

PREFACE

According to estimates released by the World Health Organization (WHO) in March 2014, **'**in 2012 around 7 million people died – one in eight of total global deaths – as a result of air pollution exposure.' And further, 'this finding (…) confirms that **air pollution is now the world's largest single environmental health risk**'. Moreover, the WHO concludes the release by saying that '**reducing air pollution could save millions of lives**' ("WHO | 7 Million Premature Deaths Annually Linked to Air Pollution" 2015).

Physical activity is an evidently health-promoting activity and increasingly recommended to maintain a healthy lifestyle. But then, what are the consequences on our health if we remain physically active - for instance by regular cycling- but get exposed to air pollution that just has been pointed out as 'the world's largest single environmental health risk'? Is physical activity in presence of air pollution still health promoting, as promoted, or rather hazardous to our health?

This thesis elaborates on this question and aims to explore the interdependencies in the health effects of physical activity in a potentially harmful environment, such as in high levels of outdoor air pollution found next to main roadways. The novelty of this study lies in its unique study design, which facilitates disentangling the effects of physical activity and those of traffic-related air pollution in a 'real-world' exposure setting.

This thesis has been written at the Centre for Research in Environmental Epidemiology (Barcelona, Spain) between 2010 and 2015 and supervised by Dr. Mark Nieuwenhuijsen. It presents the work of the study entitled '**TAPAS Experimental'** which was conducted as part of this thesis within the framework of the European project called ***TAPAS*** *(**T**ransportation, **A**ir pollution and **P**hysical **A**ctivite**S**; An integrated health risk assessment programme of climate change and urban policies)*. Project TAPAS was initiated and coordinated by Mark J Nieuwenhuijsen (PI) and Audrey de Nazelle, in collaboration with Michelle Mendez, Judith Garcia-Aymerich, Jan-Paul Zock, Josep M. Antó, and collaborating partners in Paris, Basel, Copenhagen, Prague

and Warsaw, and funded by the Coca-Cola Foundation. The purpose of this project was to help decision-makers to design urban policies that would address climate change and promote other health-related outcomes.

This work comprises a compilation of scientific publications in agreement with the regulation of the Doctoral Programme in Biomedicine in the Department of Experimental and Health Science at Pompeu Fabra University in Barcelona. This thesis includes an abstract, a general introduction, and the rationale and the objectives of the project "TAPAS Experimental", as well as the methods used in this study, and the results as a compilation of three research publications. It closes with an overall discussion section and final conclusions.

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1. INTRODUCTION

1.1. Real-world traffic-related air pollution

Air pollution is one of the most important environmental and public health issues (Chen and Kan 2008). The World Health Organisation ranked urban outdoor air pollution among the 19 leading global risks for mortality affecting countries across all income groups (World Health Organization 2009). During the last few years a range of observational and experimental studies have emerged, showing adverse health effects, such as increased morbidity and mortality, associated with both short and long-term exposure to air pollutants (Hoek et al. 2013; Peters et al. 2012; Raaschou-Nielsen et al. 2012; Maynard et al. 2007; McCreanor et al. 2007; Beelen et al. 2014).

People spend a substantial proportion of their time in traffic. In Europe the average daily time is one to one-and-a-half hours (Kaur, Nieuwenhuijsen, and Colvile 2007). In Barcelona, Spain the average travel time spent in transit throughout the day is two hours (de Nazelle et al. 2013). Most of the journeys are made during rush hour when the traffic-related air pollution levels are the highest.

1.1.1. Monitoring of traffic-related pollution

Particulate matter (PM) is commonly used as indicator pollutant for fuel combustion and traffic-related air pollution (Chen and Kan 2008). Particulate air pollution is a mixture of contaminants from a range of sources such as smoke, fumes, soot, and other combustion by-products, as well as natural particles such as windblown dust, sea salt, pollen, and spores. It includes primary particles coming directly out of exhaust stacks and tailpipes. It can also include secondary particles such as sulphates and nitrates, which are formed by the condensation of vaporized materials or from the by-products derived from the oxidation of the atmospheric gases (Dockery 2009). The particles are identified by their aerodynamic diameters, for instance PM_{10} (aerodynamic diameter smaller than 10µm), $PM_{2.5}$ (aerodynamic diameter smaller than 2.5µm) and ultrafine particles (aerodynamic diameter smaller than 0.1µm). Another common surrogate of traffic-related pollution is NO_2. Most NO emitted by traffic rapidly transforms to NO_2,

which decreases exponentially with distance to the source (Singer 2004). NO_2, together with sunlight, contributes to the generation of tropospheric ozone concentrations. Black carbon/ elemental carbon (BC/EC) is considered an indicator for emissions from mainly diesel vehicles and it is rapidly gaining importance in health risk assessments (Janssen et al. 2011). Other gaseous pollutants that are used to determine outdoor air quality are carbon dioxide (CO_2) and carbon monoxide (CO).

1.2. Acute health effects of traffic-related air pollution

1.2.1. Health effects assessment in real-world conditions

It has been suggested that the exposure to single pollutants -for instance, particulate matters in exposure chambers- does not represent the adverse health effects of the simultaneous exposure to different pollutants, such as PM and gaseous co-pollutants, and their possible synergistic interactions that are usually found in the environments (Y.-C. T. Huang et al. 2012). There is evidence that ambient factors can potentiate the adverse health responses compared with those shown in chamber studies (Spektor et al. 1988). However, most of the previous investigations have been performed in controlled chamber studies. Only a few studies have simultaneously assessed the exposures and acute health markers associated with traffic-related air pollution under real-world conditions, which involves the simultaneous exposure to multiple pollutants.

1.2.2. The importance of ultrafine particles in health effects assessments

It is well established that exposure to particulate matter of varying size fractions has toxic effects (Pope and Dockery 2006). Epidemiological studies show significant positive relations between air pollutions particles with a 50% cut-off in aerodynamic diameter of 10µm (PM_{10}) or less and increased morbidity and mortality. Ultrafine particles are of particular interest when exploring the health effects of particulate air pollution. UFP may be more hazardous because of their small size as well as their capability to depose in the lower respiratory tract and cross the air-blood barrier, thus entering the systemic circulation (Daigle et al. 2003; Nemmar et al. 2002).

1.2.3. The multitude of acute health effects associated with short-term exposure to TRAP with and without physical activity

Exposure to ambient air pollution has been associated with a range of adverse health effects such as subclinical effects in blood biomarkers, and physiological changes in pulmonary functions and the cardiovascular system. Clinical symptoms and hospital admissions, outpatient and emergency-room visits and premature death have also been associated with exposure to TRAP. A few epidemiology studies have found that exposures occurring during the short-term commuting or other outdoor activities may lead to clinical health outcomes due to short bursts of pollutants intake, which could have telling health impacts (Peters et al. 2004; von Klot et al. 2008).

There is a body of research to determine the effects of air pollution on acute health markers in controlled experimental settings. The majority of the recent evidence focuses on respiratory and cardiovascular effects associated with short and long-term exposures to air pollution. Sub-groups under high-risks to the TRAP effects include young children, elderly people, and persons with pre-disposed diseases such as asthma.

Most of such studies that have used relatively non-invasive methods have shown the effects of short-term exposures on lung function, airway responsiveness, and respiratory symptoms mostly in asthmatics, although a few also demonstrated such effects in healthy subjects too (McCreanor et al. 2007; Strak et al. 2010). More invasive controlled experimental studies have demonstrated the impacts of short-term particulate exposure in healthy adults (e.g. lung inflammation, depletion of airway antioxidant defence, and impairment of vascular and fibrinolytic functions), (Mills et al. 2005; Stenfors et al. 2004; Behndig et al. 2006).

<u>Effects of traffic-related air pollution in combination with physical activity</u>

One notable study that simultaneously assessed exposures and acute health markers associated with traffic-related air pollution under real-world conditions is the Oxford Street study. In this study asthmatics had pulmonary function tests performed before and after walking for two hours along a heavy-traffic street and a in a park (Hyde Park) in London, UK (McCreanor et al. 2007). The authors of this study detected FEV_1

impairments and airway acidosis after the exposure to predominantly diesel traffic. Strak and colleagues found non-significant FEV_1 decrements and an increased airway inflammation in healthy adults after cycling in TRAP (Strak et al. 2010). Besides pulmonary effects, systemic effects have been observed in real-world air pollution studies involving physical activity. Vinzents et al. studied oxidative DNA damage in 15 healthy subjects after their exposure to ultrafine particles (UFP) while cycling and found that such particles were significant predictors of purine oxidation in DNA (Vinzents et al. 2005). Broeckaert and colleagues showed in healthy subjects, after cycling in an episode of photochemical smog (Ozone) in Italy, an increase of Clara Cell 16 protein (CC16) in serum, a marker indicating increased lung epithelial barrier permeability (Broeckaert et al. 2000). More recently, systemic effects in relation to TRAP were observed Bos and colleagues. A 12-week aerobic training program in an urban environment was - besides an association with exhaled Nitric Oxide - also associated with increases in blood neutrophils and leucocytes (Inge Bos et al. 2013). A Canadian crossover study recently showed ozone (O_3) associated increases in systolic and diastolic blood pressure, and $PM_{2.5}$ associated decreases in heart rate variability (HRV) in healthy women after cycling for two hours on different traffic routes in Montreal (Weichenthal, Hatzopoulou, and Goldberg 2014). Moreover, the exposure to TRAP may alter physiological responses such as the exercise-induced release of brain-derived neurothrophic factor (BDNF), a neurothrophin playing a key role in cognition. A Belgium study showed increased serum BDNF concentration after cycling in an air-filtered room but not after cycling in TRAP (I Bos et al. 2011).

Effects of traffic-related air pollution in resting condition

A Swedish study with asthmatic subjects sitting in a car in a street tunnel indicated an association between NO_2 and FEV_1 change. Spirometry was measured before and during the exposure, and after an allergen challenge (Svartengren et al. 2000). Zuurbier and colleagues compared the health effects in different travel modes. Healthy volunteers travelled by bus, car and bicycle. A with soot and particle number counts (PNC) associated increase in airway inflammation marker FeNO (Fraction of exhaled nitric oxide) could be observed in bus and car drivers (Zuurbier et al. 2010). In an experiment conducted in a tunnel in Sweden changes in nuclear expression in bronchial epithelium

collected by means of a bronchoscopy and biopsy were detected (Larsson et al. 2007). In a study by Adar et al., elderly subjects free of specific cardiac disorders but often overweighed and partly asthmatics and COPD patients travelled by bus. An increased airway inflammation, indicated by increases in FeNO, and changes in heart rate variability were observed post-exposure (Adar, Adamkiewicz, et al. 2007; Adar, Gold, et al. 2007). More recently, associations between TRAP and heart rate variability (HRV) indices could be observed also in a Chinese study in young healthy adults. Moreover, the authors showed effect amplification through high noise levels (J. Huang et al. 2013). An association of in-vehicle $PM_{2.5}$ and changes in blood biomarkers (lymphocytes, neutrophils, C-reactive protein, von Willebrand factor), as well as in electrocardiographic markers, ectopic beats and also heart rate variability, could be observed after the working shift of highway patrol troopers (Riediker et al. 2004). Dales and colleagues showed through measurements of flow-mediated vasodilatation (FMD) impairments in microvascular vasodilatation capacity in healthy volunteers after sitting for two hours at bus stops in Ottawa (Dales et al. 2007). Moreover, the exposure to traffic-related air pollution exposure has been linked to adverse effects on the brain such as neuroinflammation and neuropathology (Guxens and Sunyer 2012; Genc et al. 2012; Calderón-Garcidueñas et al. 2014).

1.3. Mechanisms of health effects through traffic-related air pollution

In the following section the entry ways of air pollutants, mainly those related to particulate matter, are presented along with hypothetical pathways and mechanisms for their effects on different organ systems that are particularly relevant with respect with regard to the outcomes shown in this thesis.

1.3.1. Pulmonary system

The Inhalation of particulate matter components, particularly those which has been derived from combustion and carry ultrafine particles, transition metals, organic compounds (Mills et al. 2009; Brook et al. 2010) and potentially gaseous pollutants

(Brook et al. 2010), and generate oxidative stress, causes pulmonary inflammation, and lung damage (Pope and Dockery 2006), (Brook et al. 2010), (Simkhovich, Kleinman, and Kloner 2008; Riva et al. 2011; Valavanidis et al. 2013).

1.3.2. Systemic response

Direct translocation of ultrafine particles to the blood circulation: Systemic effects could be induced through a direct and rapid translocation of ultrafine particles and soluble compounds from the alveoli to the capillary circulation. The air pollutants enter directly in the blood stream, and similarly induce oxidative stress and pro-inflammatory responses such as reactive oxygen species (ROS), pro-inflammatory cytokines release, and the activation of white blood cells.

Indirect induction of a systemic response through a local oxidative stress production: Another hypothesis suggests the inhaled pollutants induce a production of reactive oxygen species (ROS) in the airways and lung alveoli promoting a local inflammatory reaction in the lungs as described above. This local oxidative stress reaction also leads to the activation of pro-inflammatory cytokines and white blood cells and their release into the blood stream (Pope and Dockery 2006; Simkhovich, Kleinman, and Kloner 2008; Brook et al. 2010).

1.3.3. Cardiovascular system

Based on experimental evidence, different interrelated biological mechanisms and pathways have been proposed for the short-term effects of air pollution on cardiovascular events that are presented in the following:

a) **Direct effect through autonomic nervous system (ANS) imbalance:** The inhalation of PM components may cause an immediate vascular responses mediated by the autonomic nervous system (ANS) imbalance due to pulmonary neural reflexes in lung receptors. Such immediate reactions lead to a vasoconstriction and endothelial dysfunction and also to increased BP levels and reduced heart rate variability (Brook et al. 2010).

b) **Direct translocation of ultrafine particles to the blood circulation:** After the particles enter the blood circulation, as described before, they could infiltrate in the arterial walls leading to a vasoconstriction and endothelial dysfunction (either directly or through oxidative stress reactions), and, in turn, lead to increased BP levels. Furthermore, the particles could also induce cardiovascular toxic effects leading to changes in the coronary flow or heart rate variability (Brook et al. 2010; Simkhovich, Kleinman, and Kloner 2008).

c) **Indirect through pulmonary and systemic inflammation:** As described earlier, the inhalation of air pollutants causes a pulmonary inflammation, which, in turn, leads not only to the release of systemic proinflammatory cytokines and white blood cells but also the release of vasculoactive molecules, such as endothelin, and reactive oxygen species affecting the vascular system.(Mills et al. 2009; Brook et al. 2010) This systemic reaction promotes endothelial vascular dysfunction, which, in turn, contributes to increases in blood pressure (Brook et al. 2009; Brook and Rajagopalan 2009).

d) **Altered bioavailability of nitric oxide in the vessels:** A fourth hypothesised pathway suggests that increases of blood pressure are mediated through reduced NO bioavailability in the vessels. Inhalation of diesel exhaust disturbs the normal vascular homoeostasis with enhanced NO generation, which is unable to compensate for the excess consumption (Langrish et al. 2013).

1.3.4. Central nervous system (CNS)

As described earlier, the exposure to traffic-related air pollution might have negative effects on the brain such as impaired cognition, and affect the secretion of neurotrophins such as brain-derived neurotrophic factor (BDNF) levels. Airborne particulate matter may translocate directly to the CNS or trigger the release of soluble inflammatory mediators and alter the susceptibility for neuroinflammation in the CNS, which results in neuropathology through a variety of pathways and mechanisms (Genc et al. 2012).

Findings suggest that an exercise-induced release of BDNF (see also following section 'Physical activity') is inhibited through the TRAP exposure (I. Bos et al. 2011) as a consequence of the suppression of basal gene expression of BDNF (I. Bos et al. 2012). Another reason for adverse effects on the brain could be the induction of oxidative stress. The brain is extremely vulnerable to such stress because of its high metabolic demands and energy use, as well as its low levels of endogenous scavengers such as vitamin C (MohanKumar et al. 2008).

1.4. Physical activity

In the following section the beneficial health effects and their mechanisms are presented. This section aims to provide a general introduction to health effects induced by physical activity, which are particularly relevant for the contents of this thesis rather than giving a comprehensive and complete overview.

1.4.1. Health significance of physical inactivity

The public health significance of physical inactivity is enormous. Physical inactivity has been identified as the fourth leading risk factor for global mortality (6% of deaths globally). This follows high blood pressure (13%), tobacco use (9%) and high blood glucose (6%). Overweight and obesity are responsible for 5% of global mortality (World Health Organization 2009). It is well established that physical inactivity increases the risk of chronic diseases such as diabetes type 2, cardiovascular diseases, cancer and dementia, and mental diseases such as depression and contributes to what is called the 'diseasome of physical inactivity'. Physical inactivity promotes inflammatory processes. The chronic low grade systemic inflammation is involved in the pathogenesis of a number of chronic diseases through adverse health effects such as insulin resistance and arteriosclerosis (Pedersen 2009).

1.4.2. Health benefits of physical activity and their mechanisms

It has been shown that regular physical activity reduces the risk of many adverse health outcomes (Hamer and Chida 2008; Harriss et al. 2009; Pate et al. 1995). Regular physical activity generally reduces risk factors such as regulating blood pressure (Chobanian et al. 2003)(Cornelissen and Fagard 2005)(Cornelissen et al. 2011).

However, the effects of short-term physical activity and chronic physical activity diverge. The acute immunological responses of physical activity, such as the induction of chemokines and cytokines, depend upon factors such as the intensity, the duration, and the regularity in which physical activity is performed. While chronic exercise leads to a reduction in chronic inflammation, acute exercise appears to promote a pro-inflammatory response. Kasapis et al. reviewed the literature with respect to the effects of physical activity on inflammatory makers and indicated that short-term exercise on a non-regular basis produces a short-term inflammatory responses, while a long-term 'habitual' physical activity might entail an 'anti-inflammatory' effect (Kasapis and Thompson 2005). Some of the beneficial effects of physical activity may be a consequence of its influence on inflammatory processes. Evidence suggests that the protective effect of exercise may be to some extent ascribed to the anti-inflammatory effects of regular exercise through the reduction in visceral fat mass and/or by the promoting an anti-inflammatory state (Pedersen 2009). In general, the immunological benefits of regular physical activity include improvements of the immune function and anti-inflammatory effects such as increased levels of anti-inflammatory cytokines, cytokine inhibitors, and chemokines (Phillips et al. 2014). Regular training for example, induces a suppressive effect upon acute phase protein C-reactive protein (CRP), when compared with the previously untrained control participants (Dufaux et al. 1984). Increased levels of CRP, which is synthesized primarily in the liver, adipose tissue, and vascular smooth muscle cells, usually indicate an increased inflammatory activity. A similar response was found in another inflammation marker, tumor necrosis factors-alpha (TNF-α), a cytokine which performs a variety of functions in the immune system, such as cell proliferation and differentiation, and cytolysis (Phillips et al. 2014). Circulating TNF-α levels decrease after regular moderate physical activity (Tsukui et al. 2000). The cytokine Interleukin-8, for example, tend to increase rather in trained

subjects after high-intensity exercise compared with sedentary participants (Landers-Ramos et al. 2014). Interleukin-6 is a cytokine acting as anti- but also pro-inflammatory mediator, depending on what triggered its release: circulating levels of interleukin-6 synthesized in the muscle (myokine) increase as a response to physical activity of moderate intensity. IL-6 in its function as a myokine is known to activate a cascade of anti-inflammatory immunological responses, including the release of interleukin-10 with each bout of exercise (Petersen and Pedersen 2005). In addition to the altered cytokines responses, the anti-inflammatory effect of physical activity may in part be explained through the alterations in the oxidative stress induction: physical activity acutely induces oxidative stress; however, long-term physical activity increases antioxidant defences through an increase of the antioxidant enzyme activity (Powers, Ji, and Leeuwenburgh 1999). Acute exercise has direct effects on the cellular innate immune function. Both, acute and chronic physical activity alter the number and function of circulating cells of the innate immune system, such as neutrophils, monocytes, and natural killer cells (Walsh et al. 2011). During and immediately after exercise, lymphocytosis has been observed, which is proportional to exercise intensity and duration, with the number of cells (T cells and to a lesser extent B cells) falling below pre-exercise levels during the early stages of recovery, before returning to resting values normally within 24 hours.(Walsh et al. 2011) Furthermore, it has also been suggested that acute exercise leads to an alteration in the neutrophil gene-expression (Radom-Aizik et al. 2008). Acute exercise results in an initial, rapid increase in the number of blood neutrophils as a catecholamine induced response. The first increase in blood neutrophils is followed by a second increase in blood neutrophils a few hours later, which is exercise intensity and duration related. This second later increase is likely to be induced by elevated cortisol stimulating the release of neutrophils from bone marrow (Walsh et al. 2011).

It has been suggested that acute physical activity has beneficial pulmonary effects. In healthy participants, acute exercise, also of light intensity, usually has bronchodilatory effects. The degree of bronchodilation appears to be dependent on the intensity of exercise, while the mechanisms, in particular those related with light exercise, are not

fully understood. A bronchodilation during vigorous exercise occurs most likely due to an activation of β2-receptors by endogenous catecholamines (Snyder et al. 2006).

Regular physical activity is supposed to improve endothelial functions, thereby preserving nitric oxide availability, possibly through the prevention of a preceding oxidative stress production (Taddei et al. 2000), which, in turn, may explain the beneficial regulatory effects of physical activity on blood pressure.

It has been suggested that physical activity generally has beneficial effects on the brain, promoting its plasticity, and improving cognitive functions as well as memory (Hötting and Röder 2013; Cotman and Engesser-Cesar 2002). Physical activity is promising as a non-pharmaceutical intervention for the prevention of age-related cognitive decline and neurodegenerative diseases (Bherer, Erickson, and Liu-Ambrose 2013). A key role in the underlying process, which leads to improved learning and memory in response to exercise, seems to be mediated by an exercise-induced increase of BDNF in the brain (Cotman and Berchtold 2002). Most, but not all, epidemiological studies suggest a positive association for physical activity and higher levels of BDNF. Among the neurotrophins, BDNF is the most widely expressed in the central nervous system (CNS) with a wide repertoire of functions such as regulating neuronal survival, migration, phenotypic differentiation, axonal and dendritic growth, and synapse formation (E. J. Huang and Reichardt 2001).

BDNF is synthesized in the periphery by vascular endothelial cells, T cells, B cells, monocytes (Nakahashi et al. 2000) and skeletal muscles (Mousavi and Jasmin 2006).
BDNF in the peripheral circulation is able to cross the blood-brain barrier bi-directionally, thus resulting in a direct exchange between BDNF levels circulating in blood plasma and in the brain (Pan et al. 1998).

1.5. Physical activity in traffic-related air pollution

Regular physical activity seems to provide protection against the adverse effects of air pollution. Wong et al. showed that habitual exercise may prevent premature death

attributable to air pollution (Wong et al. 2007). However, performing exercise in polluted air appears to cause adverse health effects, as indicated by several studies, especially in vulnerable subjects such as those with existing lung impairments (McCreanor et al. 2007).

It has been shown that outdoor exercise in air pollution could contribute to the development of asthma in children. McConnell and colleagues compared asthma incidences in children in a community with higher concentrations of ozone and found a higher asthma incidence associated with heavy exercise, compared with children in the same neighbourhood who exercise less (McConnell et al. 2002). A possible explanation may be that physical activity increases the volume of inhaled air and hence potentially the inhaled dose of airborne substances, such as pollutants, and also allergenic matter (Carlisle and Sharp 2001). In a study comparing different passive and active travel modes, the inhaled doses of all air pollutants were highest in cyclists. In an active travel mode, such as cycling, the minute ventilation was 2.1 times higher than that of car passengers and 2.0 times higher than that of bus passengers (Zuurbier et al. 2009).

1.5.1. Particle deposition during exercise

During physical activity, the deposition fraction, the fraction of inhaled particles that are retained in the lungs after inhalation, increase. Daigle et al. found that the total number of deposited ultrafine particles was more than 4.5 times higher during moderate exercise on a bicycle ergometer compared with rest, because of a combined increase in the deposition fraction and minute ventilation. Daigle and colleagues also showed that the level of lung deposition of the particles increased, the finer the particles were. The combination of increased particle intake and the high deposition of UFP in the alveolar region lead to a significantly higher UFP burden to the alveolar epithelium during exercise (Daigle et al. 2003).

2. RATIONALE AND OBJECTIVES

Previous experimental studies assessed the health effects of traffic-related air pollution in combination with or without physical activity, but they were not designed to explore the contribution of the physical activity in effects that were found to be associated with TRAP. Furthermore, previous study designs did not facilitate the assessment of the presence of a potential preventive effect against adverse responses to TRAP attributed to physical activity.

It remained to be determined what acute health responses could be attributed to the traffic-related air pollution exposure, which ones could be attributed to physical activity, and what health effects are caused by a potential interaction of both, PA and TRAP, as they occur in different active commute patterns.

The underlying hypothesis of this study was that

a) short-term exposure to traffic-related air pollution may cause health impairments in healthy adults; and

b) physical activity in traffic-polluted air, such as during active commuting, may modify such health effects.

The overall purpose of this study was to determine the effects on acute health markers in a sample of healthy adult population, following a short-term exposure to traffic-related air pollution under real-world conditions and the interdependencies with physical activity by collecting data that would allow disentangling the effects of each of the two factors TRAP and PA.

3. METHODS

This chapter describes the methods used in the TAPAS Experimental study, as they are the basis for this thesis. Contents relate to the

- study design, including the exposure sites;
- the study population, including their recruitment and screening process; and
- the on-site exposure, as well as the biomarkers and procedures for monitoring of the health effects.

This chapter also explores how contextual data about the participants' health status and their pre-exposures were collected.

3.1. Study design

The study was conducted in Barcelona, Spain, between February and November 2011 and followed a well-controlled crossover study design. The Ethics Review Committee of the 'Institut Municipal d'Investigació Mèdica (IMIM)' approved the study and all subjects gave written informed consent prior to participation.

Guiding principles with regard to in the design of the experimental study were to consider 'real-world' exposure conditions whenever possible; however, priority was given to the safety of the participants and the researchers. Furthermore, non-invasive biomarkers were to be preferred.

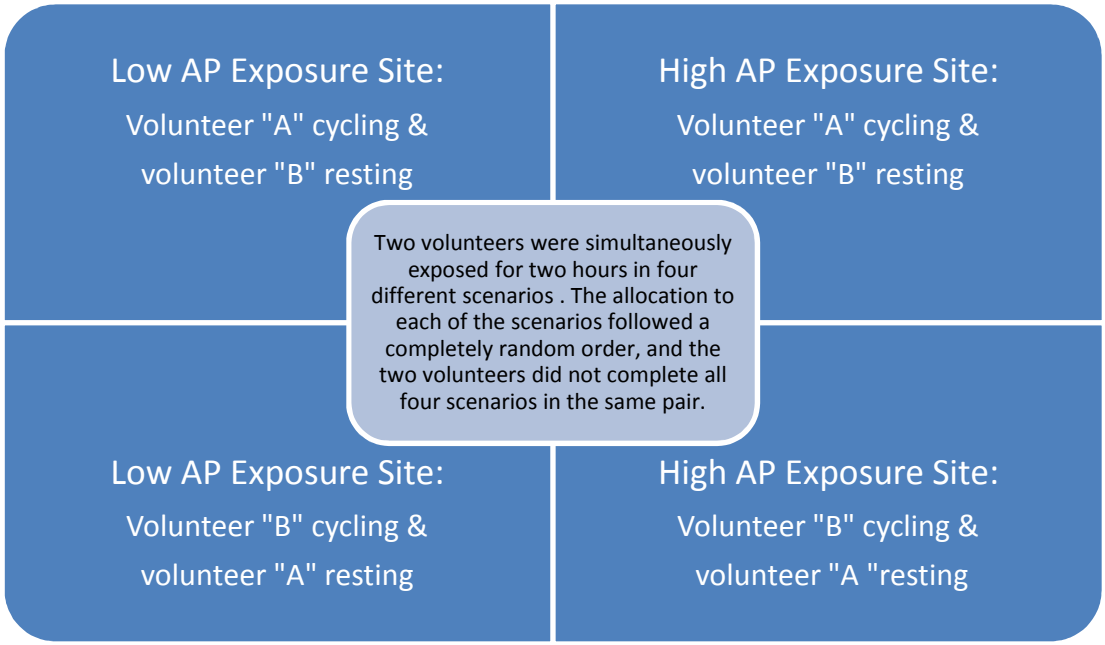
The study followed a crossover study design involving twenty-eight[2] healthy and non-smoking adults within the range of 18-60 years of age. Each volunteer served as his/her own control to exclude confounding by factors that are stable within an individual over time but vary between participants. To avoid a diurnal effect, all experiments and measurements were scheduled at the same time during the day. Thirty-one volunteers were exposed to either heavily polluted air (busy street in morning rush hour) or to

[2] Three volunteers had to be excluded from the statistical analysis because they did not participate in all exposure scenarios.

lower density traffic air pollution (control site). Two subjects were studied simultaneously on each occasion; one of them performed moderate physical activity by riding a stationary bicycle (intermittent 15 minutes cycling and 15 minutes break), while the other volunteer remained seated throughout the two hours. The time between the four exposure sessions for each volunteer was set to be at least four days. Volunteers were assigned randomly to the exposure scenarios. Each volunteer was to participate in all of the four following scenarios (**Figure 1**):

1. Physical activity in high air pollution exposure
2. Physical activity in lower air pollution exposure
3. Rest in high air pollution exposure
4. Rest in lower air pollution exposure

Figure 1. Crossover study design – The simultaneous exposure of two volunteers in four different scenarios



Each volunteer was expected to be exposed four times, resulting in 112 exposure occasions in total. To avoid a diurnal effect, all exposures and measurements were conducted at the same time during the day as well as during the same days of the week

to account for variations in traffic characteristics (Tuesdays and Thursdays during morning rush hours between 8 a.m. to 10 a.m.)

3.2. Physical activity intensity during the exposure sessions

Each volunteer was required to reach his/her individual range of moderate physical activity intensity, which was controlled through their heart rate monitored continuously by a fingertip pulse oximeter (Konica Minolta, Japan). For moderate-intensity physical activity, the volunteers´ target heart rate was 50% to 70% of their maximum heart rate, which was estimated for each volunteer on the basis of their age and sex (Gulati et al. 2010). The heart rate was constantly observed by the research team and the physical activity intensity was adjusted accordingly by adapting the participants´ pedalling speed, and in a few cases, the pedalling resistance. The participants were supposed to feel fine during all their 15-minute physical activity intervals. Therefore, in addition to the monitored heart rate, further indicators were constantly observed and inquired, such as muscular exertion (thigh or leg tiredness or pain), sweating, breathlessness, general physical tiredness and exertion, and general well-being.

3.3. Exposure and control sites

According to the principles of this study, to be close to real-world conditions, the exposure sites should be two places that are commonly used by Barcelona´s citizens. The high level TRAP exposure sessions took place on a bridge above a main roadway, – Ronda Litoral). As a 'control site' with lower levels of TRAP, a place in the 'Mercat de la Barceloneta' square was chosen. It's a place with market activities where people usually linger, walk and/or ride a bicycle; with considerably less motorized traffic compared with the traffic site. The safety of volunteers and research staff were respected and protected. The experimental exposures did not involve unusual risks since these are conditions and pollution levels that citizens potentially encounter during their activities in daily life or when commuting.

3.4. Participants´ pre- and post-experimental exposure

The volunteers 'pre-exposure' to physical activity and air pollution is a critical issue in this study design. To account for that, three days before each of the four experiment days, all the participants filled out a half-hourly **activity diary** asking for time, activity, location, travel mode, and perceived exposure to environmental tobacco smoke or other pollutants, which allowed us to estimate the volunteers' air pollutant pre-exposure and energy expenditure due to physical activity for the preceding three days. Additionally, at the beginning of each study day, the volunteers filled out a brief questionnaire asking about their current health status, food intake in the last 24 hours, and the mode and duration of travel to the study centre.

Furthermore, to keep minimal the exposure to volatile pollutants before the baseline measurements, the participants were required to arrive at the research centre in early morning hours (before 6:45 a.m.). Before this time, traffic activity is very low in Barcelona. Measurements of ultrafine particles in the underground in Barcelona showed relatively low (around 20,000pt/cc) levels.

The high density and low density traffic exposure sites were relatively close to each other in order to approximate pre-exposure. The unit where health measurements took place was located in the clinical research facility of the 'Biomedical Research Park Barcelona', which was in approximately 10 minute driving distance from both exposure sites. The volunteers were transported from the health research unit to the exposure sites by a car (taxi) and accompanied by one person from the research team.

To avoid additional exposures volunteers remained resting within the clinical research unit after their return from the exposure sites until all follow-up health measurements were completed.

3.5. The study population

It was meant to include a balanced group of volunteers in terms of age groups and sex. The male to female ratio was meant to be close to one.

3.5.1. Eligibility of participants

Participants were eligible if they were healthy, non-smoking adults in the age range of 18-60 years, and not exposed to potentially influencing airborne particles during working hours; **(Table 1).**

Table 1. Inclusion and exclusion criteria

Indicator	*Specific criteria*
Age	• Between 18 and 60 years
Smoking status	• Non-smokers or former smokers (smoking cessation minimum one year prior to the study)
Weight	• Normal weight and overweight people (BMI 18.5-29.9)
Health status	• No intake of any kind of medications* (two months before experiment start) • No chronic conditions diagnosed in the past, which could influence the health responses (e.g. diabetes, pulmonary, or cardiovascular diseases) • No symptoms of a recent (within the last month) or acute infection reported or observed • Not taking any medications (exception: birth control pills) during the last three months and during the experiment. • Normal pulmonary function (indicated by spirometry above the lower limit of normal for FEV_1 and the FEV_1/FVC ratio > 0.70 according the ERS/ATS COPD guidelines). • Females: Not-pregnant
Occupational exposure status	• No worksite exposure to industrial dust, fumes, and/or gases

*Volunteers reporting allergies were not excluded as long as antihistaminic treatment (e.g. antihistamines or corticosteroids) was not indicated during the last three months.

3.5.2. Recruitment and screening procedures

First contact was made with the potential participants was mainly via telephone or email. The first contact aimed at providing the potential participants with an outline of the study and facilitated the pre-selection of potential participants through exploring their temporal availability for participation, their personal and health-related data such as age, smoking status, first information about health status (e.g., chronic diseases), and medication intake. The potential participants were invited for a screening visit. All screening visits were conducted by a professional nurse who informed the participants in depth about the study procedures, potential risks, samples storage, and data protection, and also requested them to sign a statement of informed consent. The participants were asked to complete a detailed questionnaire asking about specific information regarding their health status, including acute and past health conditions, medications intake, physical symptoms, smoking history, diet and eating behaviour, physical activity, as well as home and work-related exposures. Furthermore, the participants' weight and height were measured, and a first spirometry was performed in order to test the eligibility, and to familiarise the volunteer with the procedure for the measurement of pulmonary function to assure a good-quality baseline measurement on the first experiment day. The participants were instructed not to start or to omit taking any vitamin or herbal supplementation, and to inform research personnel as soon as possible in case of any changes in health status or a new indication for medical treatment. Furthermore, the participants were instructed to abstain from alcohol for 24 hours (except one glass of wine or beer for dinner) and from caffeine-containing drinks for at least four hours before the baseline measurements. Furthermore, they were asked not to perform heavy exercises (such as extensive running) for three days preceding the exposure occasions. Also, the participants were informed to remove any kind of nail polish (or artificial nails) for the exposure sessions in order to ensure correct readings of the fingertip pulse oximeter. Moreover, the participants were provided with activity diaries and instructed how to fill them out during three preceding days before each of the four exposure occasions. The activity diaries were to complete on a half-hourly basis, and they included the participants´ activities, the location, in which the activities took place, travel times and modes, and the documentation of any perceived environmental exposures (environmental tobacco smoke etc.).

3.6. Health response monitoring

The selection of the used markers and their time points in the post-exposure follow-up were based on the findings of published previous controlled exposure studies, and epidemiological studies that focussed on short-term changes in exposures. In addition, the biological plausibility and sensitivity of potential markers and their response times were discussed with experts in the particular field of the marker concerned. Eventually, we appreciated operative interests such as logistics and costs for the final selection. Preference was given to non-invasive biomarkers.

The strands of the health response monitoring involved self-reported symptoms, pulmonary functions and -inflammation, cardiovascular effects, systemic inflammation, oxidative stress and lipid oxidation, differential blood cell count, blood cholesterols, transaminases, and gene expression in whole blood.

Health end-points were measured before and after exposure with the exception of cardiovascular outcomes and the monitoring of symptoms that also took place during the two-hour exposure; (**Table 2**).

Table 2. Timeline for monitoring health end-points

	Pre-exposure	*Intra-exposure*	*Post-exposure*					
	Baseline (1h)	**2 hours**	**<1h**	**2h**	**3h**	**4h**	**5h**	**6h**
Blood withdrawal	X		X					
Urine sample	X		X					
Spirometry	X		X	X		X		X
FeNO	X		X		X			X
Blood Pressure	X	X	X	X		X	X	
Symptoms Score		X	X	X		X		
Holter ECG	●	→	→	→	→	→	→	●
Pulsoximetry	●	→	→	→	→	→	→	●

All measurements were carried out in a quiet and temperature-controlled clinical unit for clinical trials within the Barcelona Biomedical Research Park building. As mentioned earlier in order to minimize further exposure to air pollution, all the participants remained inside the unit and in rather quiet conditions after the exposure session until all follow-up health measurements were completed.

Blood biomarkers

A blood sample was taken before and after exposure from all subjects. Blood samples were analysed besides biomarkers with a direct relation to main research interest, and in addition, for standard clinical makers such as CRP, blood cholesterols, and transaminases to detect conditions that potentially could bias our results such as an acute inflammation; **(Table 3).**

All blood samples, with the exception of blood for the cell counts were directly after blood withdrawal processed and stored at -80°C within the laboratory facilities of the Biomedical Research Park Barcelona for later analysis.

Table 3. Blood biomarkers measured in the TAPAS experimental study

Health endpoint	*Biomarker*
Standard blood cell count	Leukocytes (and white blood cell differential), erythrocytes, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, thrombocytes, mean platelet volume
Systemic inflammation	Cytokines (IL1b, IL6, IL8, IL10)* C-Reactive Protein, TNF-alpha
Oxidative stress and systemic antioxidant defence	Myeloperoxidase, superoxide dismutase, glutathione peroxidase
Lipid oxidation	Malondialdehyde, F2-Isoprostane
Biochemistry	Blood cholesterols, uric acid
Liver cell damage	Transaminases
Neuronal growth (CNS)	Brain-derived neurotrophic factor
Gene regulation	Gene-expression analysis in whole blood

* IL= Interleukin

Pulmonary function

Lung function tests were performed before and after the exposure session. The forced expiratory flow in one second (FEV_1), forced vital capacity (FVC), forced expiratory flow 25-25% ($FEF_{25–75\%}$, and the peak expiratory flow (PEF), and the peak expiratory flow (PEF) were measured by using a portable computerized spirometer NDD EasyOne^{TM} (Andover, Massachusetts, USA) following the guidelines of the European Respiratory Society (Miller et al. 2005). Only sessions which led to at least two acceptable tests and reproducibility of the FEV_1 and FVC within 150 ml were

considered for further analysis. The ratio of FEV_1/FVC, and also the highest sum of FVC and FEV_1 were calculated to select the $FEF_{25–75\%}$ accordingly.

Fraction of exhaled Nitric Oxide (FeNO)

Airway inflammation was assessed by the measurements of airway production of NO in accordance with the guidelines of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) for standardized measurements of the exhaled nitric oxide (American Thoracic Society and European Respiratory Society 2005). FeNO was measured by using an NIOX MINO^{TM} online analyser (Aerocrine, Solna, Sweden) with the single breath method. Subjects were requested to exhale to residual volume, insert the mouthpiece, inhale to total lung capacity through an NO scrubber, and subsequently exhale into the device for approximately 10 seconds at a constant flow of 50 $mL \cdot s^{-1}$. The manoeuvre was supported by an acoustic and a visual feedback of the device.

Cardiovascular Monitoring

Upon their arrival in the research unit, the participants were allowed to rest in silence, and then were equipped with a portable three-channel Holter monitor (Cardiolight, Gem-Med, Barcelona, Spain) with the purpose of continuously monitoring the time- and frequency indices of heart rate variability (HRV), cardiac ectopy, and heart rate until the end of the experiment day. The recordings were processed by the Holter-provider company, Gem-Med in accordance with the manufacture protocol. The standards of the measurement of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology were considered (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996).

Blood Pressure:

Blood pressure (BP) and heart rate (HR) were measured before, during, and after exposure in seated position with cuffs placed on the right upper arm after a 10 minute resting interval by an automatic ambulatory blood pressure monitor (Omron Healthcare, Japan). Three readings with a two to three minute break in between were taken. The average of the three readings was considered for analysis. The participants were blinded to their blood pressure readings whenever possible.

Oxygen saturation

Oxygen saturation is a measure of how much oxygen the blood is carrying as a percentage of the maximum it could carry. The oxygen saturation, also referred to as SpO2, was monitored continuously during the complete exposure day via a small blood-oxygen monitor (Konica Minolta, Japan) clipped to the volunteers finger. This pulse oximeter is a convenient measurement device, as it measures indirectly and hence non-invasively the oxygen saturation of the volunteers' blood. The blood cells that are saturated with oxygen absorb and reflect light differently than those that are not. The clip shines a light through one side of the finger while a detector on the other side measures the light that comes through and gives a rough estimate of the oxygen saturation. The simultaneous and continuous indication of the heart rate was used to control the physical activity level (individual moderate intensity range) in the 'cycling' participants by adjusting their pedalling speed and also the pedalling resistance in a few cases, as mentioned earlier.

Urine sampling

Urine was sampled for a potential analysis of the biomarkers of oxidative stress and exposure indicators. The volunteers were instructed to empty their bladder in the morning after waking up, in order to avoid an influence of the day before the baseline urine sampling. The baseline urine sample was collected just before they left to the exposure sites. Another sample was collected from the first micturition after the tow-hour exposure session. Urine samples were directly after their collection processed and stored at -20°C within the facilities of the Biomedical Research Park Barcelona.

Symptoms recording

Moreover, a standard questionnaire was used to explore the possible symptoms and the volunteers´ subjective state of health during and after exposure. The volunteers were asked about their state of health before, during and after the exposure session. The sections covered pulmonary and cardiac symptoms, as well as the general state of health and well-being.

3.7. On-site air pollution exposure monitoring

The on-site exposure monitoring included particulate matters of different aerodynamic size diameters, nitric oxides, black carbon, and noise levels. The characterisation of air pollution at both experiment sites took place as the following:

1) **UFP counts** (ultrafine particle matter in the size range 0.01–1.0µm) were obtained by using an optical particle counter CPC 3007 (TSI, Minnesota, USA). Readings above 100,000 particles per cubic cm from the TSI model CPC-3007 required a correction for coincidence, which, if left unadjusted, would result in underestimation of particle counts at high concentrations. The following equation was applied: cpc_corr=38 457×(exp(cpc×0.00001)), (Westerdahl et al. 2005).

2) During the two-hour exposure period **$PM_{2.5}$ and PM_{10}** [particulate matter mass with aerodynamic diameters of less than 2.5 µm ($PM_{2.5}$) and less than 10 µm (PM_{10})] were collected by using a Harvard Impactor (HI), (Air Diagnostics and Engineering), at a flow rate of 10 L/min. The gravimetric analysis of air quality filter samples was conducted in a specialized laboratory in accordance with standard operating procedures in temperature and relative humidity-controlled conditions. We collected for a total of 112 $PM_{2.5}$ and PM_{10} mass filter samples, 18 field blanks that were equally distributed over the whole field work period in order to validate the collected data. The average blank values were subtracted from the final particulate matter mass.

3) In addition, we monitored **PM_1, $PM_{2.5}$, PM_4,** and **PM_{10}** (particulate matter mass with aerodynamic diameters of less than 1µm, less than 2.5µm, less than 4µm, and less than 10µm, respectively) by continuously using the real time aerosol monitor DustTrack™ II model 8532 (TSI, Minnesota, USA).

4) **Nitrogen oxides (NO_X)** were measured via an NOx analyser model 8532 (2B Technologies, Boulder, Colorado, USA).

5) **Black carbon (BC)** concentrations were measured by using a portable aethalometer Model AE51 (Magee Scientific, Berkeley, California, USA) and corrected for filter attenuation (Kirchstetter and Novakov 2007; Wang et al. 2011).

6) **Temperature and relative humidity** were measured by using a Q-track™ (Q-Track Corporation, USA).

7) Meteorology data including **wind direction and wind speed** were continuously monitored by using a tripod-mounted weather station (Oregon Scientific, USA)

8) **Noise levels (dba**) were monitored continuously via a sound level meter model SC160 (CESVA instruments, Barcelona, Spain.

9) Regular **counts of motorized traffic** were performed at both exposure sites during all exposure sessions. Two manual handheld traffic counters were used by a field technician with one counter in each hand for both light vehicles (cars) and heavy vehicles (buses and trucks).

Standard operating procedures were developed and followed to ensure correct exposure monitoring. Instruments were calibrated before start of the experiment period and on a regular basis in accordance with manufacturer recommendations. Before each experiment day calibrations such as zero checks, were performed and flow checks scheduled throughout the two-hour exposure session. All incidences and instrument failures during the experiment time were documented.

In addition, we obtained the pollen counts and air pollution data from fixed monitoring stations located in and around Barcelona city for the entire experiment period. This allowed us to estimate the air pollution exposure of each volunteers by using land-use regression models for the three days before his/her scheduled experiment days (Beelen et al. 2013).

3.8. Statistical analysis

A statistical analysis was performed by using STATA 12. Exposure data was summarized as means, tested for correlations, and transformed into interquartile ranges. Changes from baseline were calculated. The a priori alpha level was set at $P<0.05$ for all planned comparisons. Normality was evaluated by using simple graphical methods.

We applied mixed effect models for repeated measurements with both, baseline values and individuals as random effects, in order to account for intra-individual variability.

We included sex, age, body mass index (BMI), on-site temperature and relative humidity, and the time of the measurement as covariates in all main models. To account for volunteers ´exposure for the day before the experiments took place, we included the estimated time during which each volunteer was exposed to environmental tobacco smoke (ETS), their energy expenditure from physical activities expressed in Metabolic Equivalent of Task (MET) based on Ainsworth et al.´s compendium of physical activities(Ainsworth et al. 2000) and their NO_2 exposure using time adjusted land use regression models (Beelen et al. 2013).

We tested in separate models for physical activity (yes/no), high air pollution (yes/no), and the four exposure scenarios (low and high air pollution with and without physical activity). Single pollutant models included continuous pollutant levels with additional adjustment for physical activity (yes/no).

Moreover, we tested for interactions between physical activity (yes/no) and high air pollution exposure (yes/no). Furthermore, we tested for interaction between physical activity (yes/no) and all measured pollutants, and additionally tested for interaction with the time of the health measurements.

Analysis of variance (ANOVA) for repeated measures was applied to test for differences between the baselines of the four exposure scenarios.

4. RESULTS

The study 'TAPAS Experimental' included a variety of biomarkers for short-term health effects determination as presented in the previous section. The following results refer to the publication coming out of the first analysis covering pulmonary and inflammatory responses, as well as the effects on blood pressure and brain-derived neurotrophic factor. Further publications are to be expected after further analysis of the remaining biomarkers collected in this project.

Paper I:
Respiratory and inflammatory responses to short-term exposure to traffic-related air pollution with and without moderate physical activity (published)

Paper II:
Arterial blood pressure responses to short-term exposure to low and high traffic-related air pollution with and without moderate physical activity (published)

Paper III:
Brain-derived neurotrophic factor (BDNF) serum levels decrease after intermitted moderate physical activity in high levels of traffic-related air pollution (to be submitted)

4.1. Paper I: Respiratory and inflammatory responses to short-term exposure to traffic-related air pollution with and without moderate physical activity

Kubesch NJ, de Nazelle A, Westerdahl D, Martinez D, Carrasco-Turigas G, Bouso L, et al. Respiratory and inflammatory responses to short-term exposure to traffic-related air pollution with and without moderate physical activity. Occup Environ Med. 2015 Apr;72(4):284–93. DOI: 10.1136/oemed-2014-102106

4.2. Paper II: Arterial blood pressure responses to short-term exposure to low and high traffic-related air pollution with and without moderate physical activity

Kubesch N, De Nazelle A, Guerra S, Westerdahl D, Martinez D, Bouso L, et al. Arterial blood pressure responses to short-term exposure to low and high traffic-related air pollution with and without moderate physical activity. Eur J Prev Cardiol. 2015 May 17;22(5):548–57. DOI: 10.1177/2047487314555602

4.3. Paper III: Brain-derived neurotrophic factor (BDNF) serum levels decrease after physical activity in high levels of traffic-related air pollution

Kubesch, Nadine Janet, Stefano Guerra, Audrey de Nazelle, David Martinez, Laura Bouso, Gloria Carrasco-Turigas, Dane Westerdahl, and Mark J. Nieuwenhuijsen.

To be submitted

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) SERUM LEVELS DECREASE AFTER PHYSICAL ACTIVITY IN HIGH LEVELS OF TRAFFIC-RELATED AIR POLLUTION

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COMPETING INTERESTS

The authors declare that they have no competing interests.

KEY WORDS: Brain-derived Neutrotrophic Factor, Outdoor Air Pollution, Physical Activity, Short-term Health Effects, Fine Particulate Matter, Traffic-related air pollution, Traffic exposure

WORD COUNT: 3922

CONTRIBUTORSHIPS:

NK was involved in the conception, hypotheses delineation, and design of the study, the acquisition of the data, data analysis and interpretation, and wrote the article. SG was involved in the conception, hypotheses delineation, and design of the study, the data analysis and interpretation, and was substantially involved in article revision prior to submission. AdN obtained the funding, was involved in the conception, hypotheses delineation, and design of the study, and was substantially involved in article revision prior to submission. DW was involved in the acquisition of the data and data analysis. DM was involved in the data analysis. GC and LB were involved in the acquisition of the data. MN obtained the funding, was involved in the conception, hypotheses delineation, and design of the study, the data analysis and interpretation, and was substantially involved in article revision prior to submission.

WHAT THIS PAPER ADDS

- Our study shows that intermittent moderate acute physical activity experimental setting and also habitual physical activity can decrease serum BDNF in high levels of TRAP, and, furthermore this study shows that PA and TRAP have independent effects.
- Our study stresses the importance of assessing both the exposure to air pollution and physical activity levels when assessing physiological responses of BDNF.

ABSTRACT

Objectives:

Short-term physical activity has been associated with increase in serum brain-derived neurotrophic factor in serum. We aimed to determine in healthy subjects the short-term health effects of physical activity in different levels of traffic-related air pollution (TRAP).

Methods:

Crossover real-world exposure study comparing in twenty-eight healthy subjects BDNF serum levels in response to four different exposure scenarios: two-hour exposure in a high and low TRAP environment, each in rest and in combination with intermittent moderate PA, consisting of four 15 minutes rest and cycling intervals. In addition, each volunteer´s metabolic equivalent task (MET) for the three consecutive days prior to each exposure session was calculated. Data was analysed using mixed effect models for repeated measures.

Results:

Intermittent PA compared with rest, irrespective of the TRAP exposure status, decreased statistically significantly serum BDNF (-13.65%, p=0.026). Accumulated physical activity expressed in metabolic equivalent task (MET) during three days before the blood withdrawal decreased BDNF statistically significantly (-57.92pg/ml, p=0.027). We did not find a statistically significantly association between TRAP and serum BDNF. We found no evidence for an interaction between TRAP and PA for changes in serum BDNF.

Conclusions:

In a healthy population, intermittent moderate physical activity decreases serum BDNF when performed in a high and very high polluted environment. Our study also suggests that habitual PA in high air pollution decreases circulating BDNF.

INTRODUCTION

Background

Regular physical activity reduces the risk of many adverse health outcomes such as cancer, cardiovascular disease, and diabetes.[1,2,3,4] Physical activity is known to have also health promoting effects on the brain and psychological health, and enhances cognitive function.[5,6,7,8] It has been proposed that the beneficial effects of physical activity on brain function could be mediated by brain-derived neurotrophic factor (BDNF), a neurotrophin of the family of growth factors supposed to enhance neural plasticity and improving cognitive function.[9,10]

Some studies suggest that short episodes of exercise increase serum levels of BDNF. A study by Nofuji et al. 2012 showed an increase in serum BDNF 30 minutes after moderate and vigorous exercise.[11] Similar responses were found in athletes and healthy sedentary participants after aerobic and anaerobic activity.[12,13] However, these studies show also that the BDNF increase can be transient and responses may be altered by factors such as the type and intensity of the physical activity.

The exposure to TRAP has been linked to adverse effects on the brain such as neuroinflammation and neuropathology".[14,15,16] Physical activity increases the volume of inhaled air[17] and the increased minute ventilation during physical activity in an polluted environment may result in substantial increases in the inhaled dose of traffic-related air pollutants[18,19,20] and potentially lead to adverse acute health effects. The increased air pollutant uptake during the physical activity may inhibit the positive effect of exercise on cognition[21], and alter the post-exercise response of circulating BDNF. Bos et al. showed increased serum BDNF concentration after cycling in an air-filtered room but not after cycling in TRAP.[22] Traffic-related air pollutants enter the blood stream shortly post-exposure[23] inducing different systemic responses[24,25], and may also directly translocate to the central nervous system[15], hence alterations in the BDNF response pattern after TRAP exposure are conceivable.

Although these studies have shown the effects of physical activity and also the role of air pollution as a potentially altering factor, they were generally not designed to disentangle the effects of air pollution from those caused by physical activity, nor their possible interaction.

We hypothesized that short-term physical activity induces a release of BDNF in serum and that short-term exposure to TRAP may modify these response in healthy adults. Our objective was to determine in a healthy population in a real-world situation the BDNF responses to physical activity in different levels of traffic-related air pollution (TRAP).

METHODS

Study Design

The study was conducted in Barcelona, Spain, between February and November 2011 and followed a controlled crossover study design. The Ethics Review Committee of the "Institut Municipal d'Investigació Mèdica (IMIM)" approved the study and all subjects gave written informed consent prior to participation.

We recruited 31 volunteers for this study. Eligible subjects were non-smoking adults in the age range of 18 to 60 years, healthy and without history or findings of pulmonary or cardiovascular disease, or other acute or chronic conditions (including infections, fever, cold) except sporadic nasal allergies but without medication-based treatment. Volunteers abstained from taking over-the-counter medications (e.g. pain relievers), vitamins, and herbal supplements before the experiment days. Alcohol consumption on the evening before the experiment was limited to one glass of wine or beer.

Two hours was found to be the average time that residents in Barcelona spent in transit throughout the day.[26] Therefore, all volunteers were exposed for two hours to either heavily polluted air (located on a pedestrian bridge approximately five meters above a main transit roadway for motorised traffic (often diesel powered) - Ronda Litoral) or to a lower TRAP (pedestrian friendly market square - Barceloneta market square) between 8 a.m. and 10 a.m. during morning rush hours; **(Supplementary appendix figure S1).**

Two subjects were studied simultaneously on each occasion. During the two-hour exposure time, one subject performed intermittent exercise consisting of 15 minutes cycling on a cycle ergometer alternating with 15 minutes of rest while the second volunteer rested throughout the two-hour exposure. Each volunteer participated in each of the four exposure scenarios in a narrow time period to exclude confounding by seasonal variation. The exercise interval was repeated four times summing up a total of one hour exercise during the two-hour exposure period. Each volunteer was required to reach his/her individual range of moderate physical activity intensity, which was controlled through their heart rate monitored continuously by a fingertip pulse oximeter (Konica Minolta, Japan). For moderate-intensity physical activity, the volunteers´ target heart rate was 50 to 70% of their maximum heart rate, which was estimated for each volunteer on the basis of their age and sex [Males: HRmax=220-age; Females: HRmax=206–0.88(age)].[27] During each of the PA intervals all participants were instructed and supervised constantly by the same technician to pedal at a speed and resistance level that brought them close to their upper level of their estimated moderate physical activity pulse range (70%) of their individual maximum heart. Volunteers completed the four exposure scenarios in a random order. Each volunteer was to participate in all four scenarios: low level and high level air pollution exposure, each in combination with and without physical activity. To avoid a diurnal effect, all exposure sessions and blood sampling were scheduled at the same hours during the day as well as during the same days of the week to account for variations in traffic characteristics.

Volunteers filled out a half-hourly activity diary asking for time, activity, location, travel mode, and perceived exposure to environmental tobacco smoke or other pollutants during the three days before each experiment day. That allowed us to estimate the volunteers' air pollution pre-exposure and energy expenditure due to physical activity for the preceding three days. All participants received identical meals during all exposure days.

Health Measurements

The baseline and post-exposure blood withdrawal took place in the clinical research facilities within the study centre, a five- minute drive away from the study sites. To

keep the volunteers' exposure to TRAP before baseline measurements minimal, volunteers were required to arrive in the morning before 6:45 a.m. in the study centre. Before that time the TRAP is still low in Barcelona. No restrictions were made on the volunteers' travel mode choice to the study centre. Upon volunteers arrival they were given time to rest before the baseline blood sample was taken. After returning from the exposure site all subjects remained in a quiet and temperature-controlled clinical research facility.

A blood sample was taken before and on average 30 minutes post-exposure from all subjects. All venepunctures for blood withdrawal were undertaken by a registered nurse according to SOP. Blood samples collected in serum vacutainers (BD Vacutainer^{TM}). Serum samples for the measurement of BDNF were processed shortly after blood withdrawal and instantly after alliquotation stored at -80ºC and later analysed in duplicates using high sensitivity LUMINEX^{TM} technology (Merck Millipore, U.K.) following standard procedures. BDNF concentrations were analysed according to the manufacturer's protocol.

Exposure Monitoring

The on-site exposure monitoring included continuous reading of UFP counts (ultrafine particle matter in the size range 0.01 to 1.0 µm), using an optical particle counter CPC 3007 (TSI, Minnesota, USA). Readings above 100,000 particles per cubic cm (#/cm3) from the TSI model 3007 CPC required a correction for coincidence, which if left unadjusted would result in under-estimation of particle counts at high concentrations.[28] The following equation was applied: cpc_corr=38457*(exp(cpc*0,00001)).[28] Nitrogen oxides (NOx) were measured via a NOx analyzer (2B technologies, Boulder, USA). During the two-hour exposure period $PM_{2.5}$ and PM_{10} [particulate matter mass with aerodynamic diameters of less than 2.5 µm ($PM_{2.5}$) and less than 10 µm (PM_{10})] were collected using a Harvard Impactor (HI), (Air Diagnostics and Engineering, USA)) at a flow rate of 10 l/min. The gravimetric analysis of air quality filter samples was conducted in a specialized laboratory according standard operating procedures in temperature and relative humidity controlled conditions. We collected a total of 112 $PM_{2.5}$ and PM_{10} mass filter samples 18 field blanks that were equally distributed over

the whole field work period in order to validate the collected data. The average blank values were subtracted from the final particulate matter mass. Black carbon (BC) concentrations were measured using a portable aethalometer (Magee Scientific, Berkeley, USA), and corrected for filter attenuation.[29,30]

Statistical Analysis

Statistical analyses were performed using STATA 12. Exposure data were summarized as means, tested for correlations, and transformed to interquartile ranges. The a priori alpha level was set at $P<0.05$ for all planned comparisons. Normality was evaluated using simple graphical methods and Kolmogorov-Smirnov test.

We applied mixed effect models for the analysis of repeated measurement data with both baseline values and individuals as random effects to account for intra-individual variability in all health outcomes. We calculated percent changes from baseline for use in main mixed model regression analysis. In addition, all models were adjusted for on-site ambient temperature and relative humidity, sex, age, and body mass index (BMI). To account for volunteers exposure for the day before the experiments took place we included the estimated time that each volunteer was exposed to environmental tobacco smoke (ETS), the energy expenditure for their physical activities expressed in Metabolic Equivalent of Task (MET) based on Ainsworth et al. compendium of physical activities[31] and the volunteers' NO_2 exposure using time adjusted land use regression models.[32]

We tested in separate models for physical activity versus rest, irrespective the TRAP site, and high TRAP versus low TRAP site irrespective the physical activity status. Another model included the four exposure scenarios (low and high air pollution with and without physical activity). Single pollutant models included continuous pollutant levels with additional adjustment for physical activity (yes/no). Interaction between physical activity and the TRAP site, and PA and each pollutant separately was tested by mixed effect models including an interaction term.

Furthermore, we tested the effect of the self-reported[3] habitual physical activity during the preceding three consecutive days (MET time) on the baseline BDNF levels before each of the four exposure sessions. These models were also adjusted for the estimated time that each volunteer was exposed to environmental tobacco smoke (ETS), and their estimated NO_2 exposure for the three preceding days. An interaction term was included for the habitual physical activity during the preceding three consecutive days (MET time) and the volunteers estimated NO_2 exposure for the three preceding days before baseline BDNF sampling.

The differences between the BDNF baseline levels were tested by an analysis of variance (ANOVA) for repeated measures.

RESULTS

Subject Characteristics

Overall 31 volunteers were recruited for study. All subjects except three completed the four different exposure scenarios. Two of these three volunteers had to be excluded due to predefined exclusion criteria diagnosed after entry into the study, and one volunteer left the study after participating in one trial day due to personal reasons. The remaining 28 volunteers completed all four trial days and are therefore included in the analysis; **(Table 1).**

[3] Data based on a half- hourly self-reported physical activity diary

Table 1. Baseline Characteristics of the Study Population	
Characteristic	**(n=28)**
Baseline BDNF [pg/mL (min-max)]	21.3 (6.8– 36.,7)
Female [n (%)]	15 (53.6)
Mean age [years (min-max)]	34.4 (21-53)
Mean body-mass index * [kg/m^2 (min-max)]	23.1 (18.2-29.6)
Ex-smoker [n (%)]	8 (28.6)
Allergies † [n (%)]	8 (28.6)
Resident in Barcelona city [n (%)]	20 (71.4)
Mean distance between participants' homes and study unit [km - (min-max)]	8.8 (<1-18.6)
* The body-mass index is the weight in kilograms divided by the square of the height in meters. †Self-reported by volunteers based on question asking for hay fever, rhinitis, and nasal allergies.	

Pollution Levels

All measured pollutants were statistically significantly higher in the high air pollution site (traffic site) compared to the low level air pollution site (market square)**.** Considerably contrasting levels could be found for NOx (which was 10 times higher in the traffic site compared to the market square site), BC (7x higher), and UFP (5x higher); **(Table 2).**

Table 2. Site Characteristics (Low vs. High Air Pollution Site) *					
Pollutant	**Study Site**	**Mean**	**[95% Conf.**	**Interval]**	**p value**
Black Carbon ($\mu g/m^3$)	Low TRAP	8.59	3.16	14.02	
	High TRAP	60.82	55.29	66.34	
	Contrast	52.23			<0.001
UFP (particles/cm^3)	Low TRAP	32,992.75	19,020.10	46,965.39	
	High TRAP	164,464.30	150,252.80	178,675.80	
	Contrast	131,471.6			<0.001
NO_x (ppb)	Low TRAP	71.62	15.71	127.53	
	High TRAP	722.18	665.31	779.04	
	Contrast	650.56			<0.001
PM_{10} ($\mu g/m^3$)	Low TRAP	67.79	55.48	80.10	
	High TRAP	129.68	117.16	142.20	
	Contrast	61.89			<0.001
$PM_{2.5}$ ($\mu g/m^3$)	Low TRAP	30.03	22.37	37.70	
	High TRAP	80.76	72.97	88.56	
	Contrast	50.73			<0.001
PM_{coarse} ($\mu g/m^3$)	Low TRAP	37.75	31.59	43.92	
	High TRAP	48.91	42.64	55.19	
	Contrast	11.16			0.014
Temperature (^0C)	Low TRAP	20.78	19.36	22.20	
	High TRAP	22.28	20.83	23.72	
	Contrast	1.50			0.144
Relative Humidity (%)	Low TRAP	61.86	58.46	65.26	
	High TRAP	55.07	51.61	58.53	
	Contrast	-6.79			0.007
*Means are based on the 2 hour measurements during morning rush hour (8 – 10 a.m.)					

There were high correlations (>0.9) between BC, NO_X and UFP concentrations, NO_X and $PM_{2.5}$ with BC, as well as $PM_{2.5}$ with NOx and PM_{10}. Moderate correlations were observed with PM_{coarse}; (**Supplementary appendix table S1).**

Health data

We compared means between the four exposure scenarios and found no statistically significant differences between the baseline values; (**Supplementary appendix table S2).** The BDNF serum level means comparing pre- and post-cycling, and pre- and post-rest (irrespective of the TRAP exposure status) are presented in **figure 1**.

Figure 1. Pre- and Post-exposure Mean Comparisons for PA and Rest

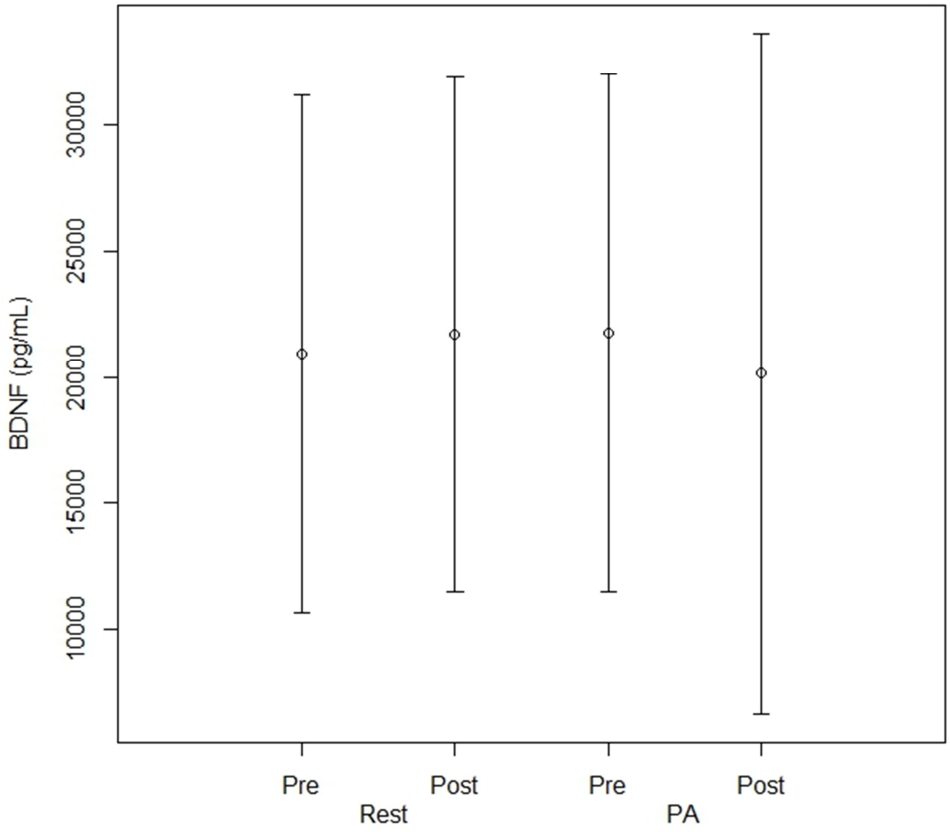

Figure 1 shows arithmetic means of the serum BDNF levels at baseline (Pre) and post-exposure (Post). Means are combined for Physical activity (PA), as well as for Rest, irrespective of the TRAP exposure status. The I bars indicate the standard deviations. The analysis of variance (ANOVA) showed that post-exposure BDNF was borderline statistically significantly lower compared to BDNF level at baseline (p=0.054).

Associations between Physical Activity, Pollution levels, and BDNF serum levels

Mixed effect models showed statistically significant decreases in post-exposure BDNF (-13.65%, p=<0.026) after intermittent physical activity (irrespective of the high/ low TRAP exposure status); **(Table 3).** Neither the different exposure scenarios that included physical activity changed circulating BDNF statistically significantly nor the interquartile increases of the individual pollutants showed significant associations with BDNF post-exposure; **(Table 3).** We found no evidence for a statistically significant interaction between physical activity and air pollution.

Table 3. Association between Physical Activity, Pollution levels, and serum BDNF*

	BDNF percent change from baseline				
	Coefficient (%)	**[95%**	**CI]**	**P Value**	**P value for Interaction with Physical activity *ƒ***
Physical Activity (PA)†	-13.65	-25.69	-1.60	**0.026**	
High TRAP Site†	4.97	-8.21	18.15	0.460	0.988
High TRAP Site & PA‡	-8.58	-26.12	8.96	0.338	
Low TRAP Site & PA‡	-13.70	-30.64	3.24	0.113	
High TRAP Site & Rest‡	5.10	-12.40	22.60	0.568	
BC (IQR)§	5.44	-6.26	17.13	0.362	0.699
UFP (IQR)§	2.36	-8.26	12.99	0.663	0.649
$N0_x$ (IQR)§	3.85	-9.38	17.08	0.568	0.593
PM_{10} (IQR)§	1.97	-6.86	10.81	0.662	0.264
$PM_{2.5}$ (IQR)§	3.16	-8.53	14.84	0.596	0.424
PM_{coarse} (IQR)§	0.63	-7.91	9.18	0.884	0.155

*Mixed effect models adjusted for on-site ambient temperature and relative humidity, age, BMI, sex, as well as total energy expenditure (MET) three days prior to exposure, environmental tobacco smoke, and NO_2 exposure for 24h before experiment.

† Pooled analysis for PA vs, Rest, and High vs. Low Exposure site, respectively.

‡ Model regressing the exposure scenarios with reference "Low TRAP site & Rest".

§ Single pollutant model in addition to above covariates adjusted for physical activity.

ƒ Interaction between Physical activity (categorical variable) and high traffic-related air pollution (TRAP) site (categorical variable) , and between Physical activity (categorical variable) and single pollutants[mean centered interquartile range (IQR)]

Associations between Physical Activity, Environmental exposures, and Baseline serum BDNF

Mixed effect models showed statistically significant decreases in BDNF (-57.92 pg/ml, p=<0.027) levels at baseline due to the physical activity accumulated in the three days prior to the experimental session. Exposure to NO_2 during the same time did not show an effect. We found no evidence for a statistically significant interaction between physical activity and air pollution; **(Table 4)**.

Table 4. Association between Physical Activity, Environmental pre-exposures, and serum BDNF at Baseline *

	Coefficient (pg/ml)	[95%	CI]	P Value	P value for Interaction *f*
MET 3 days (total) †	-57.92	-109.34	-6.50	**0.027**	
ETS 3 days (total) ‡	-122.33	-511.71	267.05	0.538	
NO_2 3 days (total) §	23.46	-39.91	86.83	0.468	0.663

*Mixed effect model adjusted for age, BMI, and sex.

† Total energy expenditure (MET) during three days prior the experiment day (based on participants' self-reported activity data). Model adjusted in addition for ETS and NO_2 exposure for the three preceding days.

‡ Total environmental tobacco smoke (ETS) exposure during three days prior the experiment day (based on participants' self-reported data). Model adjusted in addition for MET and NO_2 exposure for the three preceding days.

§ Total NO_2 exposure three days prior the experiment day [Estimations using land-use-regression models (LUR) based on participants' self-reported location data]. Model adjusted in addition for MET and ETS exposure for the three preceding days.

f Interaction between physical activity (MET) and NO_2 exposure for the three preceding days (adjusted for ETS)

DISCUSSION

Summary

In this well controlled crossover design examining BDNF in response to intermittent physical activity during a two-hour real-world exposure to different levels of TRAP, we found that short-term intermittent moderate PA in an environment with high and very high TRAP in a controlled experimental conditions as well as habitual physical activity during the three days preceding the baseline blood sample can decrease serum BDNF. We found no evidence for an interaction between physical activity and TRAP.

Site characteristics

The average concentrations of ultrafine particles, BC, and NO_x levels at the high TRAP site were 5, 7, and 10 times higher than those measured at the low TRAP site, respectively. However, the high correlations among the pollutants made it difficult to separate the effects on the health endpoints. The average ultrafine particles levels found at our high traffic site were considerably higher than those found in previous real world exposure studies in the UK, Netherlands or Belgium.[33,34,35] Mean particle concentrations in our high air pollution site were comparable with exposures measured in chamber studies [36] [37] while our "low exposure site" air pollution levels were rather comparable with those categorized in other experimental studies as "High air pollution" or "traffic site".[38,39]

We found very high levels of NO_x at our high TRAP site. Few studies measure NO_x right in traffic, but levels found in this study are not unusual for roadway environments, especially where high proportion of diesel vehicles can be found. [40] Both of our study sites were in similar proximity nearby the sea coast and close to Barcelona's harbor resulting in an influence of marine sources and shipping emissions in the particulate matter composition. The warm Mediterranean climate in Barcelona tends to increase the contents of road traffic and fuel oil combustion due to re-suspension, which is typically for warm seasons.[41]

The effects of PA, TRAP, and their interaction

Association between Non-experimental Habitual Physical Activity, Environmental Air Pollution Exposures, and Baseline Serum BDNF

We showed that total energy expenditure (MET) accumulated in three consecutive days prior to the blood withdrawal lowered circulating BDNF. Physical activity during the three consecutive days before each of our exposure sessions reflected more habitual activities of daily life. Participants were merely instructed to abstain from very heavy exercise (e.g. marathon running) during that time but could else follow their routine exercise and activity habits.

Our results are partially in accordance with those obtained in previous studies who found that long-term habitual exercise can lower peripheral BDNF.[42124344] We found no evidence for an effect of NO_2 exposure during the three preceding days on BDNF baseline levels, or evidence for a statistically significant interaction between physical activity and air pollution. A study by Bos et al. compared the changes in serum BDNF in 44 untrained healthy participants following a 12-week exercise program in either an urban setting with higher levels of TRAP or in a rural environment with lower TRAP. The study showed post-exercise non-significant decreases in absolute mean BDNF concentrations in both the urban group ($1.9ng/ml^{-1}$, p=0.52) and in the rural group ($1.89ng/ml^{-1}$, p=0.08).[35]

Association between Short-term Intermitted Moderate Physical Activity, Air Pollution levels, and Post-exposure Serum BDNF

In contrast to previous findings, our results provide experimental evidence demonstrating that also short-term intermitted moderate PA in high levels of TRAP can decrease serum BDNF shortly after. We could confirm this finding by an additional analysis using mixed models comparing the change from baseline to post-exercise within the group combined for physical activity only. Mixed models confirmed that in the PA group the post-exercise compared with pre-exercise BDNF levels were statistically significantly lower (-1611.57pg/mL, p=0.048, (**Table S4, Supplementary annex**).

We can exclude an effect due to the allocation of the samples to the two batches and four plates that the Luminex™ analysis technology required. All eight samples of the four exposure occasions of each individual were tested within the same plate and batch. In the following section, factors that may have had an influence on our findings, which are partly inconsistent with previous studies, are discussed.

To begin, it is noteworthy that in our study *high levels of TRAP were found in both exposure environments* compared to other studies. In a study by Bos and colleagues physical activity performed in average levels of UFP of 28180 pt/cm^3 did not induce the BDNF release which could be shown in filtered air (3496 pt/cm^3). The levels of TRAP found in our high exposure site were six times higher than those in the Belgian study whereas the levels of UFP we measured in our "low" TRAP site were rather close to those found in the Belgian high exposure site.[22] We therefore cannot exclude that the BDNF responses shown in this work had been different when tested in an experimental setting with considerably lower TRAP levels than those found in our study. It would be recommendable to assess the responses of physical activity patterns similar to our study performed in lower levels or in an air filtered clean room.

Secondly, the *chronic exposure to high levels of TRAP* that our participants are habitually exposed to could have influenced response pattern: Our participants were mainly resident in Barcelona city, a place where TRAP is considerably elevated compared to other European cities.[45] We cannot exclude that that a chronic exposure might alter the response pattern due to potentially underlying chronic low-grade systemic inflammation or altered oxidative stress processes.[46]

Thirdly, note the *relevance of the time between physical activity and blood withdrawal*: The timing of the BDNF determination following the physical activity in TRAP might be a predicting factor as shown previously. We measured BDNF on average 30minutes after completion of the two hour exposure session and cannot rule out that we might have missed a transient initial elevation of the circulating BDNF. Heymann et al. found that BDNF levels can drop quickly within minutes after initial elevation.[47] Griffin et al. showed a significant increase in serum BDNF concentration immediately after a 30

minutes exercise protocol (cycling until max. predicted heart rate). Thirty minutes post-exercise the serum BDNF was not significantly different to pre-exercise.[13] A study by Nofuji et al. showed that in sedentary subjects BDNF levels returned to baseline during recovery within 60 min after the test. In participants performing physical activity chronically the BDNF levels even decreased below baseline.[11] The same study also suggested that responses to acute exercise differed depending on the regular physical activity pattern of the participants. The BDNF levels for the active group decreased during recovery even below the baseline level after the physical activity unlike the sedentary group whose BDNF levels return to baseline. However, the physical activity protocol in the study by Nofuji et al. followed a more intense physical activity protocol applying maximal exercise testing.

Moreover, the *type and intensity of the exercise protocol* may influence on acute responses: We used an intermitted aerobic physical activity protocol with physical activity intensity set to the upper point of the moderate range. However, it's not fully understood yet which type and exercise intensity is most appropriate to induce a serum BDNF release. A recent review by Coelho et al. focusing on the elderly could not establish a recommendation for a protocol for the type and intensity of physical exercise required to produce an increase in circulating BDNF.[48]

Finally, the *participants´ habitual exercise and eating behaviors as part of a healthy lifestyle* could have played a modifying role in our outcomes: Our participants were screened to be healthy without any acute or chronic condition and also non-smoking which also may imply that our study population followed a generally healthy lifestyle involving some type of habitual physical activity such as for instance using active transportation modes. We adjusted for the physical activity of our study population during the three preceding days (MET time) in our main models; however, we cannot rule out that a generally healthier lifestyle or health awareness including a healthier eating behavior and some kind of physical activity of our participants could have influenced our results.

The strengths of this study is its unique design; an experimental setting with volunteers exposed in real world conditions: The well-controlled crossover study design allowed us to disentangle the effects of physical activity from those attributed to air pollution, which has been to our knowledge unique in this form. Unlike in chamber studies, the exposure in our real world setting brings a mix of airborne pollutants actually present in real traffic-related air pollution. The exposure to a mix of air pollutants has been shown to lead to different health effects than those observed in chamber studies.[49] Moreover, our crossover design facilitates the exclusion of confounding by factors that are stable within an individual over time but vary between participants, since every volunteer serves as its own control.

A limitation of the study was the relatively small sample size leading to restricted statistical power, and furthermore, the relative high TRAP levels in the "low TRAP" site. Furthermore, performing the experiments in a real world environment implied less controllable study conditions. Some unknown factors including day to day variations, but also seasonal changes with a range of unclear modifiers may have influenced our study results. We cannot exclude that other factors we could not control for had potentially an effect on the post-exposure measurements. Furthermore, we measured BDNF only once and shortly after exposure. We cannot rule out that responses would differ after a longer or even shorter time post-exposure.

It is important to note that our changes were small and observed in a healthy population (screened for any chronic or acute condition) and without clinical symptoms and our participants were non-smoking and young or middle-aged. The current study was not able to provide a clear explanation for the lack of consistency with previous studies repeatedly showing that short bursts of physical activity are increasing circulating brain-derived neurotrophic factor levels. However, to the knowledge of the author no other human study has attempted before to measure the effects of physical activity on BDNF in such high air pollution levels as found in this study. Furthermore, the clinical meaning of the decreases in BDNF as a response to intermitted physical activity in the experimental setting and the more habitual physical activity of daily life remains unclear but may be a transitional physiological response.

Conclusion

To conclude, this study suggests that habitual physical activity of daily life and also short-term intermittent physical activity in an acute exposure setting when performed in high and very high levels of TRAP can decrease circulating BDNF in healthy participants.

REFERENCES

1. Hamer M, Chida Y. Walking and primary prevention: a meta-analysis of prospective cohort studies. *Br J Sports Med* 2008; 42: 238–243.

2. Harriss DJ, Atkinson G, Batterham A, et al. Lifestyle factors and colorectal cancer risk (2): a systematic review and meta-analysis of associations with leisure-time physical activity. *Colorectal Dis Off J Assoc Coloproctology G B Irel* 2009; 11: 689–701.

3. Pate RR, Pratt M, Blair SN, et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA J Am Med Assoc* 1995; 273: 402–407.

4. Nimmo MA, Leggate M, Viana JL, et al. The effect of physical activity on mediators of inflammation. *Diabetes Obes Metab* 2013; 15 Suppl 3: 51–60.

5. Winchester J, Dick MB, Gillen D, et al. Walking stabilizes cognitive functioning in Alzheimer's disease (AD) across one year. *Arch Gerontol Geriatr* 2013; 56: 96–103.

6. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol Sci* 2003; 14: 125–130.

7. Adamson BC, Ensari I, Motl RW. The effect of exercise on depressive symptoms in adults with neurological disorders: A systematic review and meta-analysis. *Arch Phys Med Rehabil* 2015.

8. Masley S, Roetzheim R, Gualtieri T. Aerobic exercise enhances cognitive flexibility. *J Clin Psychol Med Settings* 2009; 16: 186–193.

9. Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handb Exp Pharmacol* 2014; 220: 223–250.

10. Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur J Neurosci* 2004; 20: 2580–2590.

11. Nofuji Y, Suwa M, Sasaki H, et al. Different circulating brain-derived neurotrophic factor responses to acute exercise between physically active and sedentary subjects. *J Sports Sci Med* 2012; 11: 83–88.

12. Babaei P, Damirchi A, Mehdipoor M, et al. Long term habitual exercise is associated with lower resting level of serum BDNF. *Neurosci Lett* 2014; 566: 304–308.

13. Griffin ÉW, Mullally S, Foley C, et al. Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. *Physiol Behav* 2011; 104: 934–941.

14. Guxens M, Sunyer J. A review of epidemiological studies on neuropsychological effects of air pollution. *Swiss Med Wkly* 2012; 141: w13322.

15. Genc S, Zadeoglulari Z, Fuss SH, et al. The adverse effects of air pollution on the nervous system. *J Toxicol* 2012; 2012: 782462.

16. Calderón-Garcidueñas L, Calderón-Garcidueñas A, Torres-Jardón R, et al. Air pollution and your brain: what do you need to know right now. *Prim Health Care Res Dev* 2014; 1–17.

17. Carlisle AJ, Sharp NCC. Exercise and outdoor ambient air pollution. *Br J Sports Med* 2001; 35: 214–222.

18. Zuurbier M, Hoek G, van den Hazel P, et al. Minute ventilation of cyclists, car and bus passengers: an experimental study. *Environ Health Glob Access Sci Source* 2009; 8: 48.

19. Zuurbier M, Hoek G, Oldenwening M, et al. Commuters' Exposure to Particulate Matter Air Pollution Is Affected by Mode of Transport, Fuel Type, and Route. *Environ Health Perspect* 2010; 118: 783–789.

20. Int Panis L, de Geus B, Vandenbulcke G, et al. Exposure to particulate matter in traffic: A comparison of cyclists and car passengers. *Atmos Environ* 2010; 44: 2263–2270.

21. Bos I, De Boever P, Int Panis L, et al. Physical Activity, Air Pollution and the Brain. *Sports Med Auckl NZ* 2014.

22. Bos I, Jacobs L, Nawrot TS, et al. No exercise-induced increase in serum BDNF after cycling near a major traffic road. *Neurosci Lett* 2011; 500: 129–132.

23. Nemmar A, Hoet PHM, Vanquickenborne B, et al. Passage of inhaled particles into the blood circulation in humans. *Circulation* 2002; 105: 411–414.

24. Jacobs L, Nawrot TS, de Geus B, et al. Subclinical responses in healthy cyclists briefly exposed to traffic-related air pollution: an intervention study. *Environ Health Glob Access Sci Source* 2010; 9: 64.

25. Riediker M, Cascio WE, Griggs TR, et al. Particulate Matter Exposure in Cars Is Associated with Cardiovascular Effects in Healthy Young Men. *Am J Respir Crit Care Med* 2004; 169: 934–940.

26. De Nazelle A, Seto E, Donaire-Gonzalez D, et al. Improving estimates of air pollution exposure through ubiquitous sensing technologies. *Environ Pollut Barking Essex 1987* 2013; 176: 92–99.

27. Gulati M, Shaw LJ, Thisted RA, et al. Heart rate response to exercise stress testing in asymptomatic women: the st. James women take heart project. *Circulation* 2010; 122: 130–137.

28. Westerdahl D, Fruin S, Sax T, et al. Mobile platform measurements of ultrafine particles and associated pollutant concentrations on freeways and residential streets in Los Angeles. *Atmos Environ* 2005; 39: 3597–3610.

29. Kirchstetter TW, Novakov T. Controlled generation of black carbon particles from a diffusion flame and applications in evaluating black carbon measurement methods. *Atmos Environ* 2007; 41: 1874–1888.

30. Wang X, Westerdahl D, Wu Y, et al. On-road emission factor distributions of individual diesel vehicles in and around Beijing, China. *Atmos Environ* 2011; 45: 503–513.

31. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* 2000; 32: S498–504.

32. Beelen R, Hoek G, Vienneau D, et al. Development of NO2 and NOx land use regression models for estimating air pollution exposure in 36 study areas in Europe – The ESCAPE project. *Atmos Environ* 2013; 72: 10–23.

33. McCreanor J, Cullinan P, Nieuwenhuijsen MJ, et al. Respiratory effects of exposure to diesel traffic in persons with asthma. *N Engl J Med* 2007; 357: 2348–2358.

34. Strak M, Boogaard H, Meliefste K, et al. Respiratory health effects of ultrafine and fine particle exposure in cyclists. *Occup Environ Med* 2010; 67: 118–124.

35. Bos I, DE Boever P, Vanparijs J, et al. Subclinical effects of aerobic training in urban environment. *Med Sci Sports Exerc* 2013; 45: 439–447.

36. Gong H Jr, Linn WS, Clark KW, et al. Exposures of healthy and asthmatic volunteers to concentrated ambient ultrafine particles in Los Angeles. *Inhal Toxicol* 2008; 20: 533–545.

37. Samet JM, Rappold A, Graff D, et al. Concentrated ambient ultrafine particle exposure induces cardiac changes in young healthy volunteers. *Am J Respir Crit Care Med* 2009; 179: 1034–1042.

38. Jarjour S, Jerrett M, Westerdahl D, et al. Cyclist route choice, traffic-related air pollution, and lung function: a scripted exposure study. *Environ Health Glob Access Sci Source* 2013; 12: 14.

39. Weichenthal S, Kulka R, Dubeau A, et al. Traffic-related air pollution and acute changes in heart rate variability and respiratory function in urban cyclists. *Environ Health Perspect* 2011; 119: 1373–1378.

40. Carslaw DC, Beevers SD. Estimations of road vehicle primary NO2 exhaust emission fractions using monitoring data in London. *Atmos Environ* 2005; 39: 167–177.

41. Minguillón MC, Cirach M, Hoek G, et al. Spatial variability of trace elements and sources for improved exposure assessment in Barcelona. *Atmos Environ* 2014; 89: 268–281.

42. Bus B a. A, Molendijk ML, Penninx BJWH, et al. Determinants of serum brain-derived neurotrophic factor. *Psychoneuroendocrinology* 2011; 36: 228–239.

43. Jung SH, Kim J, Davis JM, et al. Association among basal serum BDNF, cardiorespiratory fitness and cardiovascular disease risk factors in untrained healthy Korean men. *Eur J Appl Physiol* 2011; 111: 303–311.

44. Nofuji Y, Suwa M, Moriyama Y, et al. Decreased serum brain-derived neurotrophic factor in trained men. *Neurosci Lett* 2008; 437: 29–32.

45. Cyrys J, Eeftens M, Heinrich J, et al. Variation of NO2 and NOx concentrations between and within 36 European study areas: Results from the ESCAPE study. *Atmos Environ* 2012; 62: 374–390.

46. Hoffmann B, Moebus S, Dragano N, et al. Chronic residential exposure to particulate matter air pollution and systemic inflammatory markers. *Environ Health Perspect* 2009; 117: 1302–1308.

47. Heyman E, Gamelin F-X, Goekint M, et al. Intense exercise increases circulating endocannabinoid and BDNF levels in humans—Possible implications for reward and depression. *Psychoneuroendocrinology* 2012; 37: 844–851.

48. Coelho FG de M, Gobbi S, Andreatto CAA, et al. Physical exercise modulates peripheral levels of brain-derived neurotrophic factor (BDNF): a systematic review of experimental studies in the elderly. *Arch Gerontol Geriatr* 2013; 56: 10–15.

49. Huang Y-CT, Rappold AG, Graff DW, et al. Synergistic effects of exposure to concentrated ambient fine pollution particles and nitrogen dioxide in humans. *Inhal Toxicol* 2012; 24: 790–797.

Supplemental Material

Paper III

Supplementary Annex

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:
Kubesch N, Guerra S, De Nazelle A, Martinez D, Bouso L, Carrasco-Turigas G, Westerdahl D, Nieuwenhuijsen MJ. Brain-derived neurotrophic factor (BDNF) serum level decrease after physical activity in high levels of traffic-related air pollution.

Contents

Figure S2: High TRAP exposure site set up: Pedestrian Bridge above main city ring roadway "Ronda Litoral" in Barcelona

a) View from the roadway during off peak time:

Copyright: Google Maps

b) View from our exposure site at the pedestrian bridge during morning rush hour:

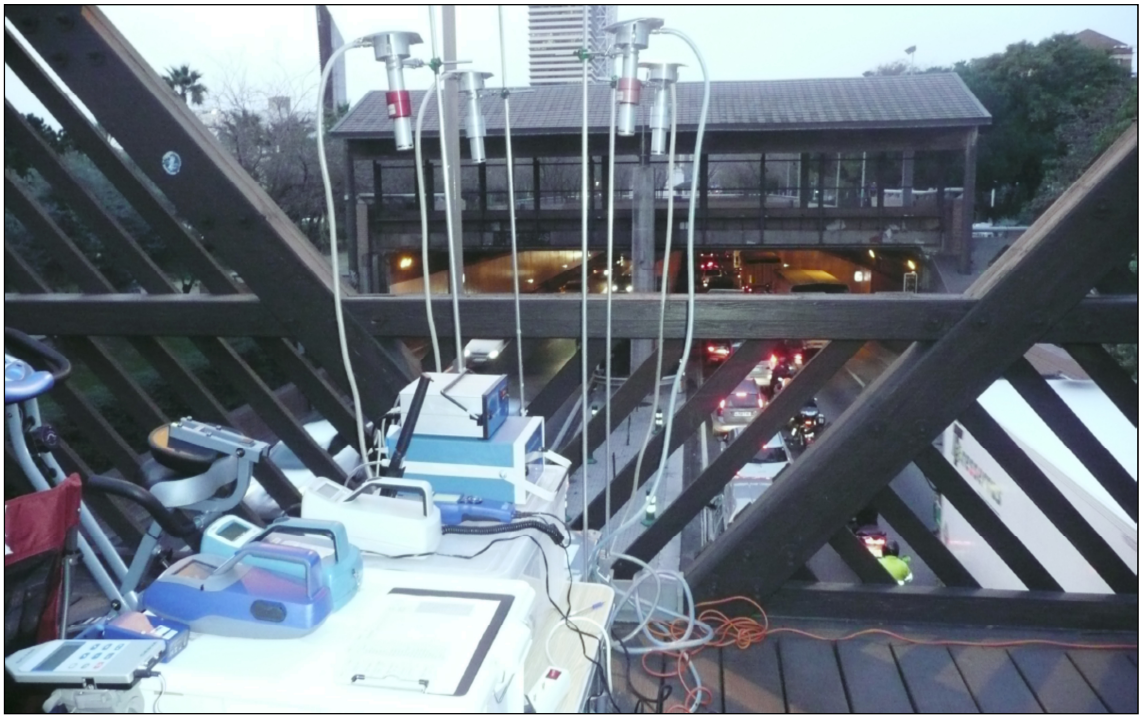

Table S2. Spearman's rank-correlation coefficients (r) between measured pollutants

	UFP	BC	NOx	PM10	PM25
UFP	-	-	-	-	-
BC	**0.92**	-	-	-	-
NO_x	**0.93**	**0.95**	-	-	-
PM_{10}	0.79	0.87	0.84	-	-
$PM_{2.5}$	0.85	**0.93**	**0.91**	**0.94**	-
PM_{coarse}*	0.41	0.50	0.45	0.80	0.58

* PM_{coarse} fraction calculated PM10 minus PM2.5

Table S3. Variance between baseline BDNF levels by the four exposure scenarios (ANOVA)

	Low TRAP & Rest§	High TRAP & PA§	Low TRAP & PA§	High TRAP & Rest§	ANOVA*
BDNF (pg/ml)	21481.50	22435.78	21041.36	20336.05	0.275

* Significance test (box corrected)
§ n=28

Table S4. Pre-post BDNF levels comparison within PA, Rest, High, and Low TRAP Exposure Groups*

	Without Adjustment for TRAP				With Adjustment for TRAP			
	Coef.(pg/mL)	[95%	CI]	P Value	Coef.(pg/mL)	[95%	CI]	P Value
PA†	-1611.57	-3251.15	28.02	0.054	-1611.57	-3211.14	-12.00	**0.048**
Rest†	770.75	-738.57	2280.07	0.317	770.75	-721.22	2262.72	0.311
	Without Adjustment for PA				**With Adjustment for PA**			
	Coef.(pg/mL)	[95%	CI]	P Value	Coef.(pg/mL)	[95%	CI]	P Value
High TRAP Site *f*	218.52	-1308.01	1745.05	0.779	218.52	-1294.20	1731.24	0.777
Low TRAP Site *f*	-1059.34	-2959.14	840.46	0.274	-1059.34	-2925.39	806.72	0.266

* Mixed effect models comparing the pre- to post-exposure BDNF level change, adjusted for on-site ambient temperature and relative humidity, age, BMI, sex, as well as total energy expenditure (MET) three days prior to exposure, environmental tobacco smoke, and NO_2 exposure for 24h before experiment.

† Pooled analysis for physical activity (PA) and Rest, irrespective of the TRAP exposure status.

f Pooled analysis for High and Low Exposure site, irrespective of the physical activity status.

5. METHODOLOGICAL DISCUSSION

5.1. Summary

The work presented in the current thesis was conducted within the framework of the TAPAS project (**T**ransportation, **A**ir **P**ollution and **P**hysical **A**ctivitie**S**: An Integrated Health Risk Assessment Programme of Climate Change and Urban Policies), which aimed to study the risks and benefits of physical activity through active commuting from policy and experimental perspectives.

The underlying hypothesis of this work was whether a short-term exposure to high levels of TRAP had adverse effects and physical activity would modify these effects. The main aim of this thesis was to contribute to the ongoing debate regarding health risks of physical activity in traffic-related air pollution, which often takes place during active travel, such as cycling, and also to disentangle the effects attributed to each of the two factors. Using a unique design with a rich panel of air pollutants and a range of health effects markers, this study was designed to contribute to the scarce knowledge about the independent health effects related to TRAP exposure and physical activity, as well as their interdependencies. In addition, this study provided some evidence that might help to resolve the puzzle of the underlying pathomechanisms of the health effects found in long-term TRAP exposure studies.

In our controlled multifactorial cross-over study design we exposed healthy volunteers to high and lower levels of TRAP, while they were cycling or at rest. In total, 117 observations were obtained from 31 volunteers on 59 experiment days, while 112 observations from 28 volunteers could be considered for analysis. The markers of pulmonary function and inflammation, systemic inflammation, blood pressure, as well the effect on brain-derived neurotrophic factor were studied for this thesis.

We found high levels of traffic-related air pollution in both exposure sites, both located near the sea coastline. Despite the fact that the TRAP levels at both exposure sites were relatively high, the levels were contrasting between the sites. The average

concentrations of ultrafine particles, BC, and NO_x levels at the high TRAP site were five, seven, and ten times higher than those measured at the low TRAP site, respectively. However, the high correlations among the pollutants made it difficult to separate the effects on the health endpoints. The average ultrafine particles levels found at our high traffic site were considerably higher than those found in previous real-world exposure studies in the UK, the Netherlands, or Belgium (McCreanor et al. 2007; Strak et al. 2010; Inge Bos et al. 2013). Mean particle concentrations in our high air pollution site were comparable with exposures measured in chamber studies (Gong et al. 2008; Samet et al. 2009) while our 'low exposure site' air pollution levels were comparable with those categorized in other experimental studies as 'high air pollution' or 'traffic site' (Jarjour et al. 2013; Weichenthal et al. 2011). The levels of NOx at our high TRAP site were very high as well. Few studies measure NOx right in traffic, but levels found in this study are not unusual for road environments, especially where high proportion of diesel vehicles can be found (Carslaw and Beevers 2005). Both of our study sites were very close to the sea-coast, with Barcelona's harbour nearby resulting in an influence of marine sources and shipping emissions on the particulate matter composition. The warm Mediterranean climate in Barcelona tends to increase the contents of road traffic and fuel oil combustion due to re-suspension, which is typical of warm seasons (Minguillón et al. 2014).

We showed that a short-term intermittent PA compared with rest, was associated with a lower SBP after exposure, irrespective of the level of TRAP. Exposure at the high-TRAP site, compared with the low-TRAP site, led to higher SBP and DBP post-exposure. Exposure to BC, UFP, NO_x, $PM_{2.5}$ and PM_{10}, and PM_{coarse} increased SBP post-exposure. Post-exposure DBP was associated with BC, UFP, NO_x, and PM_{10}. We only found evidence for an interaction between PA and PM_{10} and PM_{coarse} which lead to increased SBP post-exposure. Furthermore, we showed that short-term intermittent PA was associated with a small but statistically significant increase in FEV_1, FVC, $FEF_{25-75\%}$, exhaled NO, leucocytes, neutrophil counts, and IL-6. $PM_{2.5}$ and PM_{10} increased leucocytes and neutrophils. Ultrafine particulate matter decreased IL-8. We found limited and inconsistent evidence of an interaction between PA and UFP, as well as between $PM_{2.5}$ and PM_{10} for pulmonary and systemic inflammation. We also showed

that short-term intermittent PA in a controlled experimental setting as well as habitual physical activity during three days preceding the blood sample in a highly polluted environment could decrease serum BDNF. We found no evidence for an interaction between physical activity and TRAP.

5.2. Methodology: strengths and limitations

The strengths of this study are its well-controlled experimental design that allows to disentangle the effects of TRAP from those of physical activity, its rich dataset of air pollution components, and the determination of multiple health endpoints. This study involved a range of clinically relevant biomarkers such as blood pressure, lung function, lung inflammation, and systemic inflammation.

Furthermore, we used a real-world exposure experimental design that brings the advantage of a realistic exposure mix, including its synergies and interactions which have been shown to promote stronger health effects than those shown in exposure chamber settings. Studies conducted in a chamber have the advantage of more precise exposure assessment and fewer effects that are uncontrollable, but they have the limitation of an unreal exposure mix and the missing factors that might change the actual health responses as they would only occur in a real-world exposure setting.

The author of this work would like to acknowledge the study's limitations that are relevant for the interpretation of the results and their impacts. The two-hours intermitted physical activity summing up for one-hour of PA in total protocol during the exposure sessions may not be the most realistic physical activity protocol; However, the two-hour exposure session was chosen to a) increase the changes in the observation of health responses to TRAP exposure, b) because two-hours is the time that citizens in Barcelona spent on average in travel (de Nazelle et al. 2013), and c) to maintain comparability with previous experimental chamber and real-world studies which also used often a two-hour exposure time. However, two-hours or even one-hour continuous cycling during the exposure session seemed to be too strenuous for reflecting a moderately intense active travel mode.

High correlations between the air pollutants mixture were found at our sites. The variables must have sufficiently low correlations with each other, in particular, in two-pollutant models. Hence, we opted only for single pollutant models.

Misclassification of air pollution exposure is a well-recognized limitation in epidemiologic studies on the environment and health effects: Stronger associations are likely to be observed with air pollutants with less measurement errors (Zeger et al. 2000). Our air pollution characterization was performed on-site next to the participants; the measurement error was mainly restricted to instrumental errors that are negligible compared with errors in observational studies based on data derived from fixed monitoring stations.

We studied healthy volunteers without any pathological conditions (except atopy) who were non-smoking people only. Also, volunteers knew before recruitment that the study included outdoor physical activity. A fact that could have repelled potential participants that are generally reluctant to perform physical activity. Both factors might have contributed to our study population being more involved in healthier lifestyle in general than the average population in Spain. That might have influenced the findings of this study in general.

5.3. Further research needs

This study focusses on the short-term effects up to six hours after exposure to contribute to the understanding of the health effects that were observed in epidemiological studies. Our assessment shows the effects of a single exposure occasion because several days were left between one exposure occasion and the following. However, in a daily life setting, active travelling commuters might be exposed to similar exposures repeatedly during an entire working week. We cannot draw a conclusion with respect to the accumulating health effects that occur after several consecutive repetitions of such short-term exposures. Such a study design may reveal an impact on health, which is

different from the one found in this study due to an accumulation of exposures and their effects.

Furthermore, we assessed the health effect in a healthy population in absence of any acute or chronic conditions. The balance between the risk and benefits may be different in a diverse population. The health effects and, in particular, the interaction between the factors remain unclear in a population susceptible to certain diseases and should be assessed in a similar study design in participants with chronic conditions, elderly people, or children and would need further research.

Moreover, there may be a potential influence by the fact that our study population was mainly resident and working in Barcelona city, a place where air pollution levels are among the highest within Europe (Cyrys et al. 2012). Our study population might be adapted to the acute effects. We cannot exclude that the acute changes in a study population that does not live amid such high exposure levels found in Barcelona city may show a more pronounced response, which should be further explored.

Despite the fact that we measured a broad range of health end-points, the author would like to point out that all possible health effects of the different organ systems and similarly of susceptible populations need to be assessed before an accurate general recommendation concerning the safety and the health benefits of exercising in an environment with high TRAP levels can be given.

6. CONCLUSIONS AND IMPLICATIONS FOR PUBLIC HEALTH

6.1. Conclusions

To conclude, this study suggests that both SBP and DBP increase with TRAP exposure. The beneficial effects of PA on SBP are present at both low and high levels of TRAP, but higher in low air pollution levels, in which PA overcomes the effect of TRAP. The higher minute ventilation, and hence potentially higher intake of pollutants due to the intermittent PA, does not generally increase the adverse effects of TRAP, the only exception being coarse and larger PM. This study also provides evidence for a preventive potential of PA against the acute adverse effects of TRAP.

Furthermore, this study suggests that in healthy participants, intermittent moderate PA has beneficial effects on pulmonary functions, even when performed in a highly traffic-related polluted environment. This study also suggests that particulate air pollution is inducing airway and systemic inflammatory processes. We recommend that future studies assessing the health effects of air pollution in active travel modes should account for the effect of PA. All changes in the selected biomarkers were small and observed in a healthy population without clinical symptoms. The clinical relevance as well as the reversibility of these effects remains unclear. We propose assessment of both in similar designs in multiple repeated short-term exposures.

This study also shows that both, habitual physical activity of daily life [reflected by the total energy expenditure (MET) accumulated in three consecutive days] and short-term intermittent physical activity in an acute exposure setting, performed in high and very high levels of TRAP, can decrease circulating BDNF in healthy participants. The current study has not been able to provide a clear explanation for the lack of consistency with previous studies showing that short bursts of physical activity are increasing circulating BDNF levels. However, to the best knowledge of the author no other human study has attempted to measure the effects of physical activity on BDNF in such high air pollution levels as found in this study.

Overall**,** this study suggests that

a) The short-term exposure to traffic-related air pollution induces airway and systemic inflammatory responses, and increases blood pressure.
b) Physical activity can attenuate the effects of TRAP on SBP and induce beneficial effects on lung function. However, physical activity in high levels of TRAP also induces increases in neutrophils, leucocytes, and exhaled nitric oxide, which suggests systemic and airway inflammatory responses. Furthermore, physical activity in high levels of TRAP decreases circulating levels of BDNF. The clinical meaning of these decreases as a response to intermitted physical activity in the experimental setting and the more habitual physical activity in daily life remains unclear, but may be a transitional physiological response.
c) The health effects of traffic-related air pollution and physical activity are generally independent with the exception of the combinations with PM_{10}, and also $PM_{2.5}$, which seem to potentiate increases in SBP.
d) Summing up, real-world short-term exposure to TRAP has some adverse effects on health. Despite insular adverse interactions with pollutants and few potentially adverse but more likely physiological responses to physical activity, this study more consistently suggests that physical activity, even when performed in high levels of TRAP has a preventive potential and beneficial health effects in a healthy population few hours after exposure. A key message of this study lies in the results, which show that the different factors, TRAP and intermittent physical activity, were generally independently responsible for different health effects. The findings of this study underline the importance of assessing the levels of physical activity in the assessment of the health effects of TRAP in such studies that also involve physical activity.

6.2. Relevance for public health

We showed that physical activity has beneficial effects, even when performed in high levels of traffic-related air pollution, which supports the findings of the previous risk assessment studies concluding that positive effects of physical activity are existent even in close proximity to traffic as may occur during active commuting (de Nazelle et al.

2011; Hartog et al. 2011; Rojas-Rueda et al. 2011). Our clear and consistent associations with physical activity, which were robust against adjustment for other airborne components, such as pollen and other air pollution exposures preceding the experiment days, could be seen as a support for the recommendation to maintain sufficient physical activity levels, even when an air pollution exposure is not effortlessly avoidable. This recommendation is supported by the fact that we did not find an interaction between physical activity and the TRAP exposure despite the increased inhaled minute volume of physically active people and their increased deposition during exercise, which suggests that the effects are independent. We did not find any declines in pulmonary function and only subclinical increases in systemic inflammation. We also did not find clearly attributable adverse effects on BDNF due to high TRAP in comparison with lower TRAP exposures.

However, even though the health effects of the traffic-related air pollution exposure were limited in this study, a public health impact may still be present if we take into account that a high number of people, including a more vulnerable subpopulation, do live in urban environments under exposure to TRAP on a daily basis. In addition, the exposure coming from traffic adds on other exposures from other air pollution sources and environmental toxins. Recurrent daily exposures might be more relevant from a public health perspective since they still might lead to severe effects.

Exposure to air pollution is associated with several adverse health outcomes, including asthma attacks and abnormal heart rhythms. However, as exercise in general has clear health benefits, and appears to be relatively safe even in high levels of traffic-related air pollution. Therefore, physical activity should not be avoided unless such is recommended by a health professional after an assessment of the individual health status. In certain circumstances it may more beneficial for the individual´s health to avoid certain types of exercise in general or in certain conditions such as in episodes of higher levels of air pollution.

Considering the limitations of this study, such as the unclear health effect of an accumulating exposure or the effects in a more susceptible population, it is recommendable to consider ways to minimize the exposure and the risks of the traffic-

related air pollution in combination with or without exercise. There are several possibilities to limit personal exposure to TRAP:

a. A simple way to decrease personal exposure is to avoid physical activity or even exercises during traffic rush hours. Also, the population should be informed about the fact that certain weather conditions, such as rain and wind, decrease pollution levels due to a dilution effect.

b. Increasing the distance to traffic will substantially lower the exposure to pollutants. There is a large spatial gradient in the pollutant levels, as they decrease exponentially with distance from traffic. The latter point is of particular importance for the active commuting population, e.g., those that cycle to work. As the working population is often limited in their selection of the hour for commuting, it often occurs during rush hours when TRAP is known to be higher. Cyclists can reduce their personal exposure considerably by choosing an alternative route and cycling away from heavy traffic.

c. Workouts or exercises that are not part of commutes could be planned more carefully in terms of timing and location: Outdoor exercising could be avoided when an air quality alert has been issued and during rush hours, also it could be avoided in close proximity to a main roadway, else, the intensity and duration of this outdoor exercise could be limited.

The findings of this work could encourage the healthy population to perform outdoor physical activity and to choose an active mode for commuting. However, given the limitations of this study, awareness for TRAP exposures should be created. During work outs or for daily physical activities such as commutes, the close proximity to traffic should be avoided as mentioned earlier, and, ideally a green (or blue) environment should be chosen whenever possible.

This study showed only a few adverse effects of the short-term exposure to high levels of TRAP our findings. However, that should not limit the importance and priority for policymakers and city planners to make an effort towards infrastructural changes that

help to reduce TRAP exposure during active commuting and to improve the air quality of urban environment where citizens exercise.

Besides these infrastructural changes, the general decrease of the air pollution levels in cities with high traffic volume remains important. A variety of air pollution reduction programmes should be adopted. In addition to the need for government action, more awareness among citizens towards their individual responsibility should be raised. They must be more ambitious and try to improve air quality by reducing their own emissions.

REFERENCES

Adar, Sara Dubowsky, Gary Adamkiewicz, Diane R Gold, Joel Schwartz, Brent A Coull, and Helen Suh. 2007. "Ambient and Microenvironmental Particles and Exhaled Nitric Oxide before and after a Group Bus Trip." *Environmental Health Perspectives* 115 (4): 507–12.

Adar, Sara Dubowsky, Diane R Gold, Brent A Coull, Joel Schwartz, Peter H Stone, and Helen Suh. 2007. "Focused Exposures to Airborne Traffic Particles and Heart Rate Variability in the Elderly." *Epidemiology (Cambridge, Mass.)* 18 (1): 95–103.

Ainsworth, B E, W L Haskell, M C Whitt, M L Irwin, A M Swartz, S J Strath, W L O'Brien, et al. 2000. "Compendium of Physical Activities: An Update of Activity Codes and MET Intensities." *Medicine and Science in Sports and Exercise* 32 (9 Suppl): S498–504.

American Thoracic Society and European Respiratory Society. 2005. "ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005." *American Journal of Respiratory and Critical Care Medicine* 171 (8): 912–30.

Beelen, Rob, Gerard Hoek, Danielle Vienneau, Marloes Eeftens, Konstantina Dimakopoulou, Xanthi Pedeli, Ming-Yi Tsai, et al. 2013. "Development of NO2 and NOx Land Use Regression Models for Estimating Air Pollution Exposure in 36 Study Areas in Europe – The ESCAPE Project." *Atmospheric Environment* 72 (June): 10–23.

Beelen, Rob, Ole Raaschou-Nielsen, Massimo Stafoggia, Zorana Jovanovic Andersen, Gudrun Weinmayr, Barbara Hoffmann, Kathrin Wolf, et al. 2014. "Effects of Long-Term Exposure to Air Pollution on Natural-Cause Mortality: An Analysis of 22 European Cohorts within the Multicentre ESCAPE Project." *Lancet* 383 (9919): 785–95.

Behndig, A. F., I. S. Mudway, J. L. Brown, N. Stenfors, R. Helleday, S. T. Duggan, S. J. Wilson, et al. 2006. "Airway Antioxidant and Inflammatory Responses to Diesel Exhaust Exposure in Healthy Humans." *The European Respiratory Journal* 27 (2): 359–65.

Bherer, Louis, Kirk I. Erickson, and Teresa Liu-Ambrose. 2013. "A Review of the Effects of Physical Activity and Exercise on Cognitive and Brain Functions in Older Adults." *Journal of Aging Research* 2013: 657508.

Bos, I., P. De Boever, L. Int Panis, S. Sarre, and R. Meeusen. 2012. "Negative Effects of Ultrafine Particle Exposure during Forced Exercise on the Expression of Brain-Derived Neurotrophic Factor in the Hippocampus of Rats." *Neuroscience* 223 (October): 131–39.

Bos, I, L Jacobs, T S Nawrot, B de Geus, R Torfs, L Int Panis, B Degraeuwe, and R Meeusen. 2011. "No Exercise-Induced Increase in Serum BDNF after Cycling near a Major Traffic Road." *Neuroscience Letters* 500 (2): 129–32.

Bos, I., L. Jacobs, T.S. Nawrot, B. de Geus, R. Torfs, L. Int Panis, B. Degraeuwe, and R. Meeusen. 2011. "No Exercise-Induced Increase in Serum BDNF after Cycling near a Major Traffic Road." *Neuroscience Letters* 500 (2): 129–32.

Bos, Inge, Patrick DE Boever, Jef Vanparijs, Nathalie Pattyn, Luc Int Panis, and Romain Meeusen. 2013. "Subclinical Effects of Aerobic Training in Urban Environment." *Medicine and Science in Sports and Exercise* 45 (3): 439–47.

Broeckaert, F, K Arsalane, C Hermans, E Bergamaschi, A Brustolin, A Mutti, and A Bernard. 2000. "Serum Clara Cell Protein: A Sensitive Biomarker of Increased Lung Epithelium Permeability Caused by Ambient Ozone." *Environmental Health Perspectives* 108 (6): 533–37.

Brook, Robert D, and Sanjay Rajagopalan. 2009. "Particulate Matter, Air Pollution, and Blood Pressure." *Journal of the American Society of Hypertension: JASH* 3 (5): 332–50.

Brook, Robert D, Sanjay Rajagopalan, C Arden Pope 3rd, Jeffrey R Brook, Aruni Bhatnagar, Ana V Diez-Roux, Fernando Holguin, et al. 2010. "Particulate Matter Air Pollution and Cardiovascular Disease: An Update to the Scientific Statement from the American Heart Association." *Circulation* 121 (21): 2331–78.

Brook, Robert D, Bruce Urch, J Timothy Dvonch, Robert L Bard, Mary Speck, Gerald Keeler, Masako Morishita, et al. 2009. "Insights into the Mechanisms and Mediators of the Effects of Air Pollution Exposure on Blood Pressure and Vascular Function in Healthy Humans." *Hypertension* 54 (3): 659–67.

Calderón-Garcidueñas, Lilian, Ana Calderón-Garcidueñas, Ricardo Torres-Jardón, José Avila-Ramírez, Randy J. Kulesza, and Amedeo D. Angiulli. 2014. "Air Pollution and Your Brain: What Do You Need to Know Right Now." *Primary Health Care Research & Development*, September, 1–17.

Carlisle, A, and N Sharp. 2001. "Exercise and Outdoor Ambient Air Pollution." *British Journal of Sports Medicine* 35 (4): 214–22.

Carslaw, David C., and Sean D. Beevers. 2005. "Estimations of Road Vehicle Primary NO2 Exhaust Emission Fractions Using Monitoring Data in London." *Atmospheric Environment* 39 (1): 167–77.

Chen, Bingheng, and Haidong Kan. 2008. "Air Pollution and Population Health: A Global Challenge." *Environmental Health and Preventive Medicine* 13 (2): 94–101.

Chobanian, Aram V, George L Bakris, Henry R Black, William C Cushman, Lee A Green, Joseph L Izzo Jr, Daniel W Jones, et al. 2003. "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 Report." *JAMA: The Journal of the American Medical Association* 289 (19): 2560–72.

Cornelissen, Véronique A, and Robert H Fagard. 2005. "Effect of Resistance Training on Resting Blood Pressure: A Meta-Analysis of Randomized Controlled Trials." *Journal of Hypertension* 23 (2): 251–59.

Cornelissen, Véronique A, Robert H Fagard, Ellen Coeckelberghs, and Luc Vanhees. 2011. "Impact of Resistance Training on Blood Pressure and Other Cardiovascular Risk Factors: A Meta-Analysis of Randomized, Controlled Trials." *Hypertension* 58 (5): 950–58.

Cotman, Carl W., and Nicole C. Berchtold. 2002. "Exercise: A Behavioral Intervention to Enhance Brain Health and Plasticity." *Trends in Neurosciences* 25 (6): 295–301.

Cotman, Carl W., and Christie Engesser-Cesar. 2002. "Exercise Enhances and Protects Brain Function." *Exercise and Sport Sciences Reviews* 30 (2): 75–79.

Cyrys, Josef, Marloes Eeftens, Joachim Heinrich, Christophe Ampe, Alexandre Armengaud, Rob Beelen, Tom Bellander, et al. 2012. "Variation of NO2 and NOx Concentrations between and within 36 European Study Areas: Results from the ESCAPE Study." *Atmospheric Environment* 62: 374–90.

Daigle, Christopher C, David C Chalupa, F Raymond Gibb, Paul E Morrow, Günter Oberdörster, Mark J Utell, and Mark W Frampton. 2003. "Ultrafine Particle Deposition in Humans during Rest and Exercise." *Inhalation Toxicology* 15 (6): 539–52.

Dales, Robert, Ling Liu, Mietek Szyszkowicz, Mary Dalipaj, Jeff Willey, Ryan Kulka, and Terrence D Ruddy. 2007. "Particulate Air Pollution and Vascular Reactivity: The Bus Stop Study." *International Archives of Occupational and Environmental Health* 81 (2): 159–64.

De Nazelle, Audrey, Mark J Nieuwenhuijsen, Josep M Antó, Michael Brauer, David Briggs, Charlotte Braun-Fahrlander, Nick Cavill, et al. 2011. "Improving Health through Policies That Promote Active Travel: A Review of Evidence to Support Integrated Health Impact Assessment." *Environment International* 37 (4): 766–77.

De Nazelle, Audrey, Edmund Seto, David Donaire-Gonzalez, Michelle Mendez, Jaume Matamala, Mark J Nieuwenhuijsen, and Michael Jerrett. 2013. "Improving Estimates of Air Pollution Exposure through Ubiquitous Sensing Technologies." *Environmental Pollution (Barking, Essex : 1987)* 176 (May): 92–99.

Dockery, Douglas W. 2009. “Health Effects of Particulate Air Pollution.” *Annals of Epidemiology* 19 (4): 257–63.

Dufaux, B., U. Order, H. Geyer, and W. Hollmann. 1984. “C-Reactive Protein Serum Concentrations in Well-Trained Athletes.” *International Journal of Sports Medicine* 5 (2): 102–6.

Genc, Sermin, Zeynep Zadeoglulari, Stefan H. Fuss, and Kursad Genc. 2012. “The Adverse Effects of Air Pollution on the Nervous System.” *Journal of Toxicology* 2012: 782462.

Gong, Henry, Jr, William S Linn, Kenneth W Clark, Karen R Anderson, Constantinos Sioutas, Neil E Alexis, Wayne E Cascio, and Robert B Devlin. 2008. “Exposures of Healthy and Asthmatic Volunteers to Concentrated Ambient Ultrafine Particles in Los Angeles.” *Inhalation Toxicology* 20 (6): 533–45.

Gulati, Martha, Leslee J Shaw, Ronald A Thisted, Henry R Black, C Noel Bairey Merz, and Morton F Arnsdorf. 2010. “Heart Rate Response to Exercise Stress Testing in Asymptomatic Women: The St. James Women Take Heart Project.” *Circulation* 122 (2): 130–37.

Guxens, Mònica, and Jordi Sunyer. 2012. “A Review of Epidemiological Studies on Neuropsychological Effects of Air Pollution.” *Swiss Medical Weekly* 141: w13322.

Hamer, M, and Y Chida. 2008. “Walking and Primary Prevention: A Meta-Analysis of Prospective Cohort Studies.” *British Journal of Sports Medicine* 42 (4): 238–43.

Harriss, D J, G Atkinson, A Batterham, K George, N Tim Cable, T Reilly, N Haboubi, and A G Renehan. 2009. “Lifestyle Factors and Colorectal Cancer Risk (2): A Systematic Review and Meta-Analysis of Associations with Leisure-Time Physical Activity.” *Colorectal Disease: The Official Journal of the Association of Coloproctology of Great Britain and Ireland* 11 (7): 689–701.

Hartog, Jeroen Johan de, Hanna Boogaard, Hans Nijland, and Gerard Hoek. 2011. “Do the Health Benefits of Cycling Outweigh the Risks?” *Ciência & Saúde Coletiva* 16 (12): 4731–44.

Hoek, Gerard, Ranjini M Krishnan, Rob Beelen, Annette Peters, Bart Ostro, Bert Brunekreef, and Joel D Kaufman. 2013. “Long-Term Air Pollution Exposure and Cardio- Respiratory Mortality: A Review.” *Environmental Health: A Global Access Science Source* 12 (1): 43.

Hötting, Kirsten, and Brigitte Röder. 2013. “Beneficial Effects of Physical Exercise on Neuroplasticity and Cognition.” *Neuroscience and Biobehavioral Reviews* 37 (9 Pt B): 2243–57.

Huang, E. J., and L. F. Reichardt. 2001. “Neurotrophins: Roles in Neuronal Development and Function.” *Annual Review of Neuroscience* 24: 677–736.

Huang, Jing, Furong Deng, Shaowei Wu, Henry Lu, Yu Hao, and Xinbiao Guo. 2013. "The Impacts of Short-Term Exposure to Noise and Traffic-Related Air Pollution on Heart Rate Variability in Young Healthy Adults." *Journal of Exposure Science & Environmental Epidemiology* 23 (5): 559–64.

Huang, Yuh-Chin T, Ana G Rappold, Donald W Graff, Andrew J Ghio, and Robert B Devlin. 2012. "Synergistic Effects of Exposure to Concentrated Ambient Fine Pollution Particles and Nitrogen Dioxide in Humans." *Inhalation Toxicology* 24 (12): 790–97.

Janssen, Nicole A.H., Gerard Hoek, Milena Simic-Lawson, Paul Fischer, Leendert van Bree, Harry ten Brink, Menno Keuken, et al. 2011. "Black Carbon as an Additional Indicator of the Adverse Health Effects of Airborne Particles Compared with PM10 and PM2.5." *Environmental Health Perspectives* 119 (12): 1691–99.

Jarjour, Sarah, Michael Jerrett, Dane Westerdahl, Audrey Nazelle, Cooper Hanning, Laura Daly, Jonah Lipsitt, and John Balmes. 2013. "Cyclist Route Choice, Traffic-Related Air Pollution, and Lung Function: A Scripted Exposure Study." *Environmental Health: A Global Access Science Source* 12 (1): 14.

Kasapis, Christos, and Paul D Thompson. 2005. "The Effects of Physical Activity on Serum C-Reactive Protein and Inflammatory Markers: A Systematic Review." *Journal of the American College of Cardiology* 45 (10): 1563–69.

Kaur, S., M. J. Nieuwenhuijsen, and R. N. Colvile. 2007. "Fine Particulate Matter and Carbon Monoxide Exposure Concentrations in Urban Street Transport Microenvironments." *Atmospheric Environment* 41 (23): 4781–4810.

Kirchstetter, Thomas W., and T. Novakov. 2007. "Controlled Generation of Black Carbon Particles from a Diffusion Flame and Applications in Evaluating Black Carbon Measurement Methods." *Atmospheric Environment* 41 (9): 1874–88.

Landers-Ramos, Rian Q., Nathan T. Jenkins, Espen E. Spangenburg, James M. Hagberg, and Steven J. Prior. 2014. "Circulating Angiogenic and Inflammatory Cytokine Responses to Acute Aerobic Exercise in Trained and Sedentary Young Men." *European Journal of Applied Physiology* 114 (7): 1377–84.

Langrish, Jeremy P., Jon Unosson, Jenny Bosson, Stefan Barath, Ala Muala, Scott Blackwell, Stefan Söderberg, et al. 2013. "Altered Nitric Oxide Bioavailability Contributes to Diesel Exhaust Inhalation-Induced Cardiovascular Dysfunction in Man." *Journal of the American Heart Association* 2 (1): e004309.

Larsson, B.-M., M. Sehlstedt, J. Grunewald, C. M. Sköld, A. Lundin, A. Blomberg, T. Sandström, A. Eklund, and M. Svartengren. 2007. "Road Tunnel Air Pollution Induces Bronchoalveolar Inflammation in Healthy Subjects." *European Respiratory Journal* 29 (4): 699–705.

Maynard, Dan, Brent A. Coull, Alexandros Gryparis, and Joel Schwartz. 2007. "Mortality Risk Associated with Short-Term Exposure to Traffic Particles and Sulfates." *Environmental Health Perspectives* 115 (5): 751–55.

McConnell, Rob, Kiros Berhane, Frank Gilliland, Stephanie J. London, Talat Islam, W. James Gauderman, Edward Avol, Helene G. Margolis, and John M. Peters. 2002. "Asthma in Exercising Children Exposed to Ozone: A Cohort Study." *Lancet* 359 (9304): 386–91.

McCreanor, James, Paul Cullinan, Mark J Nieuwenhuijsen, James Stewart-Evans, Eleni Malliarou, Lars Jarup, Robert Harrington, et al. 2007. "Respiratory Effects of Exposure to Diesel Traffic in Persons with Asthma." *The New England Journal of Medicine* 357 (23): 2348–58.

Miller, M R, J Hankinson, V Brusasco, F Burgos, R Casaburi, A Coates, R Crapo, et al. 2005. "Standardisation of Spirometry." *The European Respiratory Journal* 26 (2): 319–38.

Mills, Nicholas L, Ken Donaldson, Paddy W Hadoke, Nicholas A Boon, William MacNee, Flemming R Cassee, Thomas Sandström, Anders Blomberg, and David E Newby. 2009. "Adverse Cardiovascular Effects of Air Pollution." *Nature Clinical Practice. Cardiovascular Medicine* 6 (1): 36–44.

Mills, Nicholas L, Håkan Törnqvist, Simon D Robinson, Manuel Gonzalez, Kareen Darnley, William MacNee, Nicholas A Boon, et al. 2005. "Diesel Exhaust Inhalation Causes Vascular Dysfunction and Impaired Endogenous Fibrinolysis." *Circulation* 112 (25): 3930–36.

Minguillón, María Cruz, Marta Cirach, Gerard Hoek, Bert Brunekreef, Ming Tsai, Kees de Hoogh, Aleksandra Jedynska, Ingeborg M. Kooter, Mark Nieuwenhuijsen, and Xavier Querol. 2014. "Spatial Variability of Trace Elements and Sources for Improved Exposure Assessment in Barcelona." *Atmospheric Environment* 89 (June): 268–81.

MohanKumar, Sheba M. J., Arezoo Campbell, Michelle Block, and Bellina Veronesi. 2008. "Particulate Matter, Oxidative Stress and Neurotoxicity." *Neurotoxicology* 29 (3): 479–88. doi:10.1016/j.neuro.2007.12.004.

Mousavi, Kambiz, and Bernard J. Jasmin. 2006. "BDNF Is Expressed in Skeletal Muscle Satellite Cells and Inhibits Myogenic Differentiation." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 26 (21): 5739–49.

Nakahashi, Takeshi, Hironobu Fujimura, C. Anthony Altar, Jess Li, Jun-ichi Kambayashi, Narendra N Tandon, and Bing Sun. 2000. "Vascular Endothelial Cells Synthesize and Secrete Brain-Derived Neurotrophic Factor." *FEBS Letters* 470 (2): 113–17.

Nemmar, A, P H M Hoet, B Vanquickenborne, D Dinsdale, M Thomeer, M F Hoylaerts, H Vanbilloen, L Mortelmans, and B Nemery. 2002. "Passage of Inhaled Particles into the Blood Circulation in Humans." *Circulation* 105 (4): 411–14.

Pan, W., W. A. Banks, M. B. Fasold, J. Bluth, and A. J. Kastin. 1998. "Transport of Brain-Derived Neurotrophic Factor across the Blood-Brain Barrier." *Neuropharmacology* 37 (12): 1553–61.

Pate, R R, M Pratt, S N Blair, W L Haskell, C A Macera, C Bouchard, D Buchner, W Ettinger, G W Heath, and A C King. 1995. "Physical Activity and Public Health. A Recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine." *JAMA: The Journal of the American Medical Association* 273 (5): 402–7.

Pedersen, Bente K. 2009. "The Diseasome of Physical Inactivity – and the Role of Myokines in Muscle–fat Cross Talk." *The Journal of Physiology* 587 (Pt 23): 5559–68.

Peters, Annette, Stephanie von Klot, Margit Heier, Ines Trentinaglia, Allmut Hörmann, H Erich Wichmann, and Hannelore Löwel. 2004. "Exposure to Traffic and the Onset of Myocardial Infarction." *The New England Journal of Medicine* 351 (17): 1721–30.

Peters, Annette, Stephanie von Klot, Murray A Mittleman, Christine Meisinger, Allmut Hörmann, Bernhard Kuch, and H Erich Wichmann. 2012. "Triggering of Acute Myocardial Infarction by Different Means of Transportation." *European Journal of Preventive Cardiology*, April.

Petersen, Anne Marie W, and Bente Klarlund Pedersen. 2005. "The Anti-Inflammatory Effect of Exercise." *Journal of Applied Physiology (Bethesda, Md.: 1985)* 98 (4): 1154–62.

Phillips, Cristy, Mehmet Akif Baktir, Malathi Srivatsan, and Ahmad Salehi. 2014. "Neuroprotective Effects of Physical Activity on the Brain: A Closer Look at Trophic Factor Signaling." *Frontiers in Cellular Neuroscience* 8: 170.

Pope, C Arden, 3rd, and Douglas W Dockery. 2006. "Health Effects of Fine Particulate Air Pollution: Lines That Connect." *Journal of the Air & Waste Management Association (1995)* 56 (6): 709–42.

Powers, S K, L L Ji, and C Leeuwenburgh. 1999. "Exercise Training-Induced Alterations in Skeletal Muscle Antioxidant Capacity: A Brief Review." *Medicine and Science in Sports and Exercise* 31 (7): 987–97.

Raaschou-Nielsen, Ole, Zorana Jovanovic Andersen, Steen Solvang Jensen, Matthias Ketzel, Mette Sørensen, Johnni Hansen, Steffen Loft, Anne Tjønneland, and Kim Overvad. 2012. "Traffic Air Pollution and Mortality from Cardiovascular Disease and All Causes: A Danish Cohort Study." *Environmental Health: A Global Access Science Source* 11: 60.

Radom-Aizik, Shlomit, Frank Zaldivar Jr, Szu-Yun Leu, Pietro Galassetti, and Dan M Cooper. 2008. "Effects of 30 Min of Aerobic Exercise on Gene Expression in Human Neutrophils." *Journal of Applied Physiology (Bethesda, Md.: 1985)* 104 (1): 236–43.

Riediker, Michael, Wayne E. Cascio, Thomas R. Griggs, Margaret C. Herbst, Philip A. Bromberg, Lucas Neas, Ronald W. Williams, and Robert B. Devlin. 2004. "Particulate Matter Exposure in Cars Is Associated with Cardiovascular Effects in Healthy Young Men." *American Journal of Respiratory and Critical Care Medicine* 169 (8): 934–40.

Riva, D. R., C. B. Magalhães, A. A. Lopes, T. Lanças, T. Mauad, O. Malm, S. S. Valença, P. H. Saldiva, D. S. Faffe, and W. A. Zin. 2011. "Low Dose of Fine Particulate Matter (PM2.5) Can Induce Acute Oxidative Stress, Inflammation and Pulmonary Impairment in Healthy Mice." *Inhalation Toxicology* 23 (5): 257–67.

Rojas-Rueda, David, Audrey de Nazelle, Marko Tainio, and Mark J. Nieuwenhuijsen. 2011. "The Health Risks and Benefits of Cycling in Urban Environments Compared with Car Use: Health Impact Assessment Study." *BMJ (Clinical Research Ed.)* 343: d4521.

Samet, James M, Ana Rappold, Donald Graff, Wayne E Cascio, Jon H Berntsen, Yuh-Chin T Huang, Margaret Herbst, et al. 2009. "Concentrated Ambient Ultrafine Particle Exposure Induces Cardiac Changes in Young Healthy Volunteers." *American Journal of Respiratory and Critical Care Medicine* 179 (11): 1034–42.

Simkhovich, Boris Z., Michael T. Kleinman, and Robert A. Kloner. 2008. "Air Pollution and Cardiovascular Injury Epidemiology, Toxicology, and Mechanisms." *Journal of the American College of Cardiology* 52 (9): 719–26.

Singer, Brett. 2004. "Passive Measurement of Nitrogen Oxides to Assess Traffic-Related Pollutant Exposure for the East Bay Children's Respiratory Health Study." *Atmospheric Environment* 38 (3): 393–403.

Snyder, Eric M, Kenneth C Beck, Niki M Dietz, Michael J Joyner, Stephen T Turner, and Bruce D Johnson. 2006. "Influence of beta2-Adrenergic Receptor Genotype on Airway Function during Exercise in Healthy Adults." *Chest* 129 (3): 762–70.

Spektor, D M, M Lippmann, G D Thurston, P J Lioy, J Stecko, G O'Connor, E Garshick, F E Speizer, and C Hayes. 1988. "Effects of Ambient Ozone on Respiratory Function in Healthy Adults Exercising Outdoors." *The American Review of Respiratory Disease* 138 (4): 821–28.

Stenfors, N., C. Nordenhäll, S. S. Salvi, I. Mudway, M. Söderberg, A. Blomberg, R. Helleday, et al. 2004. "Different Airway Inflammatory Responses in Asthmatic and Healthy Humans Exposed to Diesel." *The European Respiratory Journal* 23 (1): 82–86.

Strak, Maciej, Hanna Boogaard, Kees Meliefste, Marieke Oldenwening, Moniek Zuurbier, Bert Brunekreef, and Gerard Hoek. 2010. "Respiratory Health Effects of Ultrafine and Fine Particle Exposure in Cyclists." *Occupational and Environmental Medicine* 67 (2): 118–24.

Svartengren, M, V Strand, G Bylin, L Järup, and G Pershagen. 2000. "Short-Term Exposure to Air Pollution in a Road Tunnel Enhances the Asthmatic Response to Allergen." *The European Respiratory Journal: Official Journal of the European Society for Clinical Respiratory Physiology* 15 (4): 716–24.

Taddei, S., F. Galetta, A. Virdis, L. Ghiadoni, G. Salvetti, F. Franzoni, C. Giusti, and A. Salvetti. 2000. "Physical Activity Prevents Age-Related Impairment in Nitric Oxide Availability in Elderly Athletes." *Circulation* 101 (25): 2896–2901.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. 1996. "Heart Rate Variability: Standards of Measurement, Physiological Interpretation and Clinical Use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology." *Circulation* 93 (5): 1043–65.

Tsukui, S., T. Kanda, M. Nara, M. Nishino, T. Kondo, and I. Kobayashi. 2000. "Moderate-Intensity Regular Exercise Decreases Serum Tumor Necrosis Factor-Alpha and HbA1c Levels in Healthy Women." *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity* 24 (9): 1207–11.

Valavanidis, Athanasios, Thomais Vlachogianni, Konstantinos Fiotakis, and Spyridon Loridas. 2013. "Pulmonary Oxidative Stress, Inflammation and Cancer: Respirable Particulate Matter, Fibrous Dusts and Ozone as Major Causes of Lung Carcinogenesis through Reactive Oxygen Species Mechanisms." *International Journal of Environmental Research and Public Health* 10 (9): 3886–3907.

Vinzents, Peter S., Peter Møller, Mette Sørensen, Lisbeth E. Knudsen, Ole Hertel, Finn Palmgren Jensen, Bente Schibye, and Steffen Loft. 2005. "Personal Exposure to Ultrafine Particles and Oxidative DNA Damage." *Environmental Health Perspectives* 113 (11): 1485–90.

Von Klot, Stephanie, Murray A. Mittleman, Douglas W. Dockery, Margit Heier, Christa Meisinger, Allmut Hörmann, H.-Erich Wichmann, and Annette Peters. 2008. "Intensity of Physical Exertion and Triggering of Myocardial Infarction: A Case-Crossover Study." *European Heart Journal* 29 (15): 1881–88.

Walsh, Neil P., Michael Gleeson, Roy J. Shephard, Maree Gleeson, Jeffrey A. Woods, Nicolette C. Bishop, Monika Fleshner, et al. 2011. "Position Statement. Part One: Immune Function and Exercise." *Exercise Immunology Review* 17: 6–63.

Wang, Xing, Dane Westerdahl, Ye Wu, Xiaochuan Pan, and K. Max Zhang. 2011. "On-Road Emission Factor Distributions of Individual Diesel Vehicles in and around Beijing, China." *Atmospheric Environment* 45 (2): 503–13.

Weichenthal, Scott, Marianne Hatzopoulou, and Mark S. Goldberg. 2014. "Exposure to Traffic-Related Air Pollution during Physical Activity and Acute Changes in Blood Pressure, Autonomic and Micro-Vascular Function in Women: A Cross-over Study." *Particle and Fibre Toxicology* 11 (1): 70.

Weichenthal, Scott, Ryan Kulka, Aimee Dubeau, Christina Martin, Daniel Wang, and Robert Dales. 2011. "Traffic-Related Air Pollution and Acute Changes in Heart Rate Variability and Respiratory Function in Urban Cyclists." *Environmental Health Perspectives* 119 (10): 1373–78.

Westerdahl, Dane, Scott Fruin, Todd Sax, Philip M. Fine, and Constantinos Sioutas. 2005. "Mobile Platform Measurements of Ultrafine Particles and Associated Pollutant Concentrations on Freeways and Residential Streets in Los Angeles." *Atmospheric Environment* 39 (20): 3597–3610.

"WHO | 7 Million Premature Deaths Annually Linked to Air Pollution." 2015. *WHO*. Accessed April 9. http://www.who.int/mediacentre/news/releases/2014/air-pollution/en/.

Wong, Chit-Ming, Chun-Quan Ou, Thuan-Quoc Thach, Yuen-Kwan Chau, King-Pan Chan, Sai-Yin Ho, Roger Y Chung, Tai-Hing Lam, and Anthony J Hedley. 2007. "Does Regular Exercise Protect against Air Pollution-Associated Mortality?" *Preventive Medicine* 44 (5): 386–92.

World Health Organization. 2009. *Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks*. World Health Organization.

Zeger, S. L., D. Thomas, F. Dominici, J. M. Samet, J. Schwartz, D. Dockery, and A. Cohen. 2000. "Exposure Measurement Error in Time-Series Studies of Air Pollution: Concepts and Consequences." *Environmental Health Perspectives* 108 (5): 419–26.

Zuurbier, Moniek, Gerard Hoek, Marieke Oldenwening, Virissa Lenters, Kees Meliefste, Peter van den Hazel, and Bert Brunekreef. 2010. “Commuters’ Exposure to Particulate Matter Air Pollution Is Affected by Mode of Transport, Fuel Type, and Route.” *Environmental Health Perspectives* 118 (6): 783–89.

Zuurbier, Moniek, Gerard Hoek, Peter van den Hazel, and Bert Brunekreef. 2009. “Minute Ventilation of Cyclists, Car and Bus Passengers: An Experimental Study.” *Environmental Health: A Global Access Science Source* 8: 48.

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LIST OF PUBLICATIONS AND PRESENTATIONS

Articles in preparation / submitted

Kubesch N, Guerra S, A, Westerdahl D, Martinez D, Carrasco-Turigas G, Bouso L, de Nazelle A, Nieuwenhuijsen, M. Brain-derived Neurotrophic Factor (BDNF) Serum Levels decrease after Intermitted Moderate Physical Activity in High Levels of Traffic-related Air Pollution. (to be submitted)

Kubesch N, de Nazelle A, Westerdahl D, Martinez D, Carrasco-Turigas G, Bouso L, Guerra S, Nieuwenhuijsen, M. Changes in Heart Rate Variability after Short-term Exposure to Traffic-related Air Pollution with and without Moderate Physical Activity. (in preparation)

Cole-Hunter T, Weichenthal S, **Kubesch N**, Foraster M, Carrasco G, Bouso L, Westerdahl D, Martinez D, de Nazelle A, Nieuwenhuijsen M. Impact of Traffic-related Air Pollution and Noise on Acute Changes in Heart Rate Variability in Healthy Adults: Effect Modification by Sex and Physical Activity. (under review)

Peer-reviewed Journal Articles

Kubesch N, de Nazelle A, Westerdahl D, Martinez D, Carrasco-Turigas G, Bouso L, Guerra S, Nieuwenhuijsen M. Respiratory and Inflammatory Responses to Short-term Exposure to Traffic-related Air Pollution with and without Moderate Physical Activity. *Occupational and Environmental Medicine*. April 2015; 72:4 284-293, first published online on December 4, 2014.

Kubesch N, de Nazelle A, Guerra S, Westerdahl D, Martinez D, Carrasco-Turigas G, Bouso L, Hofmann B , Nieuwenhuijsen M. Arterial Blood Pressure Responses to Short-term Exposure to Traffic-related Air Pollution with and without Moderate Physical Activity. *European Journal of Preventive Cardiology*. May 2015;vol. 22, 5: pp. 548-557, first published online on October 17, 2014.

De Nazelle, A, Fruin S, Westerdahl D, Martinez D, Ripoll A, **Kubesch N**, Nieuwenhuijsen M. A Travel Mode Comparison of Commuters' Exposures to Air Pollutants in Barcelona. *Atmospheric Environment*. November 2012;151–59.

De Nazelle A, Fruin S, Westerdahl D, Martinez D, Matamala J, **Kubesch N**, Nieuwenhuijsen M. Traffic Exposures and Inhalations of Barcelona Commuters. *Epidemiology.* January 2011;22:S77–S78.

A De Nazelle , MJ Nieuwenhuijsen, JM Antó, M Brauer, D Briggs, C Braun-Fahrlander, N Cavill, AR Cooper, H Desqueyroux, S Fruin, G Hoek, LI Panis, N Janssen, M Jerrett, M Joffe, ZJ Andersen, E van Kempen, S Kingham, **N Kubesch**, K Leyden, JD Marshall, J Matamala, G Mellios, M Mendez, H Nassif, D Ogilvie, R Peiró, K Pérez, A Rabl, M Ragettli, D Rodríguez, D Rojas, P Ruiz, JF Sallis, J Terwoert, JF Toussaint, J Tuomisto, M Zuurbier, E Lebret. Improving health through policies to promote active travel: a review of evidence to support integrated health impact assessment. *Environment International,* 2011;37(4).

Moharra M, Espallargues M, **Kubesch N**, Estrada M-D, Parada A, Vondeling H, et al. Systems to support health technology assessment (HTA) in member states of the European union with limited institutionalization of HTA. *Int J Technol Assess Health Care.* December 2009;25 Suppl 2:75–83.

Books and Book Chapters

Kubesch, N (2008). Disease Management - Erwartungshaltung und Kooperations-bereitschaft der Patienten. ISBN: 978-3-8364-2547-6, VDM Verlag Dr. Müller, Saarbrücken, Germany.

Kubesch N (2008). Chapter 1 "Introduction". In Espallargues M, Estrada MD, Kubesch N, Moharra M, Parada A, Raab M, Vondeling H (editors), Handbook on HTA Capacity Building: European Network for Health Technology Assessment Work Package 8. Barcelona, Catalan Agency for Health Technology Assessment and Research. 2008. p. 1-90.
http://www.gencat.net/salut/depsan/units/aatrm/pdf/eunethta_wp8_hb_hta_capacity_building.pdf

Christofides S, **Kubesch N**, Estrada MD, Turk E, Wladysiuk M (2008). Chapter 5 "Structure". In Espallargues M, Estrada MD, **Kubesch N**, Moharra M, Parada A, Raab M, Vondeling H (editors), Handbook on HTA Capacity Building: European Network for Health Technology Assessment Work Package 8. Barcelona, Catalan Agency for Health Technology Assessment and Research. 2008. p. 1-90.
http://www.gencat.net/salut/depsan/units/aatrm/pdf/eunethta_wp8_hb_hta_capacity_building.pdf

Kubesch, N (2007). Work and Caregiving, Theory and Good Practice for a greater Reconcilability. ISBN: 978-3-639-41629-9, AV Akademikerverlag, Saarbrücken, Germany.

Meyer M, Kofahl C, **Kubesch N** (2007). Literature - State of the art, in: Döhner H, Kofahl C, Lüdecke D, Mnich E (eds). "Services for Supporting Family Carers of Older Dependent People in Europe: Characteristics, Coverage and Usage. The National Survey Report for Germany", Hamburg University Medical Centre of Hamburg Eppendorf, 16-44
http://www.uke.uni-hamburg.de/eurofamcare/documents/deliverables/nasure_de.pdf

Conference Presentations

Alonso A, **Kubesch N**, Rosenmöller M, Cash-Gibson L, Ribeiro M, Borgermans L, Marchal Y. Analysis and Description of Integrated Care Processes: Useful Hints from Project Integrate. Poster presentation. 15th International Conference for Integrated Care (ICIC15), March 2015, Edinburgh, UK

Kubesch N, de Nazelle A, Martinez D, Guerra S, Nieuwenhuijsen M. Lung function and -inflammation after short term exposures to traffic-related air pollution and physical activity. Poster discussion.23rd Annual Congress of the European Respiratory Society, September 2013, Barcelona, Spain

Cole-Hunter T, **Kubesch N**, Martinez D, Cirach M, Belmonte J, de Nazelle A, Nieuwenhuijsen M. The effect of short-term pre-exposure to ambient bioaerosols, anthropogenic pollutants and noise on cardiopulmonary health baseline parameters. Thematic poster session. 23rd Annual Congress of the European Respiratory Society, September 2013, Barcelona, Spain

Kubesch N, de Nazelle A, Westerdahl D, Martinez D, Guerra S, Nieuwenhuijsen M. Brain-derived Neurotrophic Factor (BDNF) Serum Levels Post Exposure to Traffic-related Air Pollution and Interdependencies with Physical Activity. Poster presentation. Conference of the International Society of Environmental Epidemiology (ISEE), the International Society of Exposure Science (ISES), and the International Society of Indoor Air Quality and Climate (ISIAQ), August 2013, Basel, Switzerland

Kubesch N, de Nazelle A, Westerdahl D, Martinez D, Guerra S, Nieuwenhuijsen M. Gene Expression Changes in Healthy Volunteers after Exposure to Traffic Related Air Pollution and Physical Activity" Poster presentation. Conference of the International Society of Environmental Epidemiology (ISEE), the International Society of Exposure Science (ISES), and the International Society of Indoor Air Quality and Climate (ISIAQ), August 2013, Basel, Switzerland

Kubesch, N de Nazelle A, Westerdahl D, Martinez D, Guerra S, Nieuwenhuijsen M. Respiratory and inflammatory health effects of air pollution and physical activity" Oral presentation. Conference of the International Society of Environmental Epidemiology (ISEE), the International Society of Exposure Science (ISES), and the International Society of Indoor Air Quality and Climate (ISIAQ), August 2013, Basel, Switzerland

Cole-Hunter T, **Kubesch N**, Martinez D, Cirach M, Belmonte J, de Nazelle A, Nieuwenhuijsen M. Acute effect on cardiopulmonary health baseline parameters of short-term exposure to ambient biological aerosols, air pollution and noise. Poster presentation. Conference of the International Society of Environmental Epidemiology (ISEE), the International Society of Exposure Science (ISES), and the International Society of Indoor Air Quality and Climate (ISIAQ), August 2013, Basel, Switzerland

Kubesch, N, de Nazelle A, Westerdahl D, Martinez D, Bouso L, Guerra S, Nieuwenhuijsen, M. Health Effects of Traffic Related Air Pollution and Interdependencies with Physical Activity. Electronic poster discussion. 24th Annual Conference of the International Society for Environmental Epidemiology. (ISEE), August 2012, Columbia, South Carolina, USA

Kubesch, N, de Nazelle A, Bouso L, Carrasco G, Nieuwenhuijsen M. Acute Health Effects of Traffic Related Air Pollution and Interdependencies with Physical. Poster presentation. 23rd Annual Conference of the International Society for Environmental Epidemiology (ISEE), September 2011, Barcelona, Spain

Kubesch N, Parada A, Moharra M, Estrada MD, Cortés M, Espallargues M. Information Management in HTA Organisations. Oral presentation. Health Technology Assessment-international (HTAi) Conference. June 2008. Montreal, Canada

Moharra M, **Kubesch N**, Estrada MD, Cortés M, Parada A, Espallargues M "An international comparison of health technology assessment organisations". Poster presentation. Health Technology Assessment-international (HTAi) Conference. June 2008. Montreal, Canada

Moharra M, Cortés M, **Kubesch N**, Espallargues M, Estrada MD, Aymerich M: Survey on HTA organisations: essentials elements defining HTA organisation. Poster presentation. Health Technology Assessment-international (HTAi) Conference. June 2007. Barcelona, Spain

Payne S, Large S, Smith P, **Kubesch N**, Sheffield, UK. Barriers and possibilities: Using Focus Groups to obtain Patients´ views on Written Information in Palliative Care. Poster presentation. First congress on Research and Development in Palliative Care 2000, Berlin, Germany

Editorial and Review Tasks for Scientific Publications

Peer review work for the scientific journal "Occupational and Environmental Medicine". Review of a submission related to the effects of environmental factors on blood biomarkers.

Espallargues M, Estrada MD, **Kubesch N**, Moharra M, Parada A, Raab M, Vondeling H, editors, Handbook on HTA Capacity Building: European Network for Health Technology Assessment Work Package 8. Barcelona, Catalan Agency for Health Technology Assessment and Research. 2008. p. 1-90.
http://www.gencat.net/salut/depsan/units/aatrm/pdf/eunethta_wp8_hb_hta_capacity_building.pdf

Communicating Science

Catalan news channel TV3. Peça informativa emesa al TNmigdia de TV3 el 8/12/2011**.** Short documentary about the "TAPAS Experimental" project.
http://www.youtube.com/watch?v=QZoXo7RRTG8&list=UUukulbJkh-fb979JU2ECZyA&feature=plcp

Kubesch, N. "Tapas experimental study: Respiratory and inflammatory health effects of air pollution and physical activity", Oral Presentation at the CREAL conference, Barcelona Biomedical Research Park, June 2013
http://www.creal.cat/en_noticies/230/tapas-experimental-project-air-pollution-and-physical-activity-in-the-city

Official Reports

Moharra M, **Kubesch N**, Estrada MD, Cortés M, Parada A, Espallargues M. " Handbook on HTA Capacity Building". Final report on behalf of the EUnetHTA group to the European Commission. Catalan Agency for Health Technology Assessment (CAHTA), Barcelona, Spain, October 2008 (http://www.eunethta.eu/outputs/eunethta-handbook-hta-capacity-building).

Kubesch N, Parada A, Moharra M, Estrada MD, Cortès M et al. Information management in HTA Organisations. Final report on behalf of the EUnetHTA group to the European Commission. Catalan Agency for Health Technology Assessment (CAHTA), Barcelona, Spain, May 2008. (http://www.eunethta.eu/outputs/information-management-hta-organisations-survey-report)

Grupo de trabajo de la Guía de Práctica Clínica sobre Trastornos de la Conducta Alimentaria. Guía de Práctica Clínica sobre Trastornos de la Conducta Alimentaria. Madrid: Plan de Calidad para el Sistema Nacional de Salud del Ministerio de Sanidad y Consumo. Agència d'Avaluació de Tecnologia i Recerca Mèdiques de Cataluña; 2009. Guías de Práctica Clínica en el SNS: AATRM Núm. 2006/05-01
http://www.gencat.cat/salut/depsan/units/aatrm/pdf/gpc_trastor_cond_alim_aatrm-09.pdf

Payne S, Large S, Smith P, **Kubesch N** (2000). Elderly Patients' Perceptions of the Readability and Content of Written Information in Palliative Care. Final Report to the Averil Osborn Fund, UK