Ambient Air Pollution and Birth Outcomes

Insights on Exposure Determinants and New Outcomes

Anna Schembari

TESI DOCTORAL UPF 2014

DIRECTOR:

Dr. Mark J Nieuwenhuijsen

Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain

TUTOR:

Dr. Jordi Sunyer i Deu

Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain

Alla mia famiglia,

E a tutti i bambini del mondo

To my family,

And to all the babies of the world.

Acknowledgements

When I was a child I was deeply concerned about the inequalities among children all around the world. Since ever I have been conscious about my privileges due to being raised up in such a great and open mind family. It was the '80s and around me there were images of African children dying because malnutrition and lack of access to proper health care or because being born HIV infected. Even around me I was assisting to many injustices: Sicily, neighborhoods full of trash with children playing there in search of treasures being neglected by their parents and mafia everywhere. "When I become adult I'll do my best to make the world a better place", this was my dream. It was the 2009 when I arrived at CREAL and I met a group of enthusiastic people dedicated to the improvement of children health and I soon realized that this is the place where I want to work to achieve my children dream.

Today, reading my thesis I'm sure it is only a drop in the ocean, but for me it represents the first drop in my small sea. I am deeply thankful for having met such great people that have introduced me into the world of environmental epidemiology, inspired me and encouraged me during my PhD. "Thank you! Your heart knows how much you have done for me. There are no words that can describe my gratitude towards you."

However special thanks go to:

Prof. Mark Nieuwenhuijsen for believing in me even when I was not confident in myself and for guiding me with his bright intuition and shining intelligence through the tangle of data to the clarity of the results and towards the independent thinking. He, as supervisor, was so supportive during the hard time I had raising my child alone.

Prof. Martine Vrijheid for teaching me the basics of epidemiology with striking clarity of thought; she has been the closest example I have had of a brilliant researcher, woman and mother.

Prof. Josep Maria Antó, Jordi Sunyer and Manolis Kogevinas to have been for me a constant source of inspiration and admiration with their integrity and vision, passion and rationality and humility and joy.

Audrey de Nazelle, Marie Pedersen and Payam Dadvand for being next to me during my PhD, patiently correcting my mistakes and to always smile.

To Joaquin Salvador to share with me the work of his entire life, the REDCB data.

To John Wright for his enthusiastic contribution interpreting my analyses.

To Xavier Basagaña and David Martinez for their statistical guide.

To Margarita Triguero-Más for being such an honest collaborator.

To Kyriaki Papantoniou, the colleague, the friend, the dancer and the enthusiastic woman I love.

To Magda Bosh de Basea, Marina Vafeiadi, Damaskini Valvi, Gemma Castaño, Jordi Figuerola Marcella Marinelli, Stefano Guerra, Esther Garcia, Alejandro Caceres, Marta Benet, Joana Porcel, Mari Carmen Garcia, David Donaire, Gemma Punyet and Eileen Pernot for sharing lunches, fruits, coffees, chats, laughter, tears, racing and silences.

To the ISCIII for funding my PhD.

To Gemma Perelló, Mar Ferrer and Samuel Espinal for their administrative support.

To my favorite "sala B" which literary supported me.

Before leaving you with the core of my work I want to share with you a poem: "Instants", attributed to Luis Borges (1899-1986), who certainly made it immortal.

Barcelona, September 2014

Anna Schembari

Instants

If I could I would live my life over.
This time I would try to make more mistakes.
I would try not to be so perfect,
I would laugh more.
I would be so much sillier than I have been, that I would take few things seriously.
I would be less hygienic.
I would risk more, take more trips, contemplate more sunsets, climb more mountains, swim more rivers
I would go to more places I have never been.
I would eat more ice cream and fewer beans.
I would have more real problems and fewer imaginary ones.
I was one of those people who lived every minute of life sensibly and productively.
Of course I had moments of delight.
But if I were able to go back it would be for good moments only.
Because, if you don't know it, that's what life's made of: moments.
Do not lose the now!
I was a guy who never went anywhere without a thermometer, a hot water bottle, an umbrella, and a poncho.
If I could live my life again I would travel more lightly.
If I could live again I would start going barefoot when spring comes and not stop till fall's long gone.
I would walk ride more carts, contemplate more sunrise, and play more with children, if I had my life ahead of me again.
But, now I am 85 years old, I know I am dying.

Luis Borges

Abstract

Introduction: Air pollution is a complex mixture of pollutants containing soot particles, nitrogen oxides, ozone, sulfates and other harmful minerals that are ubiquitous in the air and are identified as hazardous for human health. However the evidence linking exposure during pregnancy to air pollution and birth outcome has been so far inconsistent. The studies relied often on hospitals certificates, which include only information on residence at birth, on the one hand, and simple indicators of fetal growth such as birth weight, on the other. Thus, the exposure assessment has been often based on indicators of air quality routinely measured in each city/region, and these change over time and vary between different regions; moreover, because of the relative small number of monitors in each city, often the exposure assessment was approximate and do not represent the high spatial variability of intra-urban air pollution. The birth weight has the advantage of being often available but it is perhaps non specific enough indicator of the possible effects of air pollution on birth outcomes.

Aims: The main objective of this thesis was to improve the assessment of personal exposures to air pollution during pregnancy and to measure the effect of ambient air pollution exposure on a wider range of possible birth outcomes, such as birth defects, head circumference and mass; also elucidate the differences between ethnic groups.

Methods: Personal exposure, indoor and outdoor levels of $PM_{2.5}$, NO_2 and NO_x were simultaneously measured using Teflon filters for two days and Ogawa samplers during 7 days respectively on a sample of 54 pregnant women living in Barcelona. Potential determinants of such exposure such as house characteristics, personal behaviors and time activity pattern were also collected using personal questionnaire and diary.

LUR models developed in the context of the European Study of Cohorts for Air Pollution Effects – ESCAPE project were used to estimate exposure to NO_2, NO_x, PM_{10}, PMcoarse, $PM_{2.5}$ and $PM_{2.5}$ absorbance. The exposures were estimates at the residential address of the studies

participants in the city of Barcelona, Spain, and Bradford, England. Models were temporally adjusted for the most relevant window of susceptibility of the fetus development using the time series of each pollutant from routine monitoring stations.

In Barcelona, cases with non-chromosomal anomalies (n=2247) and controls (n=2991) were selected from the Barcelona congenital anomaly register, the Registre de Defectós Congenitos de Bacelona – REDCB, between 1994 and 2006. Cases were live births, termination of pregnancy for congenital anomalies and stillbirths. The registry collected a wide range of congenital anomalies classified according with the European definition (EUROCAT) and contained the residential address at birth which is necessary to apply the LUR exposure models. The exposure during the susceptible window for a major congenital anomaly (weeks 3 to 8 of pregnancy) was estimated on the base of the date of birth and gestational age.

In Bradford, 12,453 pregnant women (recruited at around 28 weeks) from a North of England city were recruited between 2007 and 2010 for the Born in Bradford (BiB) cohort study. The study cohort was established to examine how genetic, nutritional, environmental, behavioral and social factors impact on health and development during childhood, and subsequently adult life in a deprived multi-ethnic population. Detailed information on socio-economic characteristics, ethnicity, lifestyle factors and environmental risk factors has been collected. Information on birth weight, head circumference, triceps and subscapular skinfolds thickness was assessed at birth within the first 72 hours of life. Exposures were calculated during the whole pregnancy and during each trimester of pregnancy.

Results: Levels of personal exposure to NO_x, $PM_{2.5}$ and absorbance were slightly higher than indoor and outdoor levels while for NO_2 the indoor levels where slightly higher than the personal ones. Generally, there was a high statistically significant correlation between personal exposure and indoor levels (Spearman's r between 0.78 and 0.84). Women spent more than 60% of their time indoors at home. Ventilation of the house by opening the windows, the time spent cooking and indicators for traffic intensity were re-occurring statistically significant determinants of the

personal and indoor pollutants levels, however models for personal exposure to $PM_{2.5}$ and absorbance explained the least of the variability observed in the data.

In the study of congenital anomalies associations were estimated using the exposures estimated at spatial level and at spatio-temporal level, calculated adjusting for the temporal variation during weeks 3 to 8 of each pregnancy. The statistically significant associations consistent between the two exposures were between an IQR increase in NO_2 (12.2 $\mu g/m^3$) and coarctation of the aorta ($OR_{spatio\text{-}temporal}$=1.15; 95%CI: 1.01, 1.31) and digestive system defects ($OR_{spatio\text{-}temporal}$=1.11; 95%CI: 1.00, 1.23), and between an IQR increase in PM_{coarse} (3.6 $\mu g/m^3$) and abdominal wall defects ($OR_{spatio\text{-}temporal}$=1.93; 95%CI: 1.37, 2.73). Other statistically significant increased and decreased ORs were estimated based on the spatial model only or the spatio-temporal model only, but not both.

In the study of newborn size, in the adjusted models stratified by ethnicity, a 5 $\mu g/m^3$ increment in exposure to $PM_{2.5}$ during the third trimester was associated with birth weight among White British babies but not among Pakistani origins ($ß_{White\ British}$ = -43g; 95%CI: -76, -10 vs. $ß_{Pakistani}$ = 9g; 95%CI: -17, 35; $P_{interaction}$= 0.03), and differentially between the two ethnic groups with head circumference ($ß_{WhiteBritish}$= -0.28cm 95%CI: -0.39, -0.17 vs. $ß_{Pakistani}$= -0.08cm 95%CI: -0.17, 0.01; $P_{interaction}$<0.001), with triceps size ($ß_{WhiteBritish}$= -0.02mm 95%CI: -0.14, 0.01 vs. $ß_{Pakistani}$= 0.17mm 95%CI: 0.08, 0.25; $P_{interaction}$= 0.06) and with subscapular skinfolds ($ß_{WhiteBritish}$= 0.06mm 95%CI: -0.06, 0.18 vs. $ß_{Pakistani}$= 0.21mm 95%CI: 0.12, 0.29; $P_{interaction}$= 0.11). Association estimates were similar for PM_{10} and $PM_{2.5}$ absorbance while for NO_2 and NO_x associations were mostly non-statistically significant.

Conclusions: Results from these studies indicate that prenatal exposure to ambient air pollution overall showed little association with congenital anomalies but did show association with impaired fetal growth. However the inclusion of outcomes not commonly included in the previous studies of air pollution effects highlighted new outcomes that deserve future attention, such as congenital anomalies of the digestive system and the abdominal wall defects and skinfolds thickness at birth, an effort to further build a case-control study at European level should be undertook.

Improved exposure assessment which includes time activity pattern and information on other sources of air pollution are recommended.

Actions to regulate ambient air pollution levels in the cities and policies aiming to improve public and/or active transportation are recommended. Also in the general populations educational measures should be planned to raise the awareness of behaviors influencing air pollution exposure levels and to encourage a healthier life-style.

Riassunto

Introduzione: L'inquinamento atmosferico è una miscela complessa di sostanze inquinanti contenenti particelle di fuliggine, ossidi di azoto, ozono, solfati e altri minerali nocivi che sono onnipresenti in aria e sono ritenuti dannosi per la salute umana. Tuttavia l'effetto di tale esposizione durante la gravidanza sulla salute del neonato é stata finora contraddittoria. Gli studi sono stati spesso basati su dati presenti sui certificati ospedalieri, che includono solo informazione sulla residenza al momento della nascita, da un lato, e indicatori di crescita del feto semplici come per esempio il peso alla nascita, dall'altro. La valutazione dell'esposizione é stata quindi basata spesso su indici di qualitá dell'aria misurati di routine in ciascuna cittá/regione, questi cambiano nel tempo e variano tra le differenti regioni; non solo, visto il relativo scarso numero di monitor in ciascuna cittá, spesso la valutazione dell'esposizione risultava approssimative e non rappresentava l'alta variabilità spaziale intra-urbana dell'inquinamento dell'aria. Il peso alla nascita ha il vantaggio di essere di facile reperibilitá ma é forse un indicatore poco specifico dei possibili effetti dell'inquinamento atmosferico.

Obiettivi: L'obiettivo principale di questa tesi è stato quello di migiorare la valutazione dell'esposizione personale all'inquinamento atmosferico durante la gravidanza e misurarne l'effetto su una piú ampia gamma di possibili effetti sul neonato, quali difetti congeniti, circonferenza della testa e lo spessore della massa; inoltre delucidare le eventuali differenze tra etnie.

Metodi: L'esposizione personale, i livelli all'interno e all'esterno di $PM_{2.5}$, NO_2 e NO_x sono stati simultaneamente missurati utilizzando filtri in teflon per due giorni e campionatori Ogawa per 7 giorni, rispettivamente in un campione di 54 donne incinte che vivevano a Barcellona. Le potenziali determinanti di tale esposizione, come le caratteristiche della casa, la routine quotidiana seguita, i mezi di trasporto utilizzati e l'uso del tempo sono stati raccolti utilizzando un questionario personale e un diario.

I modelli LUR sviluppati nell'ambito dello studio europeo delle coorti per effetti dell'inquinamento atmosferico - progetto ESCAPE, sono stati usati per stimare l'esposizione a NO_2, NO_x, PM_{10}, PMcoarse, $PM_{2.5}$ e $PM_{2.5}$ assorbanza. L'esposizione é stata calcolata all'indirizzo di residenza dei partecipanti agli studi nella città di Barcellona, Spagna, e Bradford, Inghilterra. I modelli sono stati temporalmente aggiustati includendo solo la finestra più rilevante per la suscettibilità dello sviluppo del feto, utilizzando le serie storiche di ciascun inquinante dalle stazioni di monitoraggio di routine.

A Barcellona, i casi con anomalie congenite non cromosomiche (n = 2247) e i controlli (n = 2.991) sono stati selezionati dalla registro di anomalie congeite di Barcellona, il Registro del Defectós Congenitos de Bacelona - REDCB, tra il 1994 e il 2006. I casi erano nati vivi, interruzione volontaria della gravidanza per le anomalie congenite e i nati morti. Nel registro sono raccolte una vasta gamma di anomalie congenite, classificate seguendo la definizione europea (EUROCAT), e l'indirizzo di residenza al momento della nascita che è necessario applicare i modelli di esposizione LUR. L'esposizione durante la finestra di suscettibile per un difetto congenito maggiore (da 3 a 8 settimane di gravidanza) è stato stimato sulla base della data di nascita e l'età gestazionale.

A Bradford, 12.453 donne in gravidanza (reclutati a circa 28 settimane) tra il 2007 e il 2010 sono stati arruolate nello studio di coorte Born in Bradford (BiB). Lo studio è stato istituito per esaminare l'impatto della genetica, della nutrizione, di fattori ambientali, comportamentali e sociali sulla salute e lo sviluppo durante l'infanzia in una popolazione multietnica e svantaggiata. Informazioni dettagliate sulle caratteristiche socio-economiche, l'etnia, stile di vita e fattori di rischio ambientali sono state raccolte. Informazioni sul peso alla nascita, circonferenza cranica, spessore della massa nel tricipite e sottoscapolare sono stati misurati alla nascita entro le prime 72 ore di vita. L' esposizione all'inquinamento dell'aria é stata calcolata durante tutta la gravidanza e nel corso di ogni trimestre di gravidanza.

Risultati: I livelli di esposizione personale al NOx, $PM_{2.5}$ e assorbanza sono stati leggermente superiori ai livelli rilevati all'interno ed all'esterno mentre per NO_2 i livelli all'interno erano leggermente più elevati rispetto a

quelli personali. In generale, c'è stata una correlazione alta e statisticamente significativa tra esposizione personale e livelli misuratio all'interno (r di Spearman tra 0,78 e 0,84). Le donne hanno speso più del 60% del loro tempo in ambienti chiusi in casa. La ventilazione della casa aprendo le finestre, il tempo trascorso cucinando e indicatori di intensità di traffico sono state caratteristiche ricorrenti e statisticamente significative dei livelli di inquinanti personali e all'interno, ma i modelli per l'esposizione personale al $PM_{2.5}$ e assorbanza hanno spiegato il minimo della variabilità osservata nei dati.

Nello studio delle anomalie congenite le associazioni sono state stimate sia utilizzando le esposizioni a livello spaziale che a livello spazio-temporale, utilizzando modelli di regressione ligistica e calcolando odds ratio (OR). Le associazioni trovate statisticamente significative stimate usando entrambi i modelli di esposizioni erano tra un aumento del range inter-quartilico(IQR) di NO_2 (12,2 mg / m3) e la coartazione dell'aorta (ORspazio-temporale = 1,15, 95% CI: 1.01, 1.31) e difetti del sistema digestivo (ORspazio-temporale = 1,11, 95% CI: 1.00, 1.23), e tra un aumento IQR in PMcoarse (3,6 mg / m3) e difetti della parete addominale (ORspazio-temporali = 1.93, 95% CI: 1.37, 2.73). Altri OR statisticamente significativi sono stati stimati sulla base unicamente del modello spaziale o del modello spazio-temporale, ma su entrambi.

Nello studio della dimensione del neonato, nei modelli lienari aggiustati e stratificati per etnia, un incremento di 5 mg/m3 dell'esposizione a $PM_{2.5}$ durante il terzo trimestre è stato associato con peso alla nascita tra i bambini britannici, ma non tra quelli di origini pakistana ($ß_{WhiteBritish}$ = -43 g; 95% CI: -76, -10 vs $ß_{Pakistani}$ = 9 g, 95% CI: -17, 35; $P_{interaction}$= 0.03), e in modo differente tra i due gruppi etnici con circonferenza creaniale ($ß_{WhiteBritish}$ = -0.28 cm 95% CI: -0.39, -0.17 vs $ß_{Pakistani}$= -0.08 cm 95% CI: -0.17, 0.01; $P_{interaction}$ <0,001,), con la dimensione del tricipite ($ß_{WhiteBritish}$ = -0.02 mm 95% CI: -0.14, 0.01 vs $ß_{Pakistani}$ = 0,17 mm 95% CI: 0,08, 0,25; $P_{interaction}$ = 0,06) e con quella sottoscapolare ($ß_{WhiteBritish}$ = 0,06 mm 95% CI: -0.06, 0.18 vs $ß_{Pakistani}$ = 0,21 mm 95% CI: 0,12, 0,29; $P_{interaction}$ = 0.11,). Le aspciazioni stimate per il PM_{10} e $PM_{2.5}$ assorbanza erano simili, mentre quelle per NO_2 e NO_x erano per lo più non statisticamente significativa.

Conclusioni: I risultati di questi studi indicano globalmente che l'esposizione prenatale all'inquinamento atmosferico è scarsamente associato con anomalie congenite, ma con una crescita fetale alterata. Tuttavia l'inclusione di possibli effetti in salute non comunemente inclusi previamente negli studi degli effetti dell'inquinamento atmosferico ha evidenziato nuovi risultati che meritano attenzione in futuro, come anomalie congenite del sistema digestivo e difetti della parete addominale e lo spessore pliche cutanee alla nascita; inoltre bisognerebbe impulsare con maggiore forza uno studio di caso/controllo a livello Europeo . Si raccomanda valutare l'esposizione includendo informazioni sull'uso del tempo, sul tempo trascorso in trasporto e su altre fonti di inquinamento atmosferico.

Si raccomanda di sviluppare azioni per regolare i livelli di inquinamento atmosferico nelle cittá e le politiche volte a migliorare il trasporto pubblico. Inoltre nella popolazione generale dovrebbero essere previste misure più morbide volte a aumentare la consapevolezza dei comportamenti che influenzano i livelli di esposizione di inquinamento atmosferico e a incoraggiare un più sano stile di vita.

Preface

Some studies suggest that traffic-related air pollution is related to small but adverse birth outcomes, such as congenital anomalies, reduced birth weight and preterm delivery. Part of the uncertainties of the results are attributable to the error measurement of exposure which have so far relied on routine monitor stations, not capable of capture the spatial variability of traffic-related air pollution within cities and neither other determinants of the personal exposure. Furthermore the birth outcomes measured, such as birth weight or low birth weight, have been criticized to be not refined enough to capture the complex effects of such exposure. In addition, effects of ethnic differences on the association of air pollution and birth outcomes have been reported but still very little is known about the in the susceptibility to air pollution of South Asians origins populations compared to Caucasians, being very well documented the higher risk of low birth weight, diabetes and cardiovascular disease among the former.

This project aimed to understand determinants of the personal exposure to air pollution among pregnant women and to use the recently developed refined exposure assessment techniques to assess the impact of traffic-related air pollution on birth outcomes that have been less investigated such as congenital anomalies, head circumference and skinfolds thickness at birth, which are relevant outcomes to study differences between South Asians and White British newborns.

This thesis has been developed between 2010 and 2014 at the Centre for Research in Environmental Epidemiology (CREAL - Barcelona, Spain) under the supervision of Prof. Mark Nieuwenhuijsen and in collaboration with Dr. Martine Vrijheid for the congenital anomalies study. The thesis consists of three articles (2 published and 1 under review). The first article

investigates the relationship between pregnant women's personal exposures to air pollutants and the indoor and outdoor residential concentration levels, identifies predictors of personal exposure using the time activity pattern. It was based on the data from the validation study of the Air Pollution and Reproduction In Barcelona (ARIBA), which measured exposure and their determinants in 54 pregnant women in Barcelona between 2008 and 2009. The second and the third articles aim to assess the association between pregnancy exposure to air pollution and birth outcomes and rely on the exposure assessment developed during 2009/10 in the European Study of Cohorts for Air Pollution Effects - ESCAPE project. The congenital anomalies study was based on the Registre de Defectos Congenitos de Barcelona – REDCB, having recruited around 6000 cases and controls during 1994 to 2006. The newborn size study was based on birth data from the Born in Bradford – BiB study cohort which collected data on around 10000 mother-child pairs of South Asian origin and White British.

The PhD student contribution included the participation in the data collection of the ARIBA validation study on 54 pregnant women, the participation in the air pollution measurements for the ESCAPE project in Barcelona and in Catalunya, the data-base organization for the ARIBA validation study and for the congenital anomalies study, data cleaning, statistical planning and analysis, writing of the articles and communication to the scientific community at international conferences. It also included the participation and coordination of project meetings and group meetings. The Congenital anomalies study was further included in the EUROCAT report for support an European conjunct action toward surveillance of environmental pollution and linkage to congenital anomalies (Vrijheid et al 2013)

Contents

1 INTRODUCTION

As fishes live in the water, humans live in the air. Air is the human environment: the ambient where we live, find our nourishment and develop our civilizations; in simple words air is essential for human life.

During the industrial revolution in Europe and in North America, ambient air nearby the factories became heavily polluted due to the burning processes related to the mechanic production. At the same time it was there where the first urban centers were settled and have been growing. Since then the number of people exposed to air pollution has increased exponentially. Nowadays the main urban sources of air pollution have changed from the burning processes of the industrial production to the fuel combustion emitted by motor vehicles, household heating and tobacco smoke making of the air we breathe is a complex mixture of pollutants containing soot particles, nitrogen oxides, ozone, sulfates and other harmful minerals.

Exposure to air pollution at current levels in the European cities is harmful for human health. In 2012, 3% of the 3.7 million deaths attributable to outdoor air pollution occurred in children under 5 years (WHO 2014). However evidence of the effects of such exposure on birth outcomes is underrepresented in health impact assessment due to the yet non-conclusive epidemiological evidence about small adverse effect on fetus. Epidemiological studies have been conducted mainly in Europe and North America (Maisonet et al 2004, Glinianaia et al 2004, Ritz 2007, Sram et al 2005, Stieb et al 2012, Vrijheid et al 2011, Pedersen et al 2013b) and suggest that there may be small adverse effects of air pollution, specifically carbon monoxide, sulphur dioxide and particulate matter on fetal growth and pre-term delivery.

Ethnic susceptibility to air pollution has been reported in air pollution studies (Basu et al. 2014; Bell et al. 2010; Darrow et al. 2011;Geer et al

2012) but evidence is inconsistent and refers mainly to Afro-american, Hispanic and non-Hispanic ethnic groups, being Asians and South Asians are underrepresented (Basu et al. 2014; Geer et al 2012). The immigration from Sout Asia, specifically Pakistan, to Western countries has increased in recent years, being Pakistani a big community for example in England. These populations, compared to Caucasians have higher risks of obesity, diabetes and related cardiovascular disease which can be partially induced by oxidative stress. Although differences between these populations have not yet been explained by clear biological mechanisms, the oxidative stress induced by traffic-related air pollution may exacerbate certain differences.

This thesis focuses on traffic related air pollution because (I) traffic is the main source of air pollution in the cities; (II) traffic intensity is modifiable by policies regulating it but also by changing individual transportation behaviors; (III) to reduce traffic related air pollution is in the agenda of developed and developing Countries.

The importance of preserve fetal life together with a still limited epidemiological evidence of adverse effects of air pollution on fetal growth represents the motivation to choose to study birth outcomes.

1.1 Traffic-related air pollution

The WHO definition for Air pollution is: "Air pollution is contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere. Household combustion devices, motor vehicles, industrial facilities and forest fires are common sources of air pollution. Pollutants of major public health concern include particulate matter, carbon monoxide, ozone, nitrogen dioxide and sulfur dioxide. Outdoor and indoor air pollution cause respiratory and other diseases, which can be fatal". Furthermore: (I) air pollution is ubiquitous; (II) one quarter of the world population is estimated to be exposed to unhealthy concentrations of air pollutants in cities, particularly in the megacities of the developing

countries(WHO 2009); (III) babies and children are particularly at risk because of the immaturity of their respiratory system.

1.1.1 Composition and Sources

Traffic-related air pollution is a complex mixture of pollutants derived from exhaust emissions of gasses and particles from fuel combustion (primary and secondary pollutants) and non-exhaust emissions from mechanical operation of grinding and crushing. Exhaust emissions includes carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NO, NO_2 and NO_x), particulate matters (PMs), sulphur dioxide (SO_2) among others.

Non exhaust emissions are generated from brakes, tyres and road wear or from the resuspension of road dust and contribute to the formation of PM. Primary pollutants in the presence of sunlight and of determinate meteorological and geographical conditions react in the atmosphere contributing to the formation of other pollutants (secondary pollutants). For examples a secondary pollutant is NO_2, which is formed as NO combines with oxygen in the air.

NO_2 measured nearby traffic sites is considered an important indicator of traffic emissions. First because it is formed rapidly after the emission of NO from the fuel combustion and secondly because exponentially decrease with the distance to the source (Singer et al 2004). Furthermore its inexpensive sampler has facilitated the routine monitor measurements of this compound and the introduction of air quality standards.

PM measured nearby traffic sites is a complex mixture of extremely small particles and liquid droplets made up of a number of components from exhaust emissions, including acids (such as nitrates and sulfates), organic chemicals and metals, and from non exhaust emission, furthermore organic compounds such soil, dust or pollen are also present. PM is collected on filters that capture the particles, usually with aerodynamic diameter smaller than 10μm (PM_{10}) or 2.5μm ($PM_{2.5}$) or with diameter between 2.5μm and 10μm (PMcoarse). The components of the PM vary

according with the size, PM_{10} is mostly a mixture exhaust and non-exhaust gases plus a component of organic and mineral origin; PMcoarse is prevalently formed by non-exhaust emissions and resuspended road dust; $PM_{2.5}$ is characterized by a higher prevalence of traffic exhaust emissions and PAHs and by a lower proportion of road dust compared to PMcoarse (Minguillón et al 2012a). Elemental carbon content of $PM_{2.5}$, an important component of diesel exhaust, can be easily estimated with a reflectance method (Adams et al 2002). The composition of the PMs determines its toxicity, nevertheless, moreover the toxicity, their harmfulness for humans is determinate by PMs size. Indeed PM penetrates the lungs deeper and deeper as the diameter decrease: particles of diameter between 5μm and 10μm penetrate human trachea and bronchi and particles smaller than 5μm penetrate deeper till the alveoli, the smaller part of the lungs. Ultrafines have very low mass but magnitudes higher particle numbers and therefore a high surface area relative to fine and coarse particles for adsorption of toxic species (Sioutas et al 2005). Ultrafine particles have a high respiratory deposition, can escape phagocytosis by alveolar macrophages and translocate to extrapulmonary organs (Oberdorster and Utell 2002)

1.1.2 Surveillance

European and North American cities monitor air pollution levels continuously, the number of monitoring stations has varied in the time and depends on the dimension of the city. NO_2, NO_x, SO_2 and CO have been monitored since early '60s in the UK while PM collection has started later around '80s as the evidence of their harmfulness for human health has increased. Commonly, in a big European city, between 5 to 15 monitoring stations are collecting daily air pollution data, covering different area as rural, urban, urban and industrial (EIONET 2010).

The European Commission (EC) and the United States Environmental Protection Agency (EPA) are responsible to set limits for the abovementioned pollutants in order to protect human health, however these limits are not always the same and do not follow completely those

suggested by the World Health Organization (WHO), as shown in the table 1.1

Table 1.1 WHO, EC and EPA limit values for air pollutants. Values indicate annual mean concentration except for EPA PM_{10}* that are 24h mean.

	WHO[a]	EC[b]	EPA[c]
NO_2	*40 $\mu m/m^3$*	40µm/m^3	100µm/m^3
PM_{10}	*20 $\mu m/m^3$*	40µm/m^3	50µm/m^3*
$PM_{2.5}$	*10 $\mu m/m^3$*	25µm/m^3	15µm/m^3

[a] WHO 2005, guidelines, *values recommended*
http://www.who.int/mediacentre/factsheets/fs313/en/
[b] EC Directive 2008/50/EC, limits values
http://ec.europa.eu/environment/air/quality/standards.htm
[c] EPA air quality standards, limits values
http://www.epa.gov/air/criteria.html

1.1.3 Exposure assessment

In epidemiological studies it is not often feasible to measure personal exposure to traffic-relate air pollution both because it would be too expensive but also because they are based on hospital records collected retrospectively thus mostly the only information available to assign exposure is the residential address at the occurrence of the event studied (for example birth). To overcome this problem the first studies relied on the routine monitored air pollutant and the exposure was calculates as the levels itself or a combination of the levels measured at nearest monitoring station to the home address and the distance from the home (Gliniania et al 2004; Maisonet et al 2004). Such ecological inference is often subject to bias and imprecision, due to the lack of individual-level information in the data (e.g. mobility of the women), furthermore this approach may not represent the complex spatial distribution of traffic related air pollutants and it may exclude heavily exposed areas near roads, inducing strong error measurement; in particular it neglects individual exposure assessment

Modeling techniques such as geostatistical interpolation of monitoring data, dispersion models, hybrid models and Land Use Regression (LUR) models have been developed (Jerret et al 2005) to provide a refined spatial estimation of pollutants, and this permitted to assign to each study participant the estimated level of pollution at the residential address. Land use regression (LUR) modeling is a method developed in recent years to map traffic-related air pollution at the street scale. LUR uses empirical regression equation to predict outdoor concentrations at any point of the modeling area as a function of microscale urban characteristics such as traffic intensity and population density, greenness etc., which can be derived using GIS information system (Brauer et al. 2003; Briggs 2007; Briggs et al. 2000; Jerrett et al. 2007). LUR models are the fastest, simplest, most efficient and cost effective models that can be developed (Hoek et al 2008).

The European Study of Cohorts for Air Pollution Effects – ESCAPE project (www.escapeproject.eu) aims to investigate the long-term effects of exposure to air pollution on human health using health data already available from European cohort studies. Within this framework, LUR models were developed following the same protocol in 36 cities across Europe to estimate exposure of to NO_2, NO_x, PM_{10}, PMcoarse, $PM_{2.5}$ and $PM_{2.5}$ absorbance at the residential address of the study participants.

While modeled annual average concentrations are sufficient for most outcomes, such as those related to respiratory disease, cardiovascular disease, cancer incidence and mortality; pregnancy outcome studies require more detailed temporal resolution. In pregnancy outcome studies, it is common to express exposure as the average concentration per month or trimester of a specific pregnancy. The required exposure thus needs to contain a spatial and temporal component. One simple option is to develop LUR models using annual average concentrations and then use continuous routine monitoring data to produce a temporally varying component. This approach makes the assumption that the spatial pattern is constant in time, i.e. that temporal variations in the considered atmospheric pollutants were similar across the metropolitan area. Although reasonable, this assumption was likely to have induced exposure misclassification, which was believed to be minor compared with that

which would exist when temporal variations in air pollution had been ignored

1.1.4 Sources of error measurement

Errors in exposure assessment are inevitably a major source of uncertainty in epidemiological studies, and commonly act to reduce the power to detect associations with health, or bias the associations found (Nieuwenhuijsen 2003). There are several ways to reduce error in exposure assessment, the most obvious being to use more accurate and precise methods of assessment, such as personal monitoring, biomarkers and exposure diaries (Nieuwenhuijsen 2003). These methods are considered "gold standards" in exposure assessment. However, in large studies, especially, such methods are not feasible for all the participants in the study, due to high cost and burden on the participant, so alternatives must be used such as measurements from ambient monitoring stations. However, it is often possible to collect personal samples on a subset of the population along with individual characteristics and mobility data to understand factors that influence the exposures, and integrate the information with information from ecological estimates.

Currently, in the study of pregnancy exposure and birth outcomes little is known. Two studies before assessed personal exposure to air pollution among pregnant women and shown that other determinants are indoor sources such as gas cooking, tobacco smoking and wood burning from heating as well as ventilation of the house (Netherby et al 2008c, Valero et al 2010) and that the associations were different when the time activity pattern was considered (Aguilera et al 2009). However results were different according with the different study area (Canada the first and Spain the second and third), thus each study needs particular consideration of the conditions and characteristics of the population to discussed whether the measurement error occurred at random (Berkson error) or not (classical error).

1.2 Birth outcomes

Birth outcomes are a group of measures that describes the health of the baby at birth. Adverse birth outcomes are Low Birth Weight (LBW; <2500g), reduced birth weight (as continuous), preterm delivery (before 37 completed weeks), small for gestational age (SGA), intrauterine growth restriction (IUGR) and congenital anomalies, among others. These outcomes typically measure the child's current and future morbidity and mortality.

1.2.1 Congenital anomalies

Congenital anomalies, is a leading cause of fetal loss and easily remain under-detected, they contribute significantly to preterm birth and adult morbidity, represents a emotional burden for families. 3-8% of newborns worldwide have a serious structural defect, and while some can be attributed to chromosomal or syndromic disorders or known teratrogenic factors, still the cause for most of them is unknown (Weinhold 2009)

For population-based research, birth defects generally need to be actively ascertained in a registry for all live born infants and fetal deaths (typically diagnosed after 20 weeks of gestation) and live born infants and, importantly, after birth in order to capture anomalies not apparent at delivery. Furthermore, the records need to allow distinguishing between isolated, multiple, syndromic or chromosomal defects and also by anatomical sub-categories. For cardiac defects, the diagnoses should preferably be confirmed by autopsy or surgical reports, catheterization or echocardiogram. Therefore, studying the influence of air pollutants on birth defects in a valid manner will not be possible unless such a system is already in place for a given geographic area and can be linked to extensive air monitoring data for a large enough population with enough births at risk for these rare outcomes. (Ritz and Wilhelm 2008)

The susceptible window for major congenital anomalies, according with the organogenesis, is from week 3 after conception to week 8 (see figure 1), any exposure later this period is improbable to result in a major congenital anomaly.

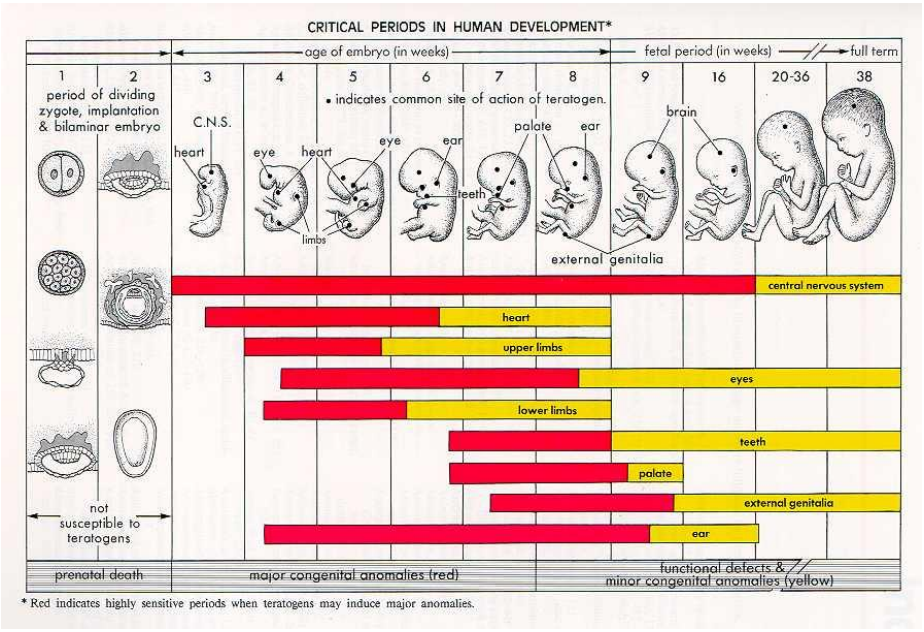

Figure 1. Fetal development and organogenesis by gestational age (in weeks).

The European Sourveillance of Congenital anomalies – Eurocat was established in 1979 by Directorate General XII (Science Research and development) as a prototype for European surveillance aiming to assess the feasibility of pooling data across national boundaries, in terms of standardization of definitions, diagnosis and terminology and confidentiality. Later in 1991 it was founded to function as a service for the surveillance of congenital anomalies in Europe. The Eurocat surveyed more than 1.7 million births per year in Europe having 43 registries in 23 countries. It covers the 29% of European birth population. Furthermore registries are used for the planning and evaluation of health services. This

includes primary prevention strategies such as periconceptional folic acid supplementation to prevent neural tube defects and vaccination against rubella to prevent congenital rubella syndrome; the so-called "secondary prevention" by prenatal screening and diagnosis, and “tertiary prevention” through pediatric, rehabilitative and other services.

1.2.2 Birth weight and newborn size

Birth weight is the results of the length of gestation and of the fetal growth, infants may be born with low birth weight (<2500g) because they were born prematurely (before 37 completed weeks of gestation) or they may be born smaller than expected given their gestational age.

According to the developmental origin of fetal disease hypothesis (Wadhawa et al 2009; Swanson et al 2009) we may expect that adverse intrauterine influences such as poor maternal nutrition and environmental exposures (including environmental tobacco smoking) lead to impaired fetal growth, resulting in low birth weight, short birth length and small head circumference. These adverse influences are postulated to also induce the fetus to develop adaptive metabolic and physiological responses which, in turn, may lead to disordered reactions to environmental challenges as the child grows, with an increased risk of glucose intolerance, hypertension and dyslipidemia in later life (Gillman 2005).

To characterize more specifically the fetal growth, hence to understand further the links between impaired fetal growth and disease development later in life other measures have been considered, such as birth length and head circumference and skinfolds thickness (Bansal et al. 2008; Gale et al 2004; Klaric et al. 2013; Krishnaveni et al. 2005).

The use of such indicators in large epidemiological studies depends on their availability on birth certificates, while birth weight is commonly reported it may not be the case for the other indicators which may further be subjects to higher error measurements. For example head circumference may be imprecise due to the deformation during the labor

and baby length may be biased by the newborn position. For specific purpose, in some birth cohort studies (Wright et al. 2013; Yajnik et al. 2002) specific protocols are implemented to standardize the measurement techniques and to further include specific outcomes such as skinfolds thickness which have been studied in England and in India to assess fat mass growth from birth onward.

In the newborns skinfolds thickness provides an estimate of the total body fat of the infant (Farmer 1985). Postnatal fat accumulation occurs predominantly in the extremities: triceps (upper arm) skinfolds thickness provides an indication of the periferical body fat mass while subscapular (upper back) skinfolds thickness reflects the visceral/subcutaneus fat (Ketel et al. 2007; Snijder et al. 2006).

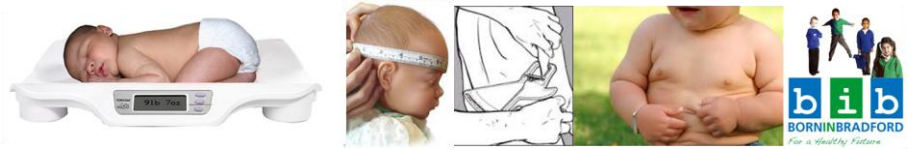

Figure 2. Images representing the birth outcomes collected in the BiB.

1.2.3 Ethnic Differences between South Asians and Caucasians

Rates of low birth weight and preterm birth can vary greatly by maternal race/ethnicity. South Asians and Indians have consistently had higher rates of low birth weight babies than Caucasians (UNICEF and WHO 2004). While it has been suggested that race is a proxy for differences in socioeconomic status, most studies that have controlled for differences in SES continue to find persistent birth outcomes differences between South Asians and White women (Margetts et al. 2002, Pearson 1991; Small 2012). In one previous study it was possible to further control for other maternal risk factors such as risky behaviors during pregnancy and use of prenatal care, however the differences in birth outcomes between White British and South Asian persisted (West et al 2013).

During childhood and adolescence, the South Asians have relatively higher subscapular skinfolds than White British (Bansal et al. 2008; Krishnaveni et al. 2005) which end-up as disproportionate central adiposity for a given body mass index (BMI) in adulthood (Snijder et al. 2006) and increased risk of adverse cardiometabolic outcomes including obesity, insulin resistance, cardiovascular risk and diabetes (Misra and Khurana 2009).

Several theories have been proposed to explain such differences in the allocation of body fat of South Asians compared to Caucasian on the premise that the storage of energy as body fat is important to survival and critical during periods of food shortage; whether this is cyclical (as in the seasons) or occasional. The "thrifty genotype" (Neel et al 1999), the "thrifty phenotype" (Hales and Barker 2001) and "El Niño"(Wells 2007) theories were developed according to the evolutionary selection of people able to store energy as fat, and especially central fat that can be mobilized quickly to release nutrients. They do not, however, explain either why central obesity is more important than generalized obesity in relation to CVD and diabetes. Sniderman et al. (2007) says that South Asians may have a reduced capacity to store fat in the relatively inactive, superficial subcutaneous, adipose tissue (primary adipose tissue compartment), which would result in earlier utilization of more metabolically active, deep subcutaneous, and intra-abdominal, adipose tissue compartments (secondary adipose tissue compartments). This theory goes under the name of "adipose tissue compartment overflow hypothesis". More recently Wells et al (2009) proposed the "variable disease selection hypothesis" suggesting that exposures to different population burdens of infectious diseases (rather than climatic or nutritional exposures per se) cause genetic ethnic variability in the anatomical location of adipose tissue compartments. There are two fundamental priorities of adipose tissue: the meeting obligatory energy needs of essential organs and the immune system maintenance. As deep adipose tissue depots are more appropriate for meeting the immediate energy demands of the immune system, the tendency of South Asians to prioritize deep visceral adipose tissue depots may therefore have resulted from chronic exposures to geographically specific, endemic diseases. Under these premises and considering that climatic, nutritional or infectious circumstances may have occurred separately or in combination Bopal and Rafnsson (2009) proposed that it

is possible that ethnic differences in energy production, allocation and storage and utilization may partly be explained by differences in mitochondrial gene structure and function.

Figure 3. White British and Pakistani origins children playing together, in Bradford.

1.4 Air pollution and Birth outcomes

1.4.1 Evidence

The link between routine measured air pollutants and adverse birth outcomes has received more attention in recent years. All routine measured air pollutants, PM_{10}, $PM_{2.5}$, SO_2, CO, O_3 and to less often NO_2, have been linked to increased risk of congenital anomalies decreased fetal weight, LBW, and preterm birth in a number of large epidemiological studies in many different countries (Glinianaia et al 2004, Stieb et al 2012; Vrjheid et al 2011). However results are heterogeneous and non conclusive. Differences in study design, sample size, population characteristics, control for confounders, air pollution measurements and exposure assessment techniques as well as the lack of knowledge on the exact biological mechanism are likely to contribute to the observed heterogeneity (Ritz and Wilhelm 2008; Slama et al. 2008; Woodruff et al. 2009). Although recent works have successfully overcome issues related to control for confounders, sample size, study design and exposure assessment (Dadvand et al. 2013; Pedersen et al. 2013) A summary is provided below distinguishing congenital anomalies from newborn size to highlights the different methodologies used to approach these outcomes.

a) Congenital anomalies

In studies of congenital anomalies, the relatively short 8-week period early in gestation, during which most organs rapidly develop in the fetus, provides a unique opportunity to study exposures acting on narrow susceptibility window. Thus, the exposure assessment should reflect the temporal and the spatial fluctuations in air pollution whit accuracy to account for the very specific etiologically relevant time windows of exposure (Ritz and Wilhelm 2008).

The evidence for an impact of ambient air pollution on congenital anomaly risk has increased in the recent years (Chen et al 2014; Vrijheid et al. 2011). Only the most recent studies (Agay-Shay et al. 2013; Marshall et al. 2010; Padula et al 2013a and 2013b) included specific traffic-related air pollutants such as $PM_{2.5}$. Exposure assessments often lacked an extensive monitor campaign, and thus did not account for the strong spatial intra-city variation that characterizes traffic-related air pollution (Cyrys et al. 2012; Eeftens et al. 2012b). Dadvand et al. (2011) estimated spatio-temporal exposure to black smoke and SO_2 with higher spatial resolution.

Cardiac anomalies or oral clefts were most frequently studied. Available evidence on other anomaly groups, such as defects of the nervous, digestive, or respiratory systems is scarce (Dolk et al. 2010; Rankin et al. 2009; Padula et al 2013b). Summary estimates from a recent meta-analysis (Chen et al 2014; Vrijheid et al. 2011) indicated that NO_2 and SO_2 were associated with two congenital heart anomalies, coarctation of the aorta (OR per 10 ppb NO_2=1.17, 95%CI 1.00-1.36; OR per 1 ppb; SO_2=1.07, 95%CI 1.01, 1.13) and tetralogy of Fallot (OR per 10ppb NO_2=1.20, 95%CI 1.02, 1.42; OR per 1 ppb SO_2=1.03, 95%CI 1.01, 1.05), and that PM_{10} was associated with atrial septal defects (OR per 10 $\mu g/m^3$=1.14, 95%CI 1.01, 1.28).

Further effort should be done harmonizing data from different registries to increase the study sample. Indeed it is unlikely that any specific agent would cause an overall increase in all types of anomalies, requiring the distinction between relevant subgroups which in turn is quite challenging since even specific defects are likely of multifactorial etiology. Finally the rarer the defect, the larger the data source needed, i.e. adequate and reliable exposure and outcome data for hundreds of thousands of pregnant women.

b) Newborn size

The evidence on the association of ambient air pollution and reduced birth size remains heterogeneous (Glinianaia et al. 2004; Stieb et al. 2012; Vrijheid et al 2011). Differences in study design, sample size, control for

confounders, air pollution measurements and exposure assessment techniques as well as the lack of knowledge on the biological mechanism has been identified as major determinant to the lack of consistency (Ritz et al 2007; Ritz and Wilhelm 2008; Slama et al. 2008; Woodruff et al. 2009; Vrijheid et al 2011).

Recent works on newborn size (Dadvand et al. 2013; Pedersen et al. 2013) have successfully outdone the issues related to study design and exposure assessment and found positive associations between exposure to traffic related air pollutants and adverse birth outcomes. For example Dadvand et al.(2013) found term LBW positively associated with 10 $\mu g/m^3$ increase in PM_{10} (OR = 1.03; 95% CI: 1.01, 1.05) and $PM_{2.5}$ (OR= 1.10; 95% CI: 1.03, 1.18) exposure during the entire pregnancy; and Pedersen et al. (2013) found increase by 5 $\mu g/m^3$ in $PM_{2.5}$ associated with a decrease in birth weight ($ß_{adj}$= -10 g; 95%CI: 0, 19) and a decrease head circumference for the same exposure ($ß_{adj}$= -0.08 cm; 95%CI: -0.12, -0.03).

There is no study separating the impact of maternal exposure to air pollution on fat mass from such an impact on fetal growth as a whole measured by birth weight. Animal studies have suggested that gestational exposure to $PM_{2.5}$ can increase the predisposition to insulin resistance and to thicker adipose tissues (Bolton et al. 2012). Also maternal smoking during pregnancy has been associated to reduced size at birth (weight, length and head circumference) but not to thinner skinfolds thickness (Bernstein et al. 2000; D'Souza et al. 1981; Luciano et al. 1998).

1.4.2 Biological mechanism

The biological mechanism linking air pollution to congenital anomalies has not yet been investigated exhaustively (Weinhold 2009). Numerous biologic pathways have been identified whereby particulate air pollutants might impact the placenta and fetus development (Kannan et al. 2006). Air pollution contributes to exacerbate systemic oxidative stress and inflammatory response which alters blood coagulation and endothelial function, affects the nutrients transportation from maternal blood through the placenta and alter the hemodynamic response. Increased

oxidative stress and oxidative damage to fetal DNA and vascular endothelium are implicated in a range of embryopathies, spontaneous abortion and perinatal deaths (Loeken 2006). In particular the neural crest cell population seems to be sensitive to toxic insults and responds by undergoing apoptosis, in part because they lack antioxidative stress proteins (Rice and Barone 2000; Hassler and Moran 1986). Normal migration and differentiation of neural crest cells play an important role in the heart development (Keyte and Huston 2012). Van Beynum et al (2008) suggested gene-environment interaction effect leading to an increased risk of congenital heart defects in mothers exposed to nitric oxide and smoking.

Birth weight, LBW, small for gestational age and also preterm birth can be caused by maternal, fetal or placental factors or a combination; for example, air pollution may affect maternal respiratory or general health, and, in turn, impair uteroplacental and umbilical blood flow, transplacental glucose and oxygen transport, and total insulin and its trophic effects on the fetus, all known as major determinants of fetal growth (Vorherr 1982). A recent review article summarized potential biologic pathways that, in some animal or cell culture models or in human beings, have been shown to be affected by particulate air pollutants, including systemic oxidative stress and inflammation, changes in blood coagulation, endothelial function and haemodynamic responses. The authors hypothesized that these might be potential mechanisms also impacting the placenta and fetus in turn (Kannan et al 2006). These pathways may or may not act independently; for example, an increase in maternal blood pressure and an impaired trophoblast invasion of the spiral arteries may induce uteroplacental hypoperfusion and a state of relative hypoxia surrounding the trophoblast, such that the resulting oxidative stress might compromise nutrient delivery to the fetus and impair fetal growth throughout pregnancy.

Moreover, exposure to fine and ultrafine particulate matter has been associated with altered placental mitochondrial function in humans (Janssen et al. 2012) and to mitochondrial damage in human tissues(Li et al. 2003), which in turn has been proposed to be associated with the susceptibility to adiposity (Bophal and Rafnssons 2009) among South Asians. In particular the authors suggested that mitochondrial efficiency

has a key role in the capacity of fat storage making south Asians more prone to store fat in deep visceral adipose tissue depots than Caucasians.

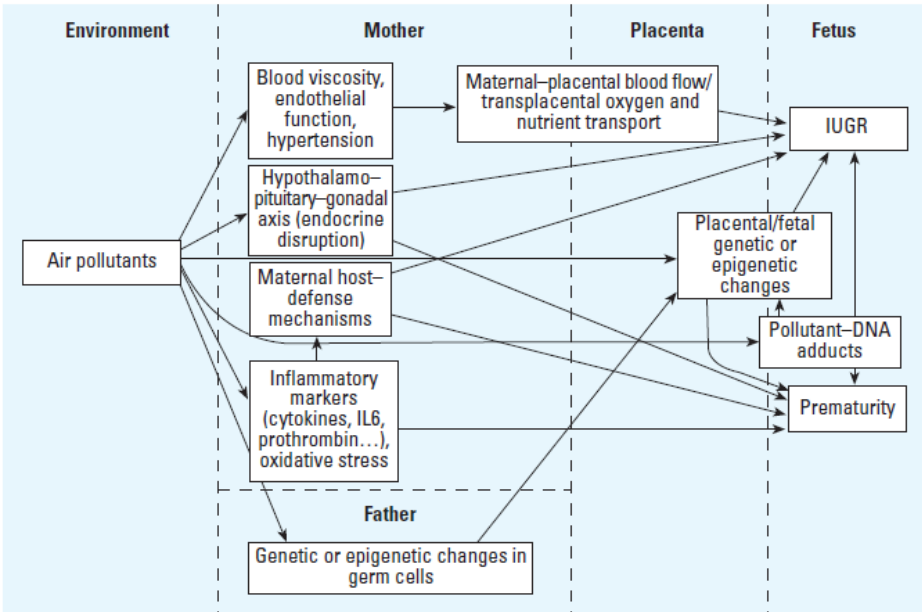

Figure 4. Possible biological mechanisms by which air pollutants could influence intra-uterine growth retardation (IUGR).

1.4.3 Current gaps

a) Personal exposure during pregnancy

Only two studies to date (Netherby et al 2008a, Valero et al 2010) have assessed pregnant women´s time activity pattern and the impact of other sources of air pollution on the total personal exposure, and results differed among geographical region reasonably because of the different weather conditions, behaviors, and occupational levels. Furthermore only Netherby et al (2008a) undertook measurements of PMs.

b) Congenital anomalies

Vrijheid et al (2011) in the meta-analysis recommended (I) to improve the exposure assessment, due to the short relevant window for the etiology of congenital anomalies and to the issues related to the use ecological assessment. (II) To harmonize the outcome definition thus to make easier to compare among studies. (III) To include the assessment of congenital anomalies others than cardiac defects and oro-facials defects such as defects of the nervous, digestive, or respiratory systems for which some evidence of association with environmental exposures has been shown (Dolk et al. 2010; Rankin et al. 2009; Padula et al 2013b).

c) Newborn size

Many steps forward have been done to improve the quality of the long term exposure assessment to air pollution and to address the health impact in European birth cohorts (Beelen et al. 2013; Cyrys et al. 2012; Eeftens et al. 2012; Pedersen et al 2013b). However, more effort is needed to understand further the possible biological mechanism, for example identifying new reproductive endpoints (Ritz and Wilhelm 2008; Woodruff et al 2009). Furthermore ethnic differences between South Asians and Whites have been poorly documented in the studies of air pollution and birth outcomes, becoming South Asians one of the largest immigrant community in the UK and in Europe.

2 RATIONALE

The epidemiology of air pollution and birth outcomes has taken a number of steps further to improve the study quality exposure assessment: refining the spatial resolution to account for the high intra-urban variability of traffic-related air pollutants, standardizing exposure techniques allowing comparisons among studies and the establishments of large scale studies such as those in the ESCAPE project. However, most studies have estimated the exposure for the residential address, without taking into the mobility of the pregnant women. This, even if small, may lead to measurement error and attenuation of risk estimates (Aguilera et al 2009). Thus it is important to disentangle the complex process that determines the personal exposure of pregnant women.

Most studies focused on birth weight as outcome which only partially account for the fetal growth (Ballester et al 2010; Ritz and Wilhelm 2008, Woodruff et al 2009). Indeed these authors warmly encourage to apply such refined exposure techniques to a wider and more refined pregnancy outcomes such for example congenital anomalies, head circumference and skinfolds thickness among others.

South Asians are at higher risk, compared to Caucasians, of low birth weight, diabetes and cardiovascular disease which can be partially explained by an excess of cellular oxidative stress (Matata and Elahi 2011). Since we assist to a large scale migration of South Asians towards western cities, they represent an important minority to include in epidemiological studies to understand if these populations are a more susceptible group even to environmental factors.

3 OBJECTIVES

General objectives:

a) To assess the determinants of the personal exposure of pregnant women.

b) To evaluate the association of exposure during pregnancy to traffic-related air pollution and birth outcomes applying refined exposure assessment techniques to various birth outcomes, including congenital anomalies, birth weight, head circumference and skinfolds thickness.

Specific objectives:

1. To investigate the relationship between pregnant women's personal exposures to NO_x, NO_2, $PM_{2.5}$ concentration and absorbance and the indoor and outdoor concentration levels at their residence in Barcelona. To identify predictors of personal exposure and indoor levels and to understand how the personal exposure levels are influenced by the time activity pattern.

2. To estimated associations between exposure to NO_x, NO_2, PM_{10}, $PM_{2.5}$ concentration and absorbance estimated using a temporally adjusted land use regression model, and congenital anomalies in Barcelona.

3. To investigate ethnic differences in the association between exposure during pregnancy to NO_x, NO_2, PM_{10}, $PM_{2.5}$ concentration and absorbance, estimated using a temporally adjusted land use regression model, and birth weight, head circumference, triceps and subscapular skinfolds thickness among White British and Pakistani origins babies, resident in Bradford.

4 METHODS

This section provides (I) an overall view of the ARIBA validation study, (II) detailed information on the exposure assessment developed in the ESCAPE project and used in the articles II and III, and (III) information on the study populations used to assess the health effects for the articles II and III. Further methodological details regarding each analysis are provided in each of the article presented in the Results section.

4.1 Personal monitoring

We recruited 54 pregnant women attending her visit at the Hospital Clinic of Barcelona. We asked the subjects to keep a 7-day diary of activities related to air pollution exposures, including mobility data. Simultaneous indoor, outdoor and personal air pollution monitoring have been conducted for NOx and $PM_{2.5}$. Nitrogen dioxide (NO and NO_2 referred to as NOx) (OgawaTM samplers, Ogawa & Co V3.98, USA, Inc) as markers for exposure to traffic emissions. Participants have been instructed to commence wearing the NOx tube, for 7 days, on the day they commence the exposure diary. We also put one tube inside their house (in living room) and one tube outside their house (e.g. close to front door). To measure $PM_{2.5}$ exposure, the women were asked to wear a small backpack containing a personal particle monitor during the daytime hours for 2 consecutive days and to place the monitor near the bed at night (BGI400 sampler with GK2.05 sampling head) (Lai et al 2004). Furthermore, one set of monitors measuring $PM_{2.5}$ have been placed inside and outside the house allowing for elemental analysis (as well as mass). The personal air sampling pumps operated continuously over this period. Filters have been weighed before and after analyses on a 6 figure balance, and we determined the light absorbance of the filter as a measure of the level of elemental carbon.

Figure 5. Pictures of the personal indoor and outdoor air pollution samples collected during the study.

4.2 Exposure assessment

The ESCAPE study started in 2008 and provided exposure assessment models around December 2010, collecting air pollution during June 2008 to June 2009. A mixture of measurements and modeling was used to

estimate exposure of study participants to ambient air pollution. Specifically, was performed a spatially resolved measurements of PM_{10}, $PM_{2.5}$, the soot content of $PM_{2.5}$ and NOx. PM measurements were conducted at 20 monitoring sites per city or area; NOx measurements were conducted at 40 monitoring sites per city or area. Measurements were conducted for three periods of two weeks per site in the cold, warm and one intermediate temperature season. Sites were selected to represent the anticipated spatial variation of air pollution at home addresses of participants in the epidemiologic studies.

Annual average concentrations were used to develop exposure models (land use regression models). Development of land use regression models was conducted by collecting potentially important predictor variables for air pollution for the geographical coordinates of each of the monitoring sites using GIS analyses. First, the locations of monitoring sites were geocoded. For each coordinate, values for potential predictor variables were collected, and geographic information system (GIS) data was collected. These variables were then linked to measurements in a statistical model to develop land use regression models. Before the air pollution measurements have been finished, GIS information were collected (e.g. information on land use, address density /number of inhabitants) as these variables were used as predictor variables in the land use regression models.

Linear regression models were thus developed using a supervised stepwise selection procedure, first evaluating univariate regressions of the corrected annual average concentrations with all available potential predictors. The predictor giving the highest adjusted explained variance (adjusted R^2) was selected for inclusion in the model if the direction of effect was as defined a priori. We then evaluated which of the remaining predictor variables further improved the model adjusted R^2, selected the one giving the highest gain in adjusted R^2, and the right direction of effect. Subsequent variables were not selected if they changed the direction of effect of one of the previously included variables. This process continued until there were no more variables with the right direction of effect, which added at least 0.01 (1%) to the adjusted R^2 of the previous model.

LUR models for NO_2 and NO_x were developed for around 40 European cities or regions, while PMs models were for 20 European cities or regions, among them Barcelona, Bradford and London/Oxford area. LUR models coefficients for PMs developed in London/Oxford area were used to estimate PMs exposure in Bradford. The final R^2 for each model are presented in the Table 2, below.

Table 2. Adjusted R^2 for the models of air pollutants developed in the ESCAPE project in UK and in Barcelona.

Area	NO_x	NO_2	PM_{10}	PMcoarse	$PM_{2.5}$	$PM_{2.5}$abs
UK*	90%	83%	90%	68%	82%	96%
Barcelona	73%	75%	90%	75%	83%	86%

* UK LUR models are based on Bradford measurements of NO_2 and NO_x, and on London and Oxford region measurements of PMs.

The model development and final formulae are detailed by Beelen et al (2013) and by Eeftens et al (2012), an example is:

$PM_{2.5}$ in Barcelona, Spain= $16.21 - 4.08 \times 10^{-6} \times$ GREEN_1000m + $2.04 \times 10^{-7} \times$ TRAFLOAD_100m + $6.82 \times 10^{-3} \times$ INTINVDIST

Where

GREEN_1000m is: urban green and natural land combined in a buffer of 1000m from the residential address

TRAFLOAD_100m = heavy traffic (vehicles·day^{-1}·m) for all roads in a buffer of 100m,

INTINVDIST is the product of inverse distance to the nearest road and the traffic intensity on this road (vehicles·day^{-1}m^{-1}).

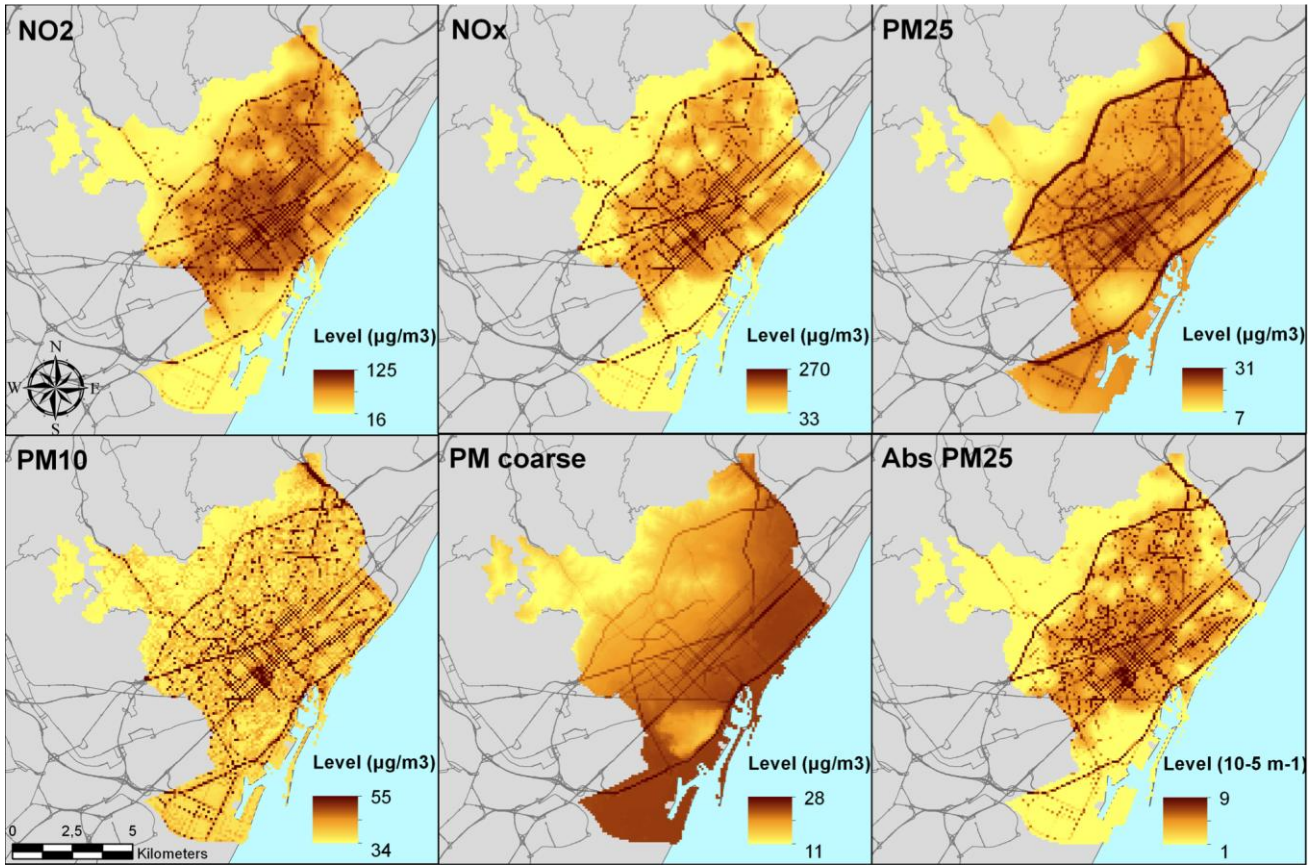

Figure 6. ESCAPE Maps of pollutants distribution in Barcelona.

4.3 Registre de Defectes Congènits de Barcelona – REDCB

The Barcelona Birth Defects Registry (Registre de Defectes Congènits de Barcelona –REDCB) was initiated in 1990 but reached a population-based status by 1992, when it became a member of the Eurocat. The REDCB is part of the Service of Health Information Systems (Servei deSistemes d'Informacio´ Sanitaria) in the Public Health Agency of Barcelona (Age`ncia de Salut Pública de Barcelona). It is funded by Regional (Generalitat de Catalunya) and Local (Ajuntament de Barcelona) Administrations.

The REDCB covers pregnancies of women resident in Barcelona City ending in live or stillborn babies of 22+ weeks of gestation or in induced abortions (IAs) due to prenatal detection of birth defects (i.e., the registry does not include birth defects in spontaneous abortions <22 weeks of gestation, or IAs due to other reasons than prenatal detection of birth defects). Some 3% of residents' newborns occur at maternity units out of the city limits, the nurses of the REDCB controls 50% of these newborns (1.5%). Since Barcelona City is a pole of attraction for at risk pregnancies, the remaining 1.5% of newborns not controlled by the registry probably provides <1% of cases. In 2008, the REDCB covers about 14,500 births annually. In the previous 5 years, the number of births has increased from about 12,500, mainly due to a higher fertility rate among immigrant women of developing countries coming to the city.

General information on cases and controls, as well as clinical information on cases, is collected using questionnaires specifically made for the registry. An interview with the mother is the main source of general information. Delivery units, pediatric departments, cytogenetic laboratories, pathology departments, prenatal diagnosis units, and pediatric cardiology services are the main sources of clinical information. The registry has direct access to some cytogenetic laboratories, but not to all. Some of the collaborating laboratories send their data by e-mail, but for most of them, registry staff members personally go to collect the case information. Pediatric cardiology centers covering part of the registry

population supply systematic case lists and diagnostic details to the registry.

The maximum ae at diagnosis is up to 3 days of age. When a suspicion of birth defect exists, follow-up is made until a diagnosis or normality is stated. This affects mainly birth defects as congenital heart defects (CHD).

IA after prenatal detection of a birth defect is legal in Spain until 22 weeks of gestation. Cases are defined as fetuses or newborns with at least 1 major or 21 minor anatomic birth defects or with unbalanced chromosomal anomalies. The follow-up period is 2 to 3 days after delivery. Due in part to this short follow-up period, poor ascertainment exists for birth defects as mild hypospadias, some cardiovascular defects, and other anomalies of internal organs not detected using prenatal ultrasounds. The ascertainment of unbalanced chromosomal anomalies without neonatal anatomic defects (as some sexual chromosome anomalies like XXX, XXY, XYY) is never complete and depends on the rate of karyotyped pregnancies.

A random sample (not case-matched) of about 2% of the newborns expected in each maternity unit is selected as controls. Information on maternal drug use, maternal and paternal diseases, and occupations is available for cases and controls, but it is not sending to the EUROCAT Central Registry. The REDCB systematically sends data to the EUROCAT Central Registry on all "core" variables but two (civil registration status and McKusick code), and on a "non-core" variable (karyotype). Background data on births are available from birth certificates and the Barcelona Perinatal Mortality Registry. The registry is located in a health authority setting (Barcelona Public Health Agency). Parental consent is asked for during interview with the mothers of cases and controls.

4.4 The Born in Bradford cohort study - BiB

Full description of the Born in Bradford cohort profile can be found in Wright et al (2012). In brief, the BiB cohort study was established in 2007 to examine how genetic, nutritional, environmental, behavioral and social factors impact on health and development during childhood, and subsequently adult life in a deprived multi-ethnic population. Between 2007 and 2011, detailed information on socio-economic characteristics, ethnicity and family trees, lifestyle factors, environmental risk factors and physical and mental health has been collected from 12 453 women with 13 776 pregnancies (recruited at around 28 weeks) and 3448 of their partners. Mothers were weighed and measured at recruitment, and infants have had detailed anthropometric assessment at birth and post-natally up to 2 years of age. Results of an oral glucose tolerance test and lipid profiles were obtained on the mothers during pregnancy at around 28 weeks gestation, and pregnancy serum, plasma and urine samples have been stored. Cord blood samples have been obtained and stored. Birth weight, head circumference, triceps skinfolds thickness, subscapular skinfolds thickness and abdominal circumference have been collected at birth, within the first 72h of life by trained operators.

Born in Bradford (BiB) was created in response to rising concerns about the high rates of childhood morbidity and mortality in the city of Bradford, the sixth largest city in the UK with a population of about half a million and urban areas that are among the most deprived in the UK. Around 20% of the population of Bradford is of South Asian origin (90% of whom are from Pakistan), and this constitutes a three-generation community that maintains close links with Pakistan Small et al (2012). The relatively young age of the population of Pakistani origin and their higher fertility rates, compared with the White British majority population, explain why almost half of babies born in the city have parents of Pakistani origin. Sixty percent of the babies born in the city are born into the poorest 20% of the population of England and Wales based on the British government's residential area Index of Multiple Deprivation (Townsend, Phillimor, and Alastair 1988). Infant mortality in Bradford has been consistently above the national average, peaking at 9.4 deaths/1000 live births in 2003, when the national average was 5.5

deaths/1000 live births, and levels of congenital anomalies and childhood disability are among the highest in the UK.

5 RESULTS

5.1 Article I: Personal, indoor and outdoor Air pollution levels among pregnant Women *

Schembari A, Triguero-Mas M, de Nazelle A, Dadvand P, Vrijheid M, Cirach M, et al. 2013. Personal, indoor and outdoor air pollution levels among pregnant women. Atmos Environ 64:287–295.
doi:10.1016/j.atmosenv.2012.09.053

*This article is reproduced according to the published manuscript

5.2 Article II Traffic-Related Air Pollution and Congenital Anomalies in Barcelona*

Schembari A, Nieuwenhuijsen MJ, Salvador J, de Nazelle A, Cirach M, Dadvand P et al. 2014. Traffic-related air pollution and congenital anomalies in barcelona. Environ Health Perspect 122:317-323

*This article is reproduced according to the published manuscript.

Schembari A, Nieuwenhuijsen MJ, Salvador J, de Nazelle A, Cirach M, Dadvand P,Beelen R, Hoek G, Basagaña X, Vrijheid M. Traffic-related air pollution and congenital anomalies in Barcelona. Environ Health Perspect. 2014 ;122(3):317-23. doi: 10.1289/ehp.1306802

5.3 Article III: Ambient Air Pollution and Newborn's Size at Birth: Differences by Maternal Ethnicity – Results From the Born in Bradford Study Cohort.*

Anna Schembari, Marie Pedersen, Kees de Hoogh, Payam Dadvand, David Martinez, Gerard Hoek, Emily S Petherick, John Wright and Mark J Nieuwenhuijsen

***** Under review on **Environmental Health Perpesctive** [second revision submitted 01 august 2014]

Schembari A, de Hoogh K, Pedersen M, Dadvand P, Martinez D, Hoek G, Petherick ES, Wright J, Nieuwenhuijsen MJ. Ambient Air Pollution and Newborn Size and Adiposity at Birth: Differences by Maternal Ethnicity (the Born in Bradford Study Cohort). Environ Health Perspect. 2015 Nov;123(11):1208-15. doi: 10.1289/ehp.1408675.

6 DISCUSSION

This section aims to integrate and interpret the results presented and to discuss the overall achievements of this thesis.

6.1 Contribution to the current knowledge

The epidemiological studies of exposure to ambient air pollution and birth outcomes have been many; however results are inconsistent partly due to imprecise exposure assessment and use of somehow limited outcomes (few groups in the case of congenital anomalies, not specific in the case of birth weight). In this thesis (I) the determinants of personal exposure during pregnancy and the correlation between personal exposure and indoor and outdoor air pollution levels have been investigated, providing a better understanding of the sources of exposure error. (II) Refined exposure assessment techniques, implemented in the European Union within the framework of the ESCAPE project, have been employed to estimate associations between a wide range of air pollutants and the birth outcomes, providing comparable evidence with much larger studies. (III) The studies presented have the merit to enhance the current range of outcome evaluated in the epidemiology of air pollution and congenital anomalies and newborn size. This helps, in the case of congenital anomalies, to explore further the implications of exposure to air pollutants and, in the case of newborn size, to test biological mechanisms which may concur to the determination of birth weight.

6.1.1 Personal vs. ambient exposure.

In the article I a detailed observation of which characteristics of the house or of the personal time activity pattern was carried out stressing the

importance of accounting for the time spent commuting, the house ventilation, the season and exposure to smoking. Personal exposure to NO_2 and NO_x was explained quite well by the characteristics described above while the variability in $PM_{2.5}$ was mainly related to the time spent commuting or cooking. The correlation between personal and outdoor levels was moderate to high and the time spent indoor at home is around the 60% on a daily base. From these results we can deduce that including data on time spent commuting might improve the estimates of exposure to traffic related air pollution. Indeed further analyses of the elemental composition of the filters indicated that the mineral found in $PM_{2.5}$ filters were probably originated in the underground rail (Minguillón et al 2012b).

In large epidemiological studies it's however difficult to include personal monitors data. In particular, the registries of congenital anomalies mostly do not recollect information about the house or the time activity of the pregnant women. Mothers of babies born with congenital anomalies are emotionally compromised thus information on personal behaviors might be subject to recall bias. On the contrary, prospective birth cohort studies might collect more detailed information about time activity pattern of the future mothers. However to date only two studies where able to do so; results from the INMA birth cohort, showed stronger associations between air pollutants and birth size among women who spent more time at home (Aguilera et al 2009, Estarlich 2011). Another approach have been to combine the exposure estimated at home with that at the working place (Madsen et al 2010; Iñiguez et al 2009), which resulted in slightly stronger association among those women who do not work or spent more time at home. In the BiB cohort study the work address was not available, our results stratified by working status showed slight not statistically significant differences between workers and non-workers, however we cannot exclude exposure misclassification because employed status can be associated with other factors such as maternal or familiar diseases and poverty.

Exposure to active or passive smoking and the season of conception are, on the contrary, the only reoccurring determinants of personal air pollution commonly available in large epidemiological studies and thus it is common to see results of the association models adjusted for exposure to smoking and for season (of conception or of birth). Such elements

have been associated to birth outcomes (Bernstein et al. 2000; Chodick et al. 2009; D'Souza et al. 1981; Hackshaw et al. 2011; Luciano et al. 1998; Strand et al. 2011; Vardavas et al. 2010). However, the confounding effect of smoking assessed in the air pollution and congenital anomalies studies was little (Gilboa et al. 2005; Marshall et al. 2010), which was also confirmed in our congenital anomalies study. In our newborn size study, on the contrary, smoking during pregnancy was a statistically significant predictor. In both our studies on birth outcomes, season of conception was included as a statistically significant covariate, moreover, in the article III, the association estimated for air pollution were significantly modified when the models were not adjusted for season of conception. In previous literature season of conception was included itself (Aguilera et al 2010; Ballestrer et al 2010; Basu et al 2013; Pedersen et al 2013b) or taken into account by temperature (Bell et a 2012; Geer et al 2012) confirming previous results.

Pregnant women spent around the 60% of their time at home during the second trimester, as shown in our article I and in Neterby et al (2008c)and in Valero et al (2010). Moreover, as the pregnancy advances, the time time spent at home increases and as well as the time spent within the residential neighborhood (Netherby et al. 2008a); hence this suggests that the exposure assessment error decreases during the last trimester of the pregnancy.

In conclusion, collecting information on personal exposure, behaviors, time activities and mobility of pregnant women is not feasible for all the participants in large epidemiological studies because of the high costs and burden on the participants. But the collection of such data in a subsample of the study population allows a better understanding of the personal exposures and helps in the interpretation of the results.

6.1.2 Exposure assessment improvements

The Article II and article III rely on the exposure assessment models developed in the ESCAPE project framework which used one of the most advanced techniques for estimating the exposure to traffic related air

pollution within cities or small regions (Beelen et al. 2013; Eeftens et al 2012). The main strengths of these models are the comprehensive range of air pollutants included, the standardized collection of pollution levels over 3 years (2008-2011), the harmonized and detailed information collected on potential determinants of traffic (using GIS) and the standardized method used to determines models over XX cities and regions in Europe. Thus we were able to estimate small-scale spatial exposure contrasts during the pregnancy periods. Models with these characteristics were developed to provide an answer to the urgent need of improvement of air pollution exposure assessment in the epidemiological studies (Ritz and Wilhelm 2008, Woodruff et al 2009).

As discussed in detail in each of the articles, to temporally adjust exposure during the exact pregnancy periods, which occurred 1 to 14 years earlier when the ESCAPE models were development, long-term routine monitoring data were used. This approach relied on the assumption that there were no major changes in the spatial distribution of the determinants of air pollution (eg. traffic density, land-use) which has been successfully tested by (Cesaroni et al 2012; Eeftens et al 2011). Further assumptions of similar temporal trend of PM10 and $PM_{2.5}$ and of NO_x and $PM_{2.5}$ absorbance were made to back-extrapolate $PM_{2.5}$ and $PM_{2.5}$ absorbance respectively. We tested and confirmed these assumptions in the article II.

The main result of a more accurate exposure assessment is to increase the precision of the association estimates thus to increase the power of the study. Indeed, the associations presented in the articles II and III are consistent with those of meta-analysis (Stieb et al 2010; Vrijheid et al 2011) and those of larger epidemiological studies (Pedersen et al 2012), but are based on much smaller study population.

The exposure assessment was based on the residential address, thus the two possible main sources of exposure misclassification are related to the exposure occurred elsewhere and to changes during pregnancy of the residential address. The first issue was discussed in detail in the previous section. The residential mobility during pregnancy have been estimated to be around 4% to 15% in European studies (Aguilera et al 2009; Estarlich et al. 2011; Pedersen et al 2012) and between 14% and 40% in US or

Canadian based studies (Brauer et al. 2008; Canfield et al. 2006; Chen et al. 2010; Lupo et al. 2010). However all agreed that the new addresses were generally within short distances, and that it does not significantly influence the exposure, and in the case of congenital anomalies is not different between cases and controls. The studies that evaluated the changes in the association estimates (Aguilera et al 2010; Brauer et al 2008; Estarlich et al 2011; Pedersen et al 2012) suggest that indeed the error was small, which was confirmed in the presented article III.

Our congenital anomalies study has been considered as a pilot for the feasibility of an European case-control study of air pollution and risk of congenital anomalies (EUROCAT 2004). Indeed such an effort should be seen in the larger contest of the establishment of European birth cohorts studies (Gehring et al. 2013; Kogevinas et al. 2004; Vrijheid et al. 2012) which have the advantage of increase the statistical power of the studies, to easily select the samples on a range of exposure and outcomes facilitating the collaboration among studies, to have an inventory and harmonization of the data collected and to simplify the conduction of studies replication. The results of the congenital anomalies study acts as a guaranty for the success of apply the already available LUR models developed in the ESCAPE project to those European registries of congenital anomalies having at least the residential address of the mother. Such type of study is made possible because of the effort done by the Eurocat to harmonize the outcome definition and the set of covariates available.

6.1.3 Novel outcomes

a) Congenital anomalies

The majority of studies evaluating the traffic-related air pollution effects on congenital anomalies have included mainly those of the cardiac system and the oro-facial and cleft palate because these are two of the largest anomaly groups and they have been suspected to be related to other environmental exposures (Dolk and Vrijheid 2003). However, neural tube

defects, cardiac defects as well as musculoskeletal, gastrointestinal and facial defects have been associated to other environmental factors (Dolk et al 1998; Hackshaw et al 2011; EUROCAT 2004), hence the importance to include them further in this type of studies.

In developing the strategy for the article II we considered that: (I) the REDCB offers a detailed description of the major congenital anomalies groups following the Eurocat classification, thus brings the opportunity to have a detailed classification of the cases easily reproducible; (II) there were only few congenital anomalies groups with less than 100 cases in the crude analyses; (III) the refined exposure assessment from the ESCAPE models was expected to reduce the measurement error and to increase the power of the study. We therefore included congenital anomalies groups not frequently studied before in relation to exposure to air pollution (Dolk et al 2010; Padula et al 2013; Rankin et al 2009) such as: neural tube defect, cardiac defects in a detailed classification, respiratory system anomalies, digestive system anomalies, abdominal wall defects, urinary, hypospadias and limb reduction. Our results were consistent with previous literature and indicate some association between air pollution and the cardiac anomalies groups. Further we also found indication of association for abdominal wall defects and digestive system to be worthy further studied.

b) Newborn size

In the studies of air pollution and birth outcomes, birth weight has been the most investigated outcome among the indicators of birth size because it is an outcome available from birth certificate. Birth weight is indeed a very important outcome because it is the results of the developmental progression from the time of conception to birth and it has been proposed as intermediate between prenatal environmental exposures and health status later life; hence the in utero effects of environmental agents on pregnancy outcomes are of interest. However, given the current interest in the developmental and growth-related effects of air pollution, the identification of novel and more sensitive health outcomes to assess early health effects that can predict later disease, is needed.

Recent epidemiological evidence links exposure to air pollution and development of obesity during childhood (Jerret et al 2014, Rundle et al 2012). Biological plausible mechanism of such an effect may act through systemic inflammation to increase pro-obesogenic pathways (Bolton et al. 2012; Mendez et al. 2013; Sun et al 2009). Some components of traffic-related air pollution may contain endocrine disruptors that could be obesogens.

In the newborns skinfolds thickness provides an estimate of the total body fat of the infant (Farmer et al. 1985). Postnatal fat accumulation occurs predominantly in the extremities: triceps (upper arm) skinfolds thickness provides an indication of the periferical body fat mass while subscapular (upper back) skinfolds thickness reflects the visceral/subcutaneus fat (Ketel et al. 2007; Snijder et al. 2006). Subcutaneous/visceral fat mass have increasingly been related to the pathogenesis of insulin resistance and other precursors of cardiovascular disease (Patel et al. 2013; Sniderman et al. 2007). Hence, we proposed triceps and skinfolds thickness at birth as outcomes to capture such possible effect of prenatal exposure to air pollution.

In Article III our results indeed indicate a statistically significant increase in triceps or in subscapular skinfolds thickness at birth at increasing exposure to PMs. This was the first study assessing such associations; however, maternal smoking during pregnancy has been associated to reduced size at birth (weight, length and head circumference) but not to thinner skinfolds thickness (Bernstein et al. 2000; D'Souza et al. 1981; Luciano et al. 1998),confirming our results.

6.1.4 The role of Pakistani Ethnicity

Asians and in particular Pakistani are underrepresented in the epidemiological studies of air pollution and birth outcomes. The Pakistani immigration in England started in the mid nineteenth century and in Bradford, an industrial city in the north of England, this community in now at its second or third generation and represents an important part of the society. The constant increased risks of low birth weight and neonatal

mortality independently form the social class among Pakistani origins newborns together with the higher risk of obesity, diabetes and cardiovascular disease during adulthood makes this ethnic group disadvantaged regardless to the White British community and important for public health implications.

As explained earlier, many have been the possible mechanisms beyond these differences which have lead to a higher abdominal/visceral fat mass and its aetherogenic consequences among South Asians compared to Caucasians. The biological mechanism proposed may act through the efficiency of mitochondria (Bhopal and Rafnsson 2009) which in turn can be affected by air pollution exposure (Janssen et al 2012).

In the article III the effect of prenatal exposure to traffic-related air pollution on birth size were analyzed in relation to the maternal ethnicity. There was indication of a differential effect of exposure to PMs and birth weight, head circumference and skinfolds thickness which identifies Pakistani origins more susceptible to fat mass storage induced by exposure to air pollution. These results call for confirmation in other epidemiological studies on the same outcomes or on the childhood growth trajectory; furthermore attention should be dedicate to understand the biological plausibility for such an effect.

6.1.5 Confounders and effect modification

A key challenge in epidemiological studies that needs to be adequately addressed to ensure the validity, accuracy and reliability of the results is confounding. After the identification of the set of relevant covariates for each exposure-outcome pairs, the availability of such information needs to be evaluated. Key factors were identified on the base of previous evidence from other studies or on data driven associations between covariates in analyses from the BiB population. One very useful tool to identify and clarify the relationships between co-variates, exposure and outcomes are the direct acyclic graphs (DAGs) because permits to visualize the research hypothesis and to detect sources of confounding, overadjustment and unnecessary adjustment (Schisterman et al. 2009), measurement error

(VanderWeele and Hernan 2012) and selection bias (Hernan et al. 2004) that may distort effect estimates. However, due to the uncertainties related to the lack of evidence for the association between some of the covariates included in a DAGs, more than one DAG is plausible under different assumption on the same research hypothesis.

Using this approach Slama et al (2008, Figure 7) described the frame upon which researches on the air pollution and birth outcomes have been based so far. We adopted that.

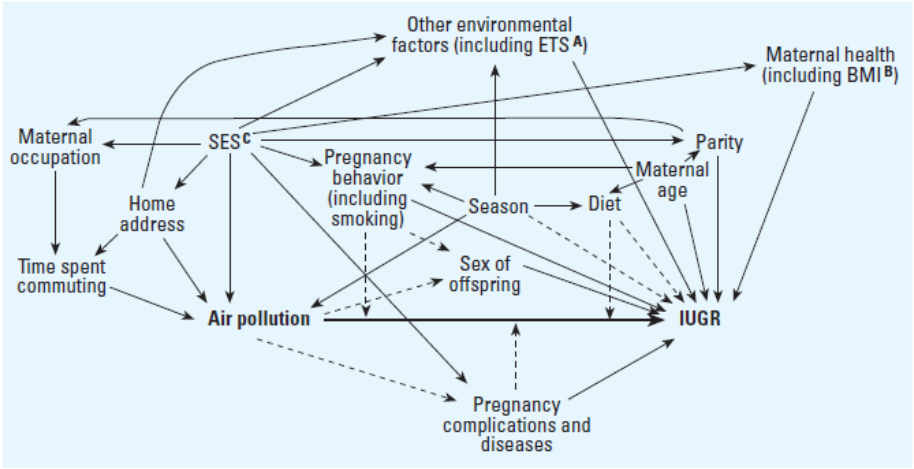

Figure 7 Hypotized relationships between air pollution, IUGR, and extraneous factors possibly acting as confounders in epidemiologic studies of air pollution and IUGR. Arrows indicates plausible effect of a factor over another not mediated by another factor present in the diagram. A dotted arrow indicates a plausible although not established relationship. An arrow form a factor A that intersects an arrow from B to C indicates that A may modify the effect of B on C.

Despite our careful consideration, we cannot rule out that other confounders that were not measured or confounders that we are not aware of, may partially explain the estimated associations, as it is discussed in more detail in the articles. Further, measurement errors in the potential confounders analyzed may have compromised our ability to control for their effect, leaving "residual" confounding.

Confounders might also be attributable to the correlation between the air pollutants itself. Such correlation is intrinsic in the nature of the pollutants

and of the exposure assessment techniques thus cannot be removed and indeed was not always negligible in article II and III. The main consequence, in this case, is the uncertainty of attributing the associations observed to one or the other exposure, as discussed in detail in the articles. In article II this issue was further mixed to the high number of comparisons made (6 pollutants and 18 congenital anomalies groups) which can also increase the probability of a false "true" association. Thus the associations found statistically significant using the spatio-temporal exposure were interpreted as "true" only if them were consistent with the associations calculated using a simpler exposure at spatial level. In article III instead, most of the statistically significant associations were found for PMs, which are indeed the same complex family of pollutants, thus there was almost no contrast in interpreting the results.

Another confounder we haven't controlled for was diet. The diet has been proposed (Kannan et al 2006) as a possible modifier of the effect of air pollution on birth outcomes in many ways: reducing oxidative stress, modifying the inflammatory response, activating coagulation or, on the contrary, improving endothelial function. There is a growing list of foods and food groups which consumption is associated with the biological pathways mentioned. Diet has been associated with birth outcomes (Pedersen et al. 2013a; Pedersen et al. 2012). However it is rare to see such interaction measured in the air pollution studies because studies based on hospital records do not report diet data thus estimate the diet and the nutrient intake is not that way straightforward. However the promise for an interaction is warranted (Sun et al 2013; Guxens et al 2012) thus we recommend to including dietary information when possible in the future studies of air pollution effect on newborns health (Mendez and Kogevinas 2011).

Another limitation is that we haven't assessed the conjunct effect of traffic-related noise and air pollution on birth outcomes. The review on the impact of noise on birth outcomes do not indicate such an impact (Hohomann et al 2013), however Gehring et al (2014) found statistically significant association between noise and birth outcomes, and in the joint models for noise and air pollutants the associations between noise and term birth weight remained largely unchanged, whereas associations decreased for all air pollutants. Indeed the main underling source of noise

and air pollution is traffic thus an adequate assessment of both is necessary to identify their independent effect.

6.2 Limitations

This paragraph aims to sharp the overall limits of the thesis and do not presents a detailed discussion of the limits of each study.

The wide range of objectives included in this project made it quite ambitious; ranging from the understanding of the determinants of exposure to air pollution to the application of refined exposure assessment techniques to study the associations with novel outcomes. Despite the carefully assessment of each of the objectives there are details which could have had straight the contribution of this project to the current gap in the knowledge.

The first limitation is the lack of the quantitative assessment and correction for the measurement error in the exposure assessment. The work presented in article I was one of the first study conducted at CREAL on personal measurements of air pollution, in particular the first measuring $PM_{2.5}$, among pregnant women. We failed in collect repeated measures in each of the trimester of pregnancy for each woman. We also faced problems of invalidity of 6 out of 60 filters, which reduced the sample size. Corrections carried out on the base of not precise information on error magnitude may increase thee bias rather than decrease it (Armstrong 1998). Indeed our publication is descriptive and further, on a practical view, we collaborated with other groups within CREAL to develop the Validation of ESCAPE Exposure EstimateS using Personal exposure Assessment-VE3SPA study, in 2010. We also collaborated with other groups to establish the validation study among pregnant women in the Helix project.

A consequence related to this is that any translation of the results of the article I into the interpretation of article II and article III and any efforts toward an overall harmonization of the results may appear speculative.

However, the articles I and II are both based in Barcelona, which increases their compatibility; overall the harmonization is recovered because under the general objective to introduce new outcomes.

6.3 Public health implications

Levels of air pollution are of public health concern and are regulated all around the developed world. However recommended values are often exceeded and the health effects appear even at levels lower than those fixed by low. The epidemiological evidence of such effects on newborns has been growing in the recent years because of the improvements in the study design and exposure assessment (Dadvand et al 2013; Pedersen et al 2013; Stieb et al 2010; Vrijheid et al 2010), and this thesis partially contributed to these achievements. A more restricted legislation of air pollution levels is recommended, however beyond that, the results of this thesis have the merit to encompass elements which can inspire a number of actions aiming to encourage a healthier lifestyle. Indeed, from my personal point of view, every epidemiological study should contain elements addressing policies recommendations and elements encouraging population behavioral changes which act synergistically towards the reduction of the exposure in the general population.

Our results suggested that increasing the time spent outdoor and in active transportation may reduce the exposure to air pollutants, we also showed that those pregnant women living in proximity to green spaces had lower air pollution and spent more time outdoor (Dadvand et al 2012). Furthermore there is a growing body of evidence for the protective effects of green spaces on birth outcomes (Dadvand et al 2012; Agay-Shay et al 2014). Finally, we encourage to reduce the indoor exposure by avoid smoking; improve the efficiency of the buildings, in particular to use clean engines to cook and warm the house use, and to efficiently ventilate the house.

Secondly, we recommend to reduce the levels of ambient air pollution developing an urban plan which includes the shifting toward cleaner heavy duty diesel vehicles and low-emissions vehicles and fuels, which

remodels the urban circulation prioritizing the active transportation such as walking and cycling and improves the fast public transportation services, which improves the safety perception in the streets and encourage the use of the public places.

If the indications from the recent emerging evidence of the impact of air pollution on birth outcomes are disregarded, the newborn health will be compromised by an increase in risk of congenital heart anomalies, a reduced birth weight and a higher risk of LBW and maybe by an increase in adiposity. In the developing countries such as India, China and Brazil the actual situation may become an emergency because on the one hand there is a rapid and some time wild urbanization and a "dirty" and inefficient vehicular traffic; on the other, unfortunately, the health care systems and the health as well as the house conditions are in many cases still deficient; hence a rapid increase in the air pollution and in the number of exposed people may represent an emergency. Some 4.3 million premature deaths were attributable to household air pollution in 2012 and almost that entire burden was in low-middle-income countries (WHO 2014)

6.4 Implications for future research

Several uncertainties remains which suggests further effort to investigate the following areas:

- Further study the toxicity of particles, including PAHs and elemental composition, both in validation studies and in models estimating the exposure.

- Conduct a more extensive personal monitoring study of air pollution levels which allows for accounting the measurement error.

- To develop and European case control study of air pollution and congenital anomalies, making use of the exposure assessment models developed in the ESCAPE project.

- To further investigate the effect of air pollution in the development of adiposity and fat storage in newborns and during childhood.

Under the hypothesis that diet and air pollution may interact in several biological pathways, information on diet should be assessed in such studies.

- In view of the Ethnic differences in the susceptibility toward air pollution, in particular between Pakistani origins and White British, we recommend future studies to try to confirm these results.

- To assess noise exposure and include it in the analyses of traffic-related air pollution to assess the eventual effect cofounder of noise exposure.

- Verify the biological pathways that may result in the increased adiposity at birth with the exposure in uterus to air pollution

- Verify the biological pathways that may result in the differential effect of air pollution between Pakistani origins and White British

7 CONCLUSIONS

✓ Personal exposure levels of air pollution among pregnant women in Barcelona were higher than indoor and outdoor estimations, and were strongly correlated with indoor levels than with outdoor.

✓ Pregnant women spent around 60% of their time indoor where exposure levels appeared influenced by human activities such as cooking or smoking, beside the outdoor levels. Furthermore using a "dirty" gas for cooking activities increased the exposure to NO_2 and NO_x. Time spent walking or biking contributed to reduce the personal exposure to particulate matters.

✓ Results on pregnant women behaviors should be considered in the light of the climate, traffic situation and hosing stock in Barcelona, a Mediterranean city with hot dry summers and mild winters, where there is often no air conditioning and where people leave windows open providing natural ventilation. Even where windows are closed there may be substantial ventilation due to bad insulation. Also, there is always a lot of traffic, with traffic density levels on average four times higher compared to London.

✓ There is small increase in risk of selected congenital anomalies and traffic-related air pollutants. However the positive association of NO_2 and NO_x with coarctation of the aorta is consistent with findings of a meta-analysis of previous studies.

✓ Associations of the digestive system anomalies with NO_2 and NO_x, and of abdominal wall defects with PMcoarse, were novel and call for confirmation.

✓ Associations of exposure to particulate matter with birth weight, head circumference and skinfolds thickness appeared differential between White British and Pakistani origins infants.

✓ Association of exposure to particulate matter and skinfolds thickness was for the first time assessed but is consistent with the recent hypothesis of the role of particles in the exacerbation of obesity.

✓ The use of refined exposure assessment technique improved the quality of results reducing the uncertainties related to the measurement error. However the assessment of some identified determinants of air pollution might improve the quality of the results.

Reference List

Adams H, Nieuwenhuijsen MJ, Colvile R, Older M, Kendall M. 2002. Assessment of road users' elemental carbon personal exposure levels, London, UK. Atmospheric •Environment 36:5335-5342.

Agay-Shay K, Friger M, Linn S, Peled A, Amitai Y, Peretz C. 2013. Air pollution and congenital heart defects. Environ Res 124:28-34.

Aguilera I, Guxens M, Garcia-Esteban R, Corbella T, Nieuwenhuijsen MJ, Foradada CM et al. 2009. Association between GIS-based exposure to urban air pollution during pregnancy and birth weight in the INMA Sabadell Cohort. Environ Health Perspect 117:1322-1327.

Armstrong BG. 1998. Effect of measurement error on epidemiological studies of environmental and occupational exposures. Occup Environ Med 55:651-656.

Ballester F, Estarlich M, Iñiguez C, Llop S, Ramón R, Esplugues A et al. 2010. Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. Environ Health 9:6.

Bansal N, Ayoola OO, Gemmell I, Vyas A, Koudsi A, Oldroyd J et al. 2008. Effects of early growth on blood pressure of infants of British European and South Asian origin at one year of age: the Manchester children's growth and vascular health study. J Hypertens 26:412-418.

Basu R, Harris M, Sie L, Malig B, Broadwin R, Green R. 2014. Effects of fine particulate matter and its constituents on low birth weight among full-term infants in California. Environ Res 128:42-51.

Beelen R, Hoek G, Vienneau D, Eeftens M, Dimakopoulou K, Pedeli X 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 72:10-23.

Bell ML, Belanger K, Ebisu K, Gent JF, Leaderer BP. 2012. Relationship between birth weight and exposure to airborne fine particulate potassium and titanium during gestation. Environ Res 117:83-89.

Bell ML, Belanger K, Ebisu K, Gent JF, Lee HJ, Koutrakis P et al. 2010. Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources. Epidemiology 21:884-891.

Berkson J. 1950. Are there two regressions? J American Statistical Association 45:164-180.

Bernstein IM, Plociennik K, Stahle S, Badger GJ, Secker-Walker R. 2000. Impact of maternal cigarette smoking on fetal growth and body composition. Am J Obstet Gynecol 183:883-886.

Bhopal RS and Rafnsson SB. 2009. Could mitochondrial efficiency explain the susceptibility to adiposity, metabolic syndrome, diabetes and cardiovascular diseases in South Asian populations? Int J Epidemiol 38:1072-1081.

Bolton JL, Smith SH, Huff NC, Gilmour MI, Foster WM, Auten RL et al. 2012. Prenatal air pollution exposure induces neuroinflammation and predisposes offspring to weight gain in adulthood in a sex-specific manner. FASEB J 26:4743-4754.

Brauer M, Hoek G, Van Vliet P, Meliefste K, Fischer P, Gehring U et al. 2003. Estimating long-term average particulate air pollution concentrations: application of traffic indicators and geographic information systems. Epidemiology 14:228-239.

Brauer M, Lencar C, Tamburic L, Koehoorn M, Demers P, Karr C. 2008. A cohort study of traffic-related air pollution impacts on birth outcomes. Environmental health perspectives 116:680.

Briggs DJ. 2007. The use of GIS to evaluate traffic-related pollution. Occup Environ Med 64:1-2.

Briggs DJ, de Hoogh C, Gulliver J, Wills J, Elliott P, Kingham S et al. 2000. A regression-based method for mapping traffic-related air pollution: application and testing in four contrasting urban environments. Sci Total Environ 253:151-167.

Canfield MA, Ramadhani TA, Langlois PH, Waller DK. 2006. Residential mobility patterns and exposure misclassification in epidemiologic studies of birth defects. Journal of Exposure Science and Environmental Epidemiology 16:538-543.

Cesaroni G, Porta D, Badaloni C, Stafoggia M, Eeftens M, Meliefste K et al. 2012. Nitrogen dioxide levels estimated from land use regression models several years apart and association with mortality in a large cohort study. Environ Health 11:48.

Chen EK, Zmirou-Navier D, Padilla C, Deguen S. 2014. Effects of air pollution on the risk of congenital anomalies: a systematic review and meta-analysis. Int J Environ Res Public Health 11:7642-7668.

Chen L, Bell EM, Caton AR, Druschel CM, Lin S. 2010. Residential mobility during pregnancy and the potential for ambient air pollution exposure misclassification. Environ Res 110:162-168.

Chodick G, Flash S, Deoitch Y, Shalev V. 2009. Seasonality in birth weight: review of global patterns and potential causes. Hum Biol 81:463-477.

Cyrys J, Eeftens M, Heinrich J, Ampe C, Aarmengaud A, Beelen R et al. 2012. Variation of NO2 and NOx concentrations between and within 36 european study areas: results from the ESCAPE study. Atmospheric environment 62:374-390.

D'Souza SW, Black P, Richards B. 1981. Smoking in pregnancy: associations with skinfold thickness, maternal weight gain, and fetal size at birth. Br Med J (Clin Res Ed) 282:1661-1663.

Dadvand P, Parker J, Bell ML, Bonzini M, Brauer M, Darrow LA et al. 2013. Maternal exposure to particulate air pollution and term birth weight:

a multi-country evaluation of effect and heterogeneity. Environ Health Perspect 121:267-373.

Dadvand P, Rankin J, Rushton S, Pless-Mulloli T. 2011. Association Between Maternal Exposure to Ambient Air Pollution and Congenital Heart Disease: a Register-based spatio-temporal Analysis. Am J Epidemiol 173:171-182.

Darrow LA, Klein M, Strickland MJ, Mulholland JA, Tolbert PE. 2011. Ambient air pollution and birth weight in full-term infants in Atlanta, 1994-2004. Environ Health Perspect 119:731-737.

Dolk H, Armstrong B, Lachowycz K, Vrijheid M, Rankin J, Abramsky L et al. 2010. Ambient air pollution and risk of congenital anomalies in England, 1991-1999. Occup Environ Med 67:223-227.

Dolk H and Vrijheid M. 2003. The impact of environmental pollution on congenital anomalies. Br Med Bull 68:25-45.

Dolk H, Vrijheid M, Armstrong B, Abramsky L, Bianchi F, Garne E et al. 1998. Risk of congenital anomalies near hazardous-waste landfill sites in Europe: the EUROHAZCON study. The Lancet 352:423-427.

Eeftens M, Beelen R, Bellander T, Cesaroni G, Cirach M, Declerq C et al. 2012. Development of land use regression models for $PM_{2.5}$,$PM_{2.5}$ absorbance, PM10 and PMcoarse in 20 European study areas; results of the ESCAPE project. Environ Sci Technol 46:11195-11205.

Eeftens M, Beelen R, Fischer P, Brunekreef B, Meliefste K, Hoek G. 2011. Stability of measured and modelled spatial contrasts in NO2 over time. Occup Environ Med 68:765-770.

EIONET. European Topic Centre on Air Pollution and Climate Change Mitigation.

EUROCAT. 2004. EUROCAT Special Report. A Review of Environmental Risk Factors for Congenital Anomalies.

http://www.eurocat-network.eu/preventionandriskfactors/riskfactorsreview

Farmer G. 1985. Neonatal skinfold thickness. Measurement and interpretation at or near term. Arch Dis Child 60:840-842.

Gale CR, O'Callaghan FJ, Godfrey KM, Law CM, Martyn CN. 2004. Critical periods of brain growth and cognitive function in children. Brain 127:321-329.

Geer LA, Weedon J, Bell ML. 2012a. Ambient air pollution and term birth weight in Texas from 1998 to 2004. J Air Waste Manag Assoc 62:1285-1295.

Geer LA, Weedon J, Bell ML. 2012b. Ambient air pollution and term birth weight in Texas from 1998 to 2004. J Air Waste Manag Assoc 62:1285-1295.

Gehring U, Casas M, Brunekreef B, Bergstrom A, Bonde JP, Botton J et al. 2013. Environmental exposure assessment in European birth cohorts: results from the ENRIECO project. Environ Health 12:8.

Gehring U, Tamburic L, Sbihi H, Davies HW, Brauer M. 2014. Impact of Noise and Air Pollution on Pregnancy Outcomes. Epidemiology.

Gilboa SM, Mendola P, Olshan AF, Langlois PH, Savitz DA, Loomis D et al. 2005. Relation between ambient air quality and selected birth defects, seven county study, Texas, 1997-2000. Am J Epidemiol 162:238-252.

Gillman MW. 2005. Developmental origins of health and disease. N Engl J Med 353:1848-1850.

Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D. 2004. Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. Epidemiology 15:36-45.

Guxens M, Aguilera I, Ballester F, Estarlich M, Fernandez-Somoano A, Lertxundi A et al. 2012. Prenatal exposure to residential air pollution and infant mental development: modulation by antioxidants and detoxification factors . Environ Health Perspect Accpeted for Publication.

Hackshaw A, Rodeck C, Boniface S. 2011. Maternal smoking in pregnancy and birth defects: a systematic review based on 173 687 malformed cases and 11.7 million controls. Hum Reprod Update 17:589-604.

Hales CN and Barker DJ. 2001. The thrifty phenotype hypothesis. Br Med Bull 60:5-20.

Hassler JA and Moran DJ. 1986. Effects of ethanol on the cytoskeleton of migrating and differentiating neural crest cells: possible role in teratogenesis. J Craniofac Genet Dev Biol Suppl 2:129-136.

Hernan MA, Hernandez-Diaz S, Robins JM. 2004. A structural approach to selection bias. Epidemiology 15:615-625.

Hoek G, Beelen R, de Hoogh K, Vienneau D, Gulliver J, Fischer P et al. 2008. A review of land-use regression models to assess spatial variation of outdoor air pollution. Atmospheric environment 42:7561-7578.

Hohmann C, Grabenhenrich L, de Kluizenaar Y, Tischer C, Heinrich J, Chen CM et al. 2013. Health effects of chronic noise exposure in pregnancy and childhood: a systematic review initiated by ENRIECO. Int J Hyg Environ Health 216:217-229.

Janssen BG, Munters E, Pieters N, Smeets K, Cox B, Cuypers A et al. 2012. Placental mitochondrial DNA content and particulate air pollution during in utero life. Environ Health Perspect 120:1346-1352.

Jerrett M, Arain A, Kanaroglou P, Beckerman B, Potoglou D, Sahsuvaroglu T et al. 2005. A review and evaluation of intraurban air pollution exposure models. J Expo Anal Environ Epidemiol 15:185-204.

Jerrett M, Arain MA, Kanaroglou P, Beckerman B, Crouse D, Gilbert NL et al. 2007. Modeling the intraurban variability of ambient traffic pollution in Toronto, Canada. J Toxicol Environ Health A 70:200-212.

Jerrett M, McConnell R, Wolch J, Chang R, Lam C, Dunton G et al. 2014. Traffic-related air pollution and obesity formation in children: a longitudinal, multilevel analysis. Environ Health 13:49.

Kannan S, Misra DP, Dvonch JT, Krishnakumar A. 2006. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. Environ Health Perspect 114:1636-1642.

Ketel IJ, Volman MN, Seidell JC, Stehouwer CD, Twisk JW, Lambalk CB. 2007. Superiority of skinfold measurements and waist over waist-to-hip ratio for determination of body fat distribution in a population-based cohort of Caucasian Dutch adults. Eur J Endocrinol 156:655-661.

Keyte A and Hutson MR. 2012. The neural crest in cardiac congenital anomalies. Differentiation 84:25-40.

Klaric AS, Galic S, Kolundzic Z, Bosnjak VM. 2013. Neuropsychological development in preschool children born with asymmetrical intrauterine growth restriction and impact of postnatal head growth. J Child Neurol 28:867-873.

Kogevinas M, Andersen AM, Olsen J. 2004. Collaboration is needed to co-ordinate European birth cohort studies. Int J Epidemiol 33:1172-1173.

Krishnaveni GV, Hill JC, Veena SR, Leary SD, Saperia J, Chachyamma KJ et al. 2005. Truncal adiposity is present at birth and in early childhood in South Indian children. Indian Pediatr 42:527-538.

Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J et al. 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect 111:455-460.

Loeken MR. 2006. Advances in understanding the molecular causes of diabetes-induced birth defects. J Soc Gynecol Investig 13:2-10.

Luciano A, Bolognani M, Biondani P, Ghizzi C, Zoppi G, Signori E. 1998. The influence of maternal passive and light active smoking on intrauterine growth and body composition of the newborn. Eur J Clin Nutr 52:760-763.

Lupo PJ, Symanski E, Chan W, Mitchell LE, Waller DK, Canfield MA et al. 2010. Differences in exposure assignment between conception and delivery: the impact of maternal mobility. Paediatr Perinat Epidemiol 24:200-208.

Maisonet M, Correa A, Misra D, Jaakkola JJK. 2004. A review of the literature on the effects of ambient air pollution on fetal growth. Environmental research 95:106-115.

Margetts BM, Mohd YS, Al Dallal Z, Jackson AA. 2002. Persistence of lower birth weight in second generation South Asian babies born in the United Kingdom. J Epidemiol Community Health 56:684-687.

Marshall EG, Harris G, Wartenberg D. 2010. Oral cleft defects and maternal exposure to ambient air pollutants in New Jersey. Birth Defects Res A Clin Mol Teratol 88:205-215.

Matata BM, Elahi MM. The molecular basis for the link between maternal health and the origin of fetal congenital abnormalities.2011.

Mendez MA and Kogevinas M. 2011. A comparative analysis of dietary intakes during pregnancy in Europe: a planned pooled analysis of birth cohort studies. Am J Clin Nutr 94:1993S-1999S.

Mendez R, Zheng Z, Fan Z, Rajagopalan S, Sun Q, Zhang K. 2013. Exposure to fine airborne particulate matter induces macrophage infiltration, unfolded protein response, and lipid deposition in white adipose tissue. Am J Transl Res 5:224-234.

Minguillon MC, Querol X, Baltensperger U, Prévôt, ASH. 2012. Fine and coarse PM composition and sources in rural and urban sites in Switzerland: Local or regional pollution? Science of the total environment 427–428:191-202.

Misra A and Khurana L. 2009. The metabolic syndrome in South Asians: epidemiology, determinants, and prevention. Metab Syndr Relat Disord 7:497-514.

Misra A, Vikram NK, Sharma R, Basit A. 2006. High prevalence of obesity and associated risk factors in urban children in India and Pakistan highlights immediate need to initiate primary prevention program for diabetes and coronary heart disease in schools. Diabetes Res Clin Pract 71:101-102.

Neel JV. 1999. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? 1962. Bull World Health Organ 77:694-703.

Nethery E, Brauer M, Janssen P. 2008a. Time-activity patterns of pregnant women and changes during the course of pregnancy. Journal of Exposure Science and Environmental Epidemiology 19:317-324.

Nethery E, Leckie SE, Teschke K, Brauer M. 2008b. From measures to models: an evaluation of air pollution exposure assessment for epidemiological studies of pregnant women. Occup Environ Med 65:579-586.

Nethery E, Teschke K, Brauer M. 2008c. Predicting personal exposure of pregnant women to traffic-related air pollutants. Science of the total environment 395:11-22.

Nieuwenhuijsen M. Exposure Assessment in Occupational and Environmental Epidemiology.2003.

Oberdorster G and Utell MJ. 2002. Ultrafine particles in the urban air: to the respiratory tract--and beyond? Environ Health Perspect 110:A440-A441.

Padula AM, Tager IB, Carmichael SL, Hammond SK, Yang W, Lurmann F et al. 2013a. Ambient air pollution and traffic exposures and congenital heart defects in the San Joaquin Valley of California. Paediatr Perinat Epidemiol 27:329-339.

Padula AM, Tager IB, Carmichael SL, Hammond SK, Yang W, Lurmann FW et al. 2013b. Traffic-related air pollution and selected birth defects in the San Joaquin Valley of California. Birth Defects Res A Clin Mol Teratol 97:730-735.

Patel P and Abate N. 2013. Role of subcutaneous adipose tissue in the pathogenesis of insulin resistance. J Obes 2013:489187.

Pearson M. 1991. Ethnic differences in infant health. Arch Dis Child 66:88-90.

Pedersen M, Schoket B, Godschalk RW, Wright J, von Stedingk H, Tornqvist M et al. 2013a. Bulky dna adducts in cord blood, maternal fruit-and-vegetable consumption, and birth weight in a European mother-child study (NewGeneris). Environ Health Perspect 121:1200-1206.

Pedersen M, von Stedingk H, Botsivali M, Agramunt S, Alexander J, Brunborg G, Chatzi L, Fleming S, Fthenou E, Granum B, Gutzkow KB, Hardie LJ, Knudsen LE, Kyrtopoulos SA, Mendez MA, Merlo DF, Nielsen JK, Rydberg P, Segerbäck D, Sunyer J, Wright J, Törnqvist M, Kleinjans JC, Kogevinas M. 2012. Birth weight, head circumference, and prenatal exposure to acrylamide from maternal diet: the European prospective mother-child study (NewGeneris). Environ Health Perspect 120:1739-1745.

Pedersen M, Giorgis-Allemand L, Bernard C, Aguilera I, Andersen AMN, Ballester F et al. 2013b. Ambient air pollution and low birthweight: a European cohort study (ESCAPE). The Lancet Respiratory Medicine 1:695-704.

Rankin J, Chadwick T, Natarajan M, Howel D, Pearce MS, Pless-Mulloli T. 2009. Maternal exposure to ambient air pollutants and risk of congenital anomalies. Environ Res 109:181-187.

Rice D and Barone S Jr. 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108 Suppl 3:511-533.

Ritz B and Wilhelm M. 2008. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. Basic Clin Pharmacol Toxicol 102:182-190.

Ritz B, Wilhelm M, Hoggatt KJ, Ghosh JKC. 2007. Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles. American Journal of Epidemiology 166:1045-1052.

Rothman KJ, Moore LL, Singer MR, Nguyen US, Mannino S, Milunsky A. 1995. Teratogenicity of high vitamin A intake. N Engl J Med 333:1369-1373.

Rundle A, Hoepner L, Hassoun A, Oberfield S, Freyer G, Holmes D et al. 2012. Association of childhood obesity with maternal exposure to ambient air polycyclic aromatic hydrocarbons during pregnancy. Am J Epidemiol 175:1163-1172.

Schisterman EF, Cole SR, Platt RW. 2009. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 20:488-495.

Singer B, Hodgson A, Hotchi T, Kim JJ. 2004. Passive measurement of nitrogen oxides to assess traffic-related pollutant exposure for the East Bay Children's Respiratory Health Study. Atmospheric •Environment 38:393-403.

Sioutas C, Delfino RJ, Singh M. 2005. Exposure assessment for atmospheric ultrafine particles (UFPs) and implications in epidemiologic research. Environ Health Perspect 113:947-955.

Small N. 2012. Infant Mortality and Migrant Health in Babies of Pakistani Origin Born in Bradford, UK. Journal of Intercultural Studies 33:549-564.

Sniderman AD, Bhopal R, Prabhakaran D, Sarrafzadegan N, Tchernof A. 2007. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. Int J Epidemiol 36:220-225.

Snijder MB, van Dam RM, Visser M, Seidell JC. 2006. What aspects of body fat are particularly hazardous and how do we measure them? Int J Epidemiol 35:83-92.

Sram RJ, Binkova B, Dejmek J, Bobak M. 2005. Ambient air pollution and pregnancy outcomes: a review of the literature. Environ Health Perspect 113:375-382.

Stieb DM, Chen L, Eshoul M, Judek S. 2012. Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. Environ Res 117:100-111.

Strand LB, Barnett AG, Tong S. 2011. The influence of season and ambient temperature on birth outcomes: a review of the epidemiological literature. Environ Res 111:451-462.

Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB et al. 2009. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation 119:538-546.

Swanson JM, Entringer S, Buss C, Wadhwa PD. 2009. Developmental origins of health and disease: environmental exposures. Semin Reprod Med 27:391-402.

Townsend P, Phillimor P, Alastair B. Health and Deprivation: Inequality and the North.1988.

UNICEF and WHO. Low Birthweight: Country, Regional and Global Estimates.UNICEF,2004.

Valero N, Aguilera I, Llop S, Esplugues A, de Nazelle A, Ballester F et al. 2009. Concentrations and determinants of outdoor, indoor and personal

nitrogen dioxide in pregnnat women from two Spanish birth cohorts. Environ Int Submitted.

van Beynum IM, Mooij C, Kapusta L, Heil S, den Heijer M, Blom HJ. 2008. Common 894G> T single nucleotide polymorphism in the gene coding for endothelial nitric oxide synthase (eNOS) and risk of congenital heart defects. Clinical Chemistry and Laboratory Medicine 46:1369-1375.

VanderWeele TJ. 2009. On the relative nature of overadjustment and unnecessary adjustment. Epidemiology 20:496-499.

Vardavas CI, Chatzi L, Patelarou E, Plana E, Sarri K, Kafatos A, Koutis AD, Kogevinas M. 2010. Smoking and smoking cessation during early pregnancy and its effect on adverse pregnancy outcomes and fetal growth.

Vorherr H. 1982. Factors influencing fetal growth. Am J Obstet Gynecol 142:577-588.

Vrijheid M, Bianchi F, Nelen V, Thys G, Rankin J, Martos C. EUROCAT Special Report: Actions Towards European Environmental Surveillance: Feasibility of Environmental Linkage.2013.

Vrijheid M, Casas M, Bergstrom A, Carmichael A, Cordier S, Eggesbo M et al. 2012. European birth cohorts for environmental health research. Environ Health Perspect 120:29-37.

Vrijheid M, Martinez D, Manzanares S, Dadvand P, Schembari A, Rankin J et al. 2011. Ambient air pollution and risk of congenital anomalies: a systematic review and meta-analysis. Environmental health perspectives 119:598-606.

Wadhwa PD, Buss C, Entringer S, Swanson JM. 2009. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin Reprod Med 27:358-368.

Weinhold B. 2009. Environmental Factors in Birth Defects: What We Need to Know. Environ Health Perspect 117:A440-A447.

Wells JC. 2007. Commentary: Why are South Asians susceptible to central obesity?--the El Nino hypothesis. Int J Epidemiol 36:226-227.

Wells JC. 2009. Ethnic variability in adiposity and cardiovascular risk: the variable disease selection hypothesis. Int J Epidemiol 38:63-71.

West J, Lawlor DA, Fairley L, Bhopal R, Cameron N, McKinney PA et al. 2013. UK-born Pakistani-origin infants are relatively more adipose than white British infants: findings from 8704 mother-offspring pairs in the Born-in-Bradford prospective birth cohort. J Epidemiol Community Health 67:544-551.

WHO. 2014 Ambien (outdoor) air quality and health. Available: http://www.who.int/mediacentre/factsheets/fs313/en/.

WHO. 2012 Burden of disease from Ambient Air Pollution for 2012. Available: http://www.who.int/phe/health_topics/outdoorair/databases.

Wright J, Small N, Raynor P, Tuffnell D, Bhopal R, Cameron N et al. 2013. Cohort Profile: the Born in Bradford multi-ethnic family cohort study. Int J Epidemiol 42:978-991.

Yajnik CS, Lubree HG, Rege SS, Naik SS, Deshpande JA, Deshpande SS et al. 2002. Adiposity and hyperinsulinemia in Indians are present at birth. J Clin Endocrinol Metab 87:5575-5580.

Annex

About the author

Anna Schembari was born in Ragusa, Sicily in November 1982, were she lived until she was 18 years when she moved to Bologna to study statistics. Anna received her master of science in Statistics in March 2007 and since then have been working with medical doctors, for one year at the universitary hospital "S.Orsola, Malpighi" in Bologna, and then at CREAL in Barcelona where she arrived in 2008. In 2010 she received a ISCIII fellowship to carry out her PhD on air pollution and birth outcomes. As part of her PhD she has visited the Bradford Institute for Health Research, Bradford Teaching Hospitals NHS Trust, Bradford, in the United Kingdom. In 2012 she become mother of a beautiful half Italian half Nepali baby called Ananta. A big effort still needs to be done to harmonize Italian and Nepali cultures.

Picture of Anna, Ananta and Binod – may 2014

List of publications

1. Schembari A, Pedersen M, de Hoogh K, Dadvand P, Martinez D, Hoek G, Petherick E, Wright J, Nieuwenhuijsen M. Ambient Air Pollution and Newborn's Size at Birth: Differences by Maternal Ethnicity – Results From the Born in Bradford Study Cohort. Submitted to EHP, may 2014, resub. with minor revisions Aug. 2014
2. Schembari A, Nieuwenhuijsen MJ, Salvador J, de Nazelle A, Cirach M, Dadvand P et al. 2014. Traffic-related air pollution and congenital anomalies in Barcelona. Environ Health Perspect 122:317-323.
3. Schembari A, Triguero-Mas M, de Nazelle A,Dadvand P, Vrijheid M, Cirach M, Martinez D, Figueras F, Querol X, Basagaña X, Eeftens M, Meliefste K, Nieuwenhuijsen M. Personal, indoor and outdoor air pollution levels among pregnant women, Atmospheric Environment, Volume 64, January 2013, 287-295.
4. M.C. Minguillón, A. Schembari, M. Triguero-Mas, A. de Nazelle, P. Dadvand, F. Figueras, J.A. Salvado, J.O. Grimalt, M. Nieuwenhuijsen, X. Querol. Source apportionment of indoor, outdoor and personal $PM_{2.5}$ exposure of pregnant women in Barcelona, Spain, Atmospheric Environment, Volume 59, November 2012, 426-436
5. Dadvand P, de Nazelle A, Triguero-Mas M, Schembari A, Cirach M, Amoly E et al. 2012. Surrounding greenness and exposure to air pollution during pregnancy: an analysis of personal monitoring data. Environ Health Perspect 120:1286-1290.
6. Dadvand P, Figueras F, Basagana X, Beelen R, Martinez D, Cirach M et al. 2013. Ambient air pollution and preeclampsia: a spatiotemporal analysis. Environ Health Perspect 121:1365-1371.
7. Vrijheid M, Martinez D, Manzanares S, Dadvand P, Schembari A, Rankin J, Nieuwenhuijsen M. Ambient Air Pollution and Risk of Congenital Anomalies: A Systematic Review and Meta-Analysis. Environ Health Perspect. 2010
8. Aguado O, Morcillo C, Delàs J, Rennie M, Bechich S, Schembari A, Fernández F , Rosell F. Long-term implications of a single home-based educational intervention in patients with heart failure. Heart Lung. 2010;39(6 Suppl):S14-22.

Planned publications

Schembari A, Norris T, de Hoogh K, Wright J, Nieuwenhuijsen M. Ambient Air Pollution and fetal growth – Results From the Born in Bradford Study Cohort.

Schembari A, Ferrer D, Basagaña X, de Hoogh K, Pedersen M, Dadvand P, Petherick E, Wright J, Nieuwenhuijsen M. Blood pressure change during pregnancy: the role of air pollution – Results From the Born in Bradford Study Cohort.