Escola Nacional de Saúde Pública

UNIVERSIDADE NOVA DE LISBOA

Association between ambient air pollution exposure and biomarkers of cardiovascular risk: link between the first Portuguese Health Examination Survey and the air quality data

Doctoral Programme of Public Health

Specialization in Epidemiology

Vânia Isabel da Silva Gaio

Lisbon, July 2021

Escola Nacional de Saúde Pública
UNIVERSIDADE NOVA DE LISBOA

Association between ambient air pollution exposure and biomarkers of cardiovascular risk: link between the first Portuguese Health Examination Survey and the air quality data

This thesis is presented as part of the requirements for the Degree of Doctor of Public Health, under supervision of Professor Baltazar Nunes and Co-supervision of Professor Carlos Matias Dias

Lisbon, July 2021

Agradecimentos

Em primeiro lugar quero agradecer ao meu orientador, Doutor Baltazar Nunes, por ter acreditado neste tema de tese, aceitando orientar-me e motivando-me para concluir esta etapa mesmo em tempos de pandemia. Foi para mim um privilégio ser tua orientanda e agradeço todos os teus ensinamentos que foram (e ainda vão sendo) múltiplos! Tens sem dúvida um dom em conseguires fazer-te entender e ensinar! Espero que, tal como eu, muitos mais venham a ser inspirados por ti no gosto pela Epidemiologia!

Um especial agradecimento também ao Doutor Carlos Dias, não só pela coorientação desta tese, mas também por me ter permitido, como coordenador do departamento onde trabalho, ter a oportunidade de estudar esta temática e conseguir conciliá-la com os projetos de investigação de interesse do Departamento de Epidemiologia do INSA, mesmo numa altura que foi tão exigente em termos de volume de trabalho por parte de toda a equipa. Muito obrigada Doutor Carlos!

Obrigada também aos vários colegas do DEP que, de forma mais ou menos direta, também contribuíram para este trabalho. Obrigada Susana Silva, Ana Rita Torres e Constantino Caetano pelas dicas em relação ao R. Um especial agradecimento à colega e amiga Rita Roquette não só pela ajuda preciosa no que respeita à georreferenciação dos participantes do INSEF como também por ter aceitado embarcar no grande desafio que foi fazer uma revisão sistemática da literatura. Sem ti, não seria possível! Obrigada também ao Miguel Flores, funcionário da biblioteca do INSA, pela preciosa ajuda em conseguir obter determinados artigos.

Obrigada à Agência Portuguesa do Ambiente por manter disponíveis os dados da qualidade do ar, à equipa de investigação do Departamento de Ambiente e Ordenamento da Universidade de Aveiro pela partilha dos dados da modelação matemática e colaboração nas análises que foram sendo feitas, e obrigada também à equipa coordenadora do INSEF, da qual tive o privilégio de fazer parte. Sem todos vocês e sem os Portugueses que aceitaram participar no INSEF, esta tese também não existiria.

Por fim, obrigada à minha família. Obrigada aos meus pais que sempre me incutiram o gosto pelo estudo e dedicaram toda a sua vida para que as suas 3 filhas tivessem a oportunidade de ter formação superior. Este doutoramento é também uma forma que tenho de vos agradecer por tudo o que fizeram por mim, pois sei que será motivo de orgulho e sentimento de realização para vós! Um obrigado especial ao meu marido Luís, pela compreensão e por ter assegurado todas as tarefas

domésticas/parentais enquanto eu permanecia em frente ao computador. E para o meu filho, que nasceu durante este meu doutoramento, deixo uma mensagem especial: “Sebastião, espero que as noitadas em frente ao computador, ainda quando estavas na barriga da mãe, tenham servido para te mostrar como é importante lutarmos sempre por aquilo que queremos!”. E tal como a Professora Carla Nunes me disse quando engravidei no primeiro ano do programa doutoral, “Um filho será sempre um motivo maior para terminar com sucesso um doutoramento”.

Foi um gosto enorme escrever esta tese! Obrigada a todos!

Abstract

The once-surprising observation that ambient air pollution (AAP) adversely affects the cardiovascular system is now well recognized but there are yet many gaps in understanding the pathophysiologic mechanisms linking AAP and cardiovascular diseases (CVD). In this sense, epidemiological studies on the association between AAP and health conditions that increase the risk to develop CVD may shed some light on the mechanisms mediating the AAP deleterious effect on the cardiovascular system. The overall aim of this thesis is to improve our knowledge on the relationship between exposure to particulate matter and biomarkers of cardiovascular risk. Specifically, this thesis addresses the association between exposure to ambient particulate matter (PM_{10}) and biomarkers of dyslipidaemia, diabetes, hypertension and inflammatory response in the adult Portuguese mainland population, in 2015.

A systematic review and meta-analysis was firstly conducted (**Article 1**) to summarise the current state of knowledge about the association between AAP and biomarkers of dyslipidaemia prior to design the plan of analysis on this topic. Despite the few studies included, it suggested some epidemiologic evidence supporting the association between PM_{10} and increased triglycerides levels (3.14%, 95%CI: 1.36-4.95, increase per 10µg/m^3 PM_{10} increment). Subsequently, associations between PM_{10} exposure and each biomarker group was assessed through the linkage between air quality data collected through air quality monitoring network of the Environmental Portuguese Agency and individual data from the First Portuguese Health Examination Survey (**Articles 2, 3** and **4**).

Globally, the results of this thesis supports the existence of a positive association between PM_{10} exposure and some biomarkers of cardiovascular risk, namely those related to dyslipidaemia (Triglycerides, in people with abdominal obesity: 1.84%, 95%CI: 0.02-3.69, increase per 1µg/m^3 PM_{10} increment) and inflammatory response (White blood cells in females: 2.76%, 95%CI: 0.65-4.87, increase per 10 µg/m^3 PM_{10} increment and Red cell distribution width in males: 2.96%, 95%CI: 0.80-5.12, increase per 10 µg/m^3 PM_{10} increment). Consequently, it suggests that reduction of PM_{10} below current air quality standards would result in additional cardiovascular health benefits for the population. Additionally, results of this thesis contribute to provide more knowledge to establish the biologic plausibility to the previous epidemiologic studies reporting an association between air pollution and increased cardiovascular mortality and hospital admissions due to cardiovascular diseases. In future experimental studies, performed in animal models or in vitro, it would be important to explore deeply the hypothetical

biological mechanisms presented in this thesis to explain the identified associations.

In conclusion, exposure to AAP is largely beyond the control of persons and requires action by public authorities at the national and international levels. Results obtained within this thesis provide more scientific arguments for taking actions to improve air quality, even when the standard air quality limits are target. Finally, this thesis describe a methodological approach, that to my knowledge, was never performed in Portugal, linking air quality and health data, that could be used to study other environmental factors and other health outcomes in a perspective to improve the usage of data already collected and available to all the scientific community.

Key words: Air pollution, particulate matter, biomarkers of cardiovascular risk

Resumo

A observação outrora surpreendente de que a poluição do ar ambiental (PAA) afeta, de forma adversa, o sistema cardiovascular é agora bem reconhecida, mas ainda existem muitas lacunas na compreensão dos mecanismos fisiopatológicos que explicam a associação entre a exposição a PAA e as doenças cardiovasculares (DCV). Nesse sentido, os estudos epidemiológicos de associação entre PAA e as condições de saúde que aumentam o risco de desenvolver DCV podem contribuir para perceber quais os mecanismos que podem mediar o efeito deletério da PAA no sistema cardiovascular. O objetivo geral desta tese foi contribuir para melhorar o conhecimento sobre a associação entre a exposição a partículas e alguns biomarcadores de risco cardiovascular potencialmente desencadeadores de DCV fatais e não fatais. Especificamente, esta tese aborda a associação entre a exposição a partículas ambientais (PM_{10}) e biomarcadores de dislipidemia, diabetes, hipertensão e resposta inflamatória na população adulta Portuguesa do continente, em 2015.

Inicialmente, foi realizada uma revisão sistemática da literatura e meta-análise (**Artigo 1**) para sumarizar o estado atual do conhecimento sobre a associação entre a poluição do ar e os biomarcadores de dislipidemia antes de delinear a estratégia de análise sobre este tópico. Apesar dos poucos estudos incluídos, esta revisão sugeriu a existência de alguma evidência epidemiológica que sustenta a associação entre os níveis de PM_{10} e os valores dos triglicéridos (3.14%, 95%CI: 1.36-4.95, de aumento por cada incremento de 10µg/m^3 de PM_{10}). Posteriormente, através da ligação entre dados da qualidade do ar recolhidos através da rede de monitorização da qualidade do ar da Agência Portuguesa do Ambiente e os dados recolhidos no Inquérito Nacional Saúde com Exame Físico (INSEF, 2015), foram avaliadas as associações entre a exposição a PM_{10} e os biomarcadores de cada grupo estudado (**Artigo 2, 3** e **4**).

Globalmente, os resultados desta tese suportam a existência de uma associação positiva entre a exposição a PM_{10} e alguns biomarcadores de risco cardiovascular, nomeadamente aqueles relacionados com a dislipidemia (triglicéridos, em pessoas com obesidade abdominal: aumento de 1.84%, 95%CI: 0.02-3.69, por cada incremento de 1µg/m^3 de PM_{10}) e resposta inflamatória (glóbulos brancos no sexo feminino: aumento de 2.76%, 95%CI: 0.65-4.87, por cada incremento de 10µg/m^3 de PM_{10}; amplitude de distribuição dos glóbulos vermelhos no sexo masculino: aumento de 2.96%, 95%CI: 0.80-5.12, por cada incremento de 10µg/m^3 de PM_{10}).

Os resultados desta tese sugerem que a redução de PM_{10} abaixo dos padrões atuais de qualidade do ar resultaria em benefícios adicionais à saúde da população.

Além disso, contribuem para fornecer conhecimento que possa ajudar a encontrar a plausibilidade biológica para os estudos epidemiológicos anteriores que reportaram associações entre a poluição do ar e o aumento da mortalidade e internamentos hospitalares por doenças cardiovasculares. Em estudos experimentais futuros, em modelos animais ou *in vitro*, seria importante explorar em particular os mecanismos biológicos que são sugeridos nesta tese para explicar as associações identificadas.

Em conclusão, a exposição a PAA está em grande parte fora do controlo dos indivíduos e requer ação das autoridades públicas ao nível nacional e internacional. Os resultados obtidos nesta tese fornecem mais argumentos científicos para tomar ações de melhoraria da qualidade do ar, mesmo quando o limite padrão da qualidade do ar é cumprido. Adicionalmente, esta tese descreve uma abordagem metodológica, que do meu conhecimento nunca foi antes realizada em Portugal, ligando dados da qualidade do ar a dados individuais de saúde, a qual poderia ser utilizada para estudar outros fatores ambientais e outros *outcomes* de saúde numa perspetiva de otimizar a utilização de dados já recolhidos e disponíveis para toda a comunidade científica.

Palavras-chave: Poluição do ar, partículas, biomarcadores de risco cardiovascular

Table of Contents

List of abbreviations and acronyms

AAP, Ambient air pollution

AO, Abdominal obesity

APA, Environmental Portuguese Agency

APM, Ambient particulate matter

BP, Blood pressure

CAPI, Computer assisted personal interview

CAMx, Comprehensive Air Quality Model with Extensions

CI, Confidence interval

CV, Cardiovascular

CVD, Cardiovascular diseases

DALYs, Disability-adjusted life-years

DBP, Diastolic blood pressure

DM, Diabetes mellitus

EEA, European Environment Agency

EHES, European Health Examination Survey

EU, European Union

EUAAQD, European Union´s Ambient Air Quality Directives

GBD, Global Burden of Diseases

HbA1c, Glycated haemoglobin

HDL-C, High-density lipoprotein cholesterol

HEMOG, Haemoglobin

INSA, National Health Institute Doutor Ricardo Jorge

INSEF, Portuguese Health Examination Survey

LDL-C, Low-density lipoprotein cholesterol

LLE, Loss of life expectancy

NCAR, National Center for Atmospheric Research

OR, Odds ratio

PLAT, Platelets

PM, Particulate matter

RBC, Red blood cells

RDW, Red cell distribution width

RR, Relative risk

SBP, Systolic blood pressure

SD, Standard deviation

SE, Standard error

T2DM, Type 2 diabetes mellitus

TC, Total cholesterol

TG, Triglycerides

WBC, White blood cells

WHOAQG, World Health Organization´s Air Quality Guidelines

WRF-ARW, Advanced Research Weather Research and Forecasting model

WHtR, Waist-to-height ratio

List of Figures

List of Tables

1. Introduction

Ambient air pollution (AAP) is one of the world´s greatest threats, affecting not only human health but also ecosystems, the climate, society and subsequently the economy, posing numerous management challenges that requires a good understanding of its causes and effects (1). AAP can be defined as the presence of pollutants in the atmosphere from both natural or anthropogenic sources reaching concentrations that interfere with human health and well-being or that produce harmful environmental effects (2). In the last decades, increasing industrialization and urbanization has resulted in highest levels of AAP in recorded history at a global level. The majority of the world´s population now lives in cities, in close proximity to transports infrastructures, the major source of AAP in an urban context, along with the pollution resulting from heating of buildings, industrial activities and other pollution sources of human origin. Thus, air quality management has become a priority in several countries, namely in Europe, due to its influence in human health and environment (3,4).

The harmful action of AAP on health has been known for a long time, but it was only in the last few decades that its magnitude has been recognized, leading to growing public awareness of the health hazards due to air pollution exposure. A recent worldwide study about the loss of life expectancy (LLE) from air pollution compared to other risk factors concludes that AAP is one of the main global health hazards causing an estimated 8.8 million premature deaths annually and shorting lives by 3 years, especially due to cardiovascular diseases (CVD). The effects of AAP cause an LLE that rivals that of tobacco smoking (2.9 years versus 2.2 years) and it surpasses LLE due to HIV/AIDS, parasitic, vector-borne, other infectious diseases and all forms of violence by a large margin (5).

Although it may seem intuitively that exposures to air pollutants affect mostly the respiratory system, exacerbating respiratory conditions such as asthma and chronic pulmonary obstructive disease, the once-surprising observation that air pollution adversely affects the cardiovascular (CV) system is now well-recognized (6). In fact, several epidemiological studies all over the world have shown that both short and long-term exposures to environmental air pollutants are strongly associated with increased CV mortality and hospital admissions due to CVD (7–10). In Portugal, some ecological and regional studies regarding the influence of air pollution in mortality and hospital admissions have also been conducted. Significant positive associations between several air pollutants and cardiorespiratory diseases were found in 2006-2008, regarding hospital admissions in Lisbon (11). Similar results were found in Setubal

(2005-2009), considering the exposure to particulate matter and ozone (12). Another study performed in Oporto, provided evidence that exposures to ozone and particulate matter have adverse effects on the health of the general population in the summer months (13). More recently, a study performed in Madeira Island, reported associations between traffic-related pollutants and hospital admissions due to CVD and mortality (14). Another study that analyse multisite data on air pollution and mortality in 652 cities across different countries, including Lisbon and Oporto, also found associations between short-term exposure to particulate matter and daily all-cause, CV and respiratory mortality (15).

While scientific evidence supporting the association between AAP and CV mortality and morbidity is well-established at both international and national level, the pathophysiologic mechanisms linking AAP and CVD are not entirely known, being a currently research area with a lot of scientific debate. In this sense, epidemiological studies on the association between AAP and health conditions that increase the risk to develop CVD may shed some light on the mechanisms mediating the AAP deleterious effect on the CV system. However, these studies are still scarce and their evidence remains inconsistent, especially regarding the association between AAP and dyslipidaemia, diabetes (16) or hypertension (17). Consequently, more studies approaching the effect of AAP on biomarkers of subclinical and clinical CV risk conditions continue to be relevant in establishing the biologic plausibility of the association between AAP and CV mortality and morbidity.

This thesis addresses the association between exposure to ambient particulate matter (APM) and the levels of biomarkers of subclinical and clinical CV risk conditions, namely biomarkers of dyslipidaemia, diabetes, hypertension and inflammatory response that potentially trigger fatal and non-fatal CV diseases. To our knowledge, this is the first cross-sectional study linking air quality data, collected through air quality monitoring network of the Environmental Portuguese Agency (APA), and data from a Health Examination Survey, performed in a Portuguese population-based sample (First Portuguese Health Examination Survey – INSEF, 2015).

The conceptual model of the thesis is described in **Figure 1**. As can be seen, its main goal is not to study the direct association between the exposure (APM) and the final outcome (fatal and non-fatal CV events), already well supported by the scientific evidence, but instead to explore the association between APM exposure and biomarkers of subclinical and clinical CV risk conditions.

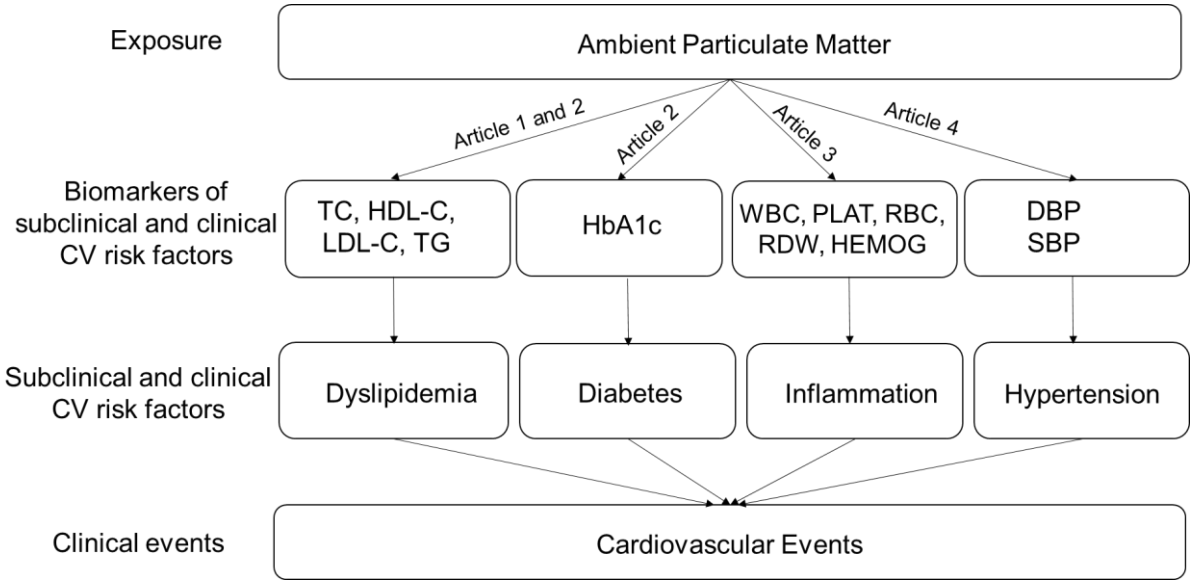

Figure 1 – Conceptual model of the thesis. Abbreviations: TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TG, Triglycerides; HbA1c, Glycated haemoglobin; WBC, White blood cells; PLAT, Platelets; RBC, Red blood cells; RDW, Red cell distribution width; HEMOG, Haemoglobin; DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

The biomarkers studied in the present work include four dyslipidaemia biomarkers (TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; and TG, Triglycerides), one diabetes biomarker (HbA1c, Glycated haemoglobin), 2 hypertension biomarkers (DBP, Diastolic blood pressure; SBP, Systolic blood pressure) and 5 biomarkers related to inflammatory response (WBC, White blood cells; PLAT, Platelets; RBC, Red blood cells; RDW, Red cell distribution width, HEMOG, Haemoglobin). One research article per each biomarker group was produced, except for the dyslipidaemia and diabetes biomarkers, which were described in the same article (**Article 2**). Moreover, regarding the association between AAP and dyslipidaemia biomarkers, a systematic review and meta-analysis was also performed, and presented in **Article 1**, with the aim to summarise the current state of knowledge prior to the design of the analysis strategy on this topic. The decision to perform the systematic review and meta-analysis on this specific topic was due to the considerable number of studies detected that were never systematized and meta-analysed. For the remaining groups of biomarkers, recent published systematic reviews and meta-analysis were used to guide the strategy of analysis on these topics.

The four articles produced in this thesis, that will be described in detail on the Results section, were the following:

Article 1: Gaio, V., Roquette, R., Dias, C. M., & Nunes, B. (2019). Ambient air pollution and lipid profile: Systematic review and meta-analysis. Environmental Pollution, 254, 113036;

Article 2: Gaio, V., Roquette, R., Monteiro, A., Ferreira, J., Lopes, D., Dias, C. M., & Nunes, B. Air pollution exposure interacts with abdominal obesity to increase blood triglycerides: a cross-sectional linkage study (submitted to European Journal of Public Health, 17/12/2020);

Article 3: Gaio, V., Roquette, R., Monteiro, A., Ferreira, J., Rafael, S., Dias, C. M., & Nunes, B. (2021). Exposure to ambient particulate matter increases blood count parameters with potential to mediate a cardiovascular event: results from a population-based study in Portugal. Air Quality, Atmosphere & Health, 1-14;

Article 4: Gaio, V., Roquette, R., Monteiro, A., Ferreira, J., Dias, C. M., & Nunes, B. Investigating the association between ambient particulate matter (PM_{10}) exposure and blood pressure values: results from the link between a Health Examination Survey and air quality data (submitted to the Journal of Public Health, 28/07/2021).

2. Literature Review

2.1. Ambient air Pollution

Ambient air Pollution (AAP) can be defined as the atmosphere contamination due to the presence of chemical substances generated from both natural (e.g. forest fires, volcanic eruptions, natural dusts, microorganisms) and anthropogenic sources (e.g. industry, transports, agriculture) with enough concentration to cause health and environmental deterioration (2,6,18).

Although AAP can result from natural sources without human intervention, it is now known that the leading source of pollution comes from large-scale human activities, such as industry, energy stations, and transports (19). According to the 2019 report of the European Environment Agency (EEA), the main sectors contributing to emissions of air pollutants in Europe are (1) transports, (2) commercial, institutional and households, (3) energy production and distribution, (4) industry, (5) agriculture and (6) waste (3).

The different sources of air pollution emit different types and amounts of pollutants and the impact of these pollutants on humans and the environment is a complex issue (18), dependent of multiple factors such as the ones described in **Figure 2** (20).

In fact, emissions are not the only factor that affects air pollutants concentrations. Primary pollutants, that are emitted directly into the atmosphere such as sulphur and nitrogen oxides, carbon monoxide or particulate matter, could interact and suffer chemical transformations, namely through sunlight reactions, to form secondary pollutants that could be also primary pollutants such as nitrogen monoxide and some particulate matter (21). Moreover, meteorological and topography conditions are involved in the dispersion of pollutants that will contribute to the final concentration of the air pollutants at a given time and place (**Figure 2**). Due to these multiple factors, there is not a linear relationship between emissions decrease and the concentrations of air pollutants observed in the air. In addition, long-distance transport of air pollutants is also possible, affecting people and the environment far away from its original sources (3,20).

Air pollution management is an extremely complex issue that requires an international cooperation in terms of research, development, administration policy, monitoring and politics to achieve effective and sustainable air pollution control (19). Over the last decades, global emissions of some gaseous air pollutants, namely sulphur dioxide and nitrogen oxides, have declined in the major emitting countries from Europe, North America and also from East Asia

as a result of the widespread implemented policies that aim to decrease air pollution exposure by setting limits and target values for the leading air pollutants (22).

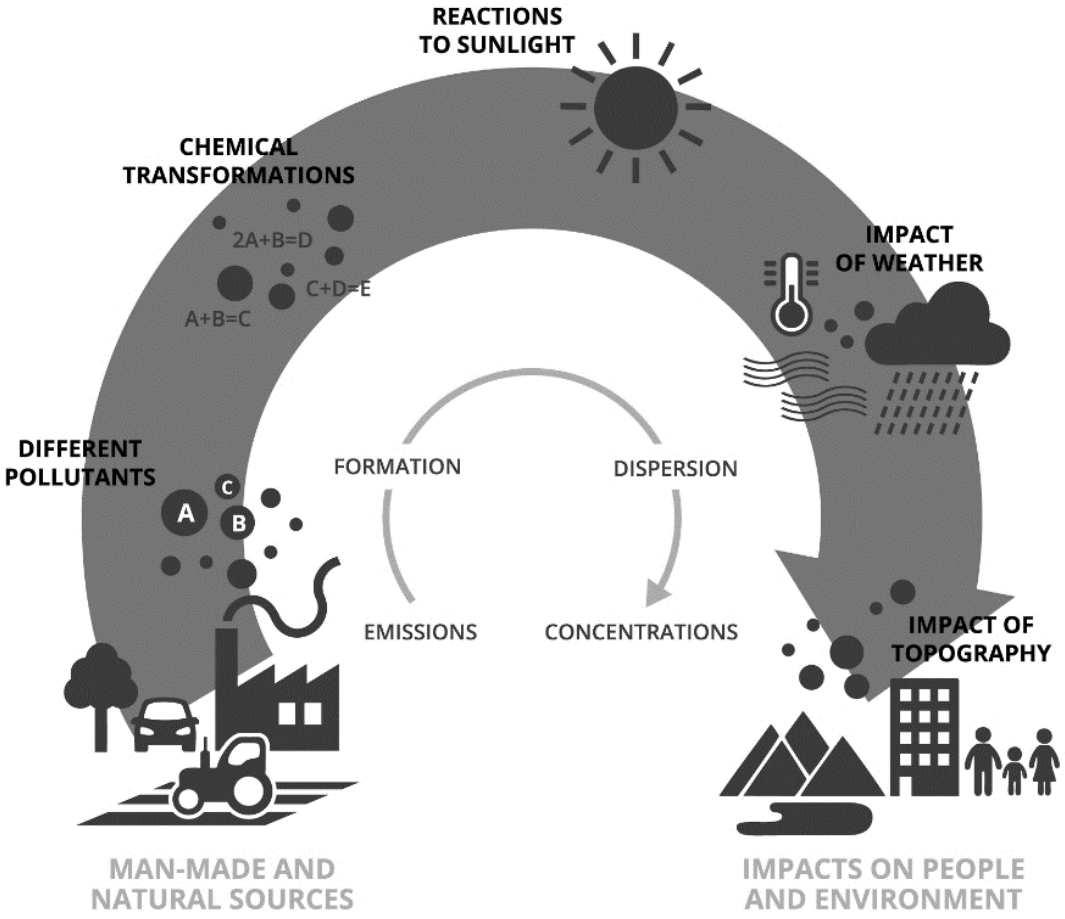

Figure 2 – Schematic illustration of the main factors contributing to air pollution concentration levels (Source: EEA, 2013 (20)).

Nevertheless, despite the decline tendency of these air pollutants emissions, a large proportion of the World's population is still exposed to air pollutants levels above the European Union´s Ambient Air Quality Directives (EUAAQD) and above the World Health Organization´s Air Quality Guidelines (WHOAQG) (3,23). It has been estimated that around 92% of people worldwide live in areas where the current WHOAQG limit values for various ambient pollutants established in 2005 are exceeded (24). Furthermore, new results from research has strengthened the evidence for adverse health effects of air pollution even at low levels of concentration, including above current air quality standards, stressing the need to add regulatory actions even in places where standards are fulfilled (25,26). Additionally, emissions of some pollutants have continued to grow, namely emissions of ammonia, an important

contributor to particulate matter (PM) formation and eutrophication, requiring the design of new effective air pollution control strategies (22).

Several air pollutants adversely affect environment and human health. The harmful effect of these pollutants substantially depends on the amount and time of exposure, pollutants' type and the accumulation of pollutants overtime (18). According to the World Health Organization, the major air pollutants that are responsible for deteriorating air quality and have strongest evidence of harmful effects on health are PM, ground-level ozone, nitrogen dioxide and sulphur dioxide (23). Detailed background information on APM will be presented in the next subsection, as it will be the pollutant under study in this thesis.

2.1.1. Particulate Matter

PM is considered a pollutant of great importance not only because it absorbs and reflects solar radiation, and therefore is an important intervenient on the climate change problem (27), but also because it is the pollutant having by far the largest impact upon public health and the scientific evidence on its harmful health effects is already quite consistent (6).

PM is a heterogeneous and complex mixture of solid and liquid particles suspended in the atmosphere, which could transport a wide variety of compounds harmful to human health, varying in particle size and chemical composition on a range of temporal and spatial scales. Regarding the chemical composition, the major groups of PM constituents that affect its toxicity properties are organic mass, elemental mass, atmospheric semi-volatile species, crustal materials and biological materials. Specific PM constituents in each of the mentioned groups are presented in **Table 1** (28).

PM could be classified as primary, when it enters directly in the atmosphere from emission sources, or secondary, when it is formed in the atmospheres through chemical reactions of primary gaseous emissions, like SO_2, NO_X, NH_3 and volatile organic compounds. According to its size, PM could be classified as PM_{10} (aerodynamic diameter of less than 10μm), $PM_{2.5\text{-}10}$ (aerodynamic diameter between 10 and 2.5 μm), $PM_{2.5}$ (aerodynamic diameter of less than 2.5 μm) and $PM_{0.1}$ (aerodynamic diameter of less than 0.1 μm).

Table 1 – Constituents of PM that affect its toxicity properties.

APM constituent	Specific constituents	Reference
Organic mass	Hydrocarbons and their derivates: PAHs, PFCs, pesticides, dioxins, furans and PCBs	(29) (30)
Elemental mass	C, Si, Be, As, Na, Ni, V, Pb, Cd, Se, Zn, Br, Co, Mn and others	(31) (32)
Atmospheric semi-volatile species	H_20, NH_4NO_3, semi-volatile organic compounds	(30)
Crustal materials	Sand, soil and dusts	(33)
Biological materials	Bacteria, spores, pollen, debris and plant fragments	(29)

Adapted from (28). **Abbreviations:** PAHs, polycyclic aromatic hydrocarbons, PFCs polyfluorinated carbons, PCBs polychlorinated biphenyls.

Smallest PM are particularly dangerous because they can easier penetrate and lodge deep inside the lungs and possibly absorbed into the bloodstream (34). In fact, PM penetrability into the respiratory system, the primary site of exposure to PM, will depend on its size as showed in **Table 4**. While PM larger than 10 μm are filtered by the nose and upper airways, smaller PM can reach the lower airways and can deposit directly in the alveolar regions of the lungs or can cross into the blood if not removed by macrophages and epithelial cells (28,35). PM chemical composition, in addition to its size, will also be decisive for their health effects determining a significant proportion of the biological response that will be triggered by PM after their inhalation (36).

Table 2 – PM penetrability according to its size.

Particle size (μm)	Penetration degree in respiratory system
>11	Passage into nostrils and upper respiratory tract
7–11	Passage into nasal cavity
4.7–7	Passage into larynx
3.3–4.7	Passage into trachea-bronchial area
2.1–3.3	Secondary bronchial area passage
1.1–2.1	Terminal bronchial area passage
0.65–1.1	Bronchioles penetrability
0.43–0.65	Alveolar penetrability

Adapted from (19).

The main sources of PM are power stations, industry, motor vehicles, domestic firewood and natural sources like wildfires and volcanoes (37,38). According to the EEA air quality data, in 2017, the largest sources of PM in Portugal were industrial processes and product use, households, energy use in industry and lastly road transports (39). Road transports has been a major contributor of PM for many years, although this is now declining in the majority of the developed countries (40).

According to the last EEA report, in 2019, concentrations of PM continued to exceed the limit value of the EU Ambient Air Quality Directives (EUAAQD) and of the World Health Organization air quality guidelines (WHOAQG) in large parts of Europe. The long-term WHOAQG for PM_{10} and $PM_{2.5}$ was exceed at 51% and 69% of the reporting stations, respectively (3). Air quality standards for the protection of health regarding PM, as given in the EUAAQD and also in the WHOAQG are presented in **Table 5**.

Table 3 – Air quality standards for the protection of health regarding PM, as given in the EU Ambient Air Quality Directives (EUAAQD) and in the World Health Organization air quality guidelines (WHOAQG). Adapted from EEA (3).

Pollutant	Averaging period	Limit value EUAAQD	Limit value WHOAQG
PM_{10}	1 day	*50 µg/m^3	** 50 µg/m^3
	Calendar year	40 µg/m^3	20 µg/m^3
$PM_{2.5}$	1 day	-	** 25 µg/m^3
	Calendar year	***25 µg/m^3	10 µg/m^3

* Not to be exceeded on more than 35 days/year; ** 99th percentile (3 days/year); *** additionally, to the limit value, there is an exposure concentration obligation of 20 µg/m_3 (Average exposure indicator in 2015) and a national exposure reduction target of 0-20% reduction in exposure (AE1 in 2020, the percentage reduction depends on the initial Average exposure indicator).

In Portugal, a Member State of the European Union (EU), the Decree-Law no.102/2010 of 23 September transposes the Directive 2008/50/EC of 21 May into the Portuguese law. This Decree-Law defines the legislative framework and establishes guidelines for the air quality management, namely the limit values of air pollutants that cannot be exceeded, during a certain period of time, in order to prevent or reduce the harmful effects on human health and environment (41). In the last report, the Portuguese Environment Agency (APA) indicated a clearly decreasing trend of the annual average concentration of PM_{10} between 2001 (45.3 µg/m^3) and 2017 (16 µg/m^3), being the values recorded in the last 16 years below the limit value imposed by the legislation (40 µg/m^3)(42).

It is important to strengthen that, despite all these legislative limit and target values currently used in air quality management, there is no evidence of a safe level of PM exposure or a threshold below which no adverse health effects occur (43) and consequently, PM levels must be always minimized even in places where limits are achieved. In the next subsection, detailed information on AAP human exposure assessment will be presented, as it is a crucial topic for an appropriate determination of possible links between AAP and health effects.

2.1.2. Assessment of human exposure

In the previous sections, some of the factors that determine the final concentration of pollutants, with a special focus on PMs, were described. After the pollutant concentration availability into the atmosphere, another very important step in the chain events from the emission of a pollutant to its harmful effects on health is the exposure (44,45).

While air pollutant concentration is a physical characteristic of the environment in a certain place and time, exposure refers to the individual's contact with a pollutant concentration, representing the interaction between the pollutant concentration and the subjects. Exposure depends not only on the pollutant concentration but also on the contact duration of the subject with a certain pollutant´s concentration. Additionally, until the health effects occurrence, dose is also an important concept, as it is the mass of the pollutant that crosses one of the body's boundaries and reaches the target tissues. When the uptake of the pollutant takes place predominantly in the respiratory system, as the case of the PM, exposure and dose may differ considerably. As a consequence, health effects may appear when a given critical or a maximum tolerable dose is exceeded (44,45). **Figure 3** describes the chain events from the emission to the health effects.

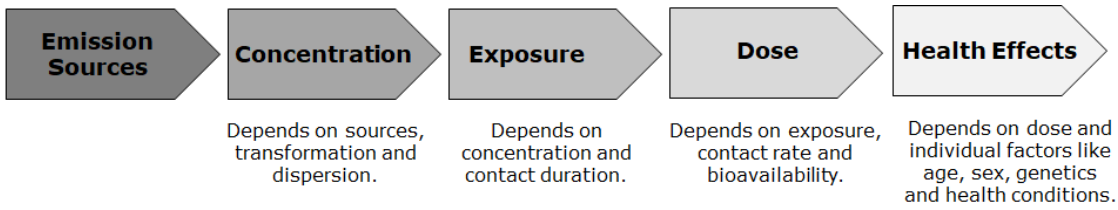

Figure 3 – Chain events from the emission to the health effects. Adapted from (46).

Taking into account that dose depends not only on exposure but also on contact rate and bioavailability, determined by individual-specific factors like respiratory, absorption and metabolic rates, it is very difficult to assess dose especially for a large number of individuals. Consequently, epidemiologic studies on AAP often use the assessment of exposure assuming that exposure is proportional to dose. Additionally, despite exposure depends on concentration and contact duration, concentration alone is often used to represent exposure not only for regulatory purposes but also on epidemiological studies assessing the association between AAP exposure and health effects (46).

The assessment of human exposure to AAP may be carried out by several approaches using direct and indirect methods. One of the direct methods is the determination of exposure biomarkers that represents the most accurate estimate of a person's "true" exposure as it provides a direct measure of the pollutant´s internal dose in the human body. However, specific exposure biomarkers of some air pollutants, namely PM, are not available or are often too costly to obtain in a large sample population (47,48).

The other direct method to assess air pollution exposure is the use of personal portable exposure monitors. This is a good methodology to assess human exposure to air pollution, as it provides a direct measurement of exposure, and small portable monitors sensitive enough for measuring ambient concentrations are now available, including measuring PM. However, it is relatively expensive to measure exposure for each individual in large population studies and time-consuming to do on many subjects (45). The development of mobile applications that estimates a personal air pollution inhaled dose based on the pollution concentrations obtained from an air pollution estimation system and on the time-activity data from the individual's on-body activity monitors is a very promising tool regarding the air pollution assessment exposure that could be used in the future (49,50).

When direct methods are not available to assess air pollution exposure, indirect methods are often used. The classical indirect method is the use of categorical exposure classifications such as type of residence or type of job. It is the simplest indirect method but its low accuracy is associated with a high exposure misclassification, which is an important source of confounding in studying the health effects of AAP. Subsequently there are two other indirect approaches that are the most commonly used nowadays and they will be described in detail below: (1) the use of air pollution data collected from routine fixed-site monitoring networks; and (2) the application of mathematical models (44).

Estimates of human air pollution exposure based on measurements obtained from ground fixed-site air monitoring stations, frequently managed by governmental agencies for regulatory purposes, are often used in epidemiological studies assuming that measurements at a single site are representative of air quality over a larger area. This is an easy approach to estimate the human air pollution exposure but it is often associated with high uncertainties due to limited spread of monitors over space and time (51). For some air pollutants, large-scale routinely collected air pollutants data remain scarce or incomplete in certain areas, resulting in lack of spatial coverage and also greater exposure assessment error (52). Additionally, air monitoring station measurements may not reflect individual-level exposure due to spatial

variability of ambient PM concentrations, the house-to-house and temporal variations in indoor infiltration of ambient PM, and personal variation in the time spent in different outdoor and indoor places (53). Furthermore, air monitoring stations are usually located in highly populated urban areas and consequently their measurements may not precisely reflect the exposure for populations from rural areas (54). In summary, to use estimates of human exposure based on measurements obtained from fixed-site air monitoring stations careful considerations are needed since stations represent the conditions for the local site and, at nearby sites, the conditions may be significantly different. Some strategies can be adopted to overcome this situation, namely some proximity-based assessments or some geo-statistical interpolation like Kriging methods but they do not account for the physiochemical characteristics of the pollutants which are determinant in the complex dispersion environments (44,51).

Finally, the application of mathematical models is a very useful tool for indirect estimation of human exposure as it aims to provide spatiotemporally resolved estimations of different air pollutants and allow improved exposure assessment. However they are usually computationally intensive, requiring detailed input data that sometimes are not available, and expertise in meteorology and climatology (51). The three main types of models are (1) land use regression models, that provides spatially continuous estimations of air pollution based on air quality monitors and multiple predictors like land use data, altitude, meteorology, traffic and other predictors (55); (2) Chemical transport models that simulate dynamics of atmospheric pollutants by combining meteorological and chemical data; and (3) hybrid models that provide spatiotemporally continuous air pollutants estimates using elements of land use alongside the effect of various temporal variables like meteorology, green space and measures of pollutants from satellite-based remote sensing or pollutants estimates from chemical transport models (51,56).

After this subsection more focused on the methodological aspects of the AAP exposure assessment, applicable specifically to PM exposure assessment, the next subsection describe the PM health effects, with a special focus on the CV related health effects as they will be the study outcomes in this thesis.

2.2. Human health effects of particulate matter

Epidemiological research concerning air pollution and health effects have been successively support the causal relationship between PM and human morbidity and mortality (28,57,58).The Global Burden of Diseases (GBD) Study in 2015 estimates that exposure to $PM_{2.5}$ caused 4.2 million deaths and 103.1 million disability-adjusted life-years (DALYs), representing 7.6% of total global deaths and 4.2% of global DALYs. Moreover, the same study also found that deaths attributable to ambient $PM_{2.5}$ increased from 3.5 million in 1990 to 4.2 million in 2015 (59).

PM is associated with a large spectrum of adverse health outcomes, ranging from death to subclinical symptoms, as described in **Figure 4**. It illustrates the inverse relationship between the severity of outcomes and the proportion of people affected by them. While the top of the pyramid is composed by well characterized health effects, mainly at CV and respiratory level, often included in the GBD estimates, the medium part of the pyramid is composed by emerging but still unquantified health effects and the basis of the pyramid includes insufficiently characterised health effects (60). This suggests that the health effects quantified in the GBD estimates represent only a small portion the total health effects caused by AAP, as well as by PM (23).

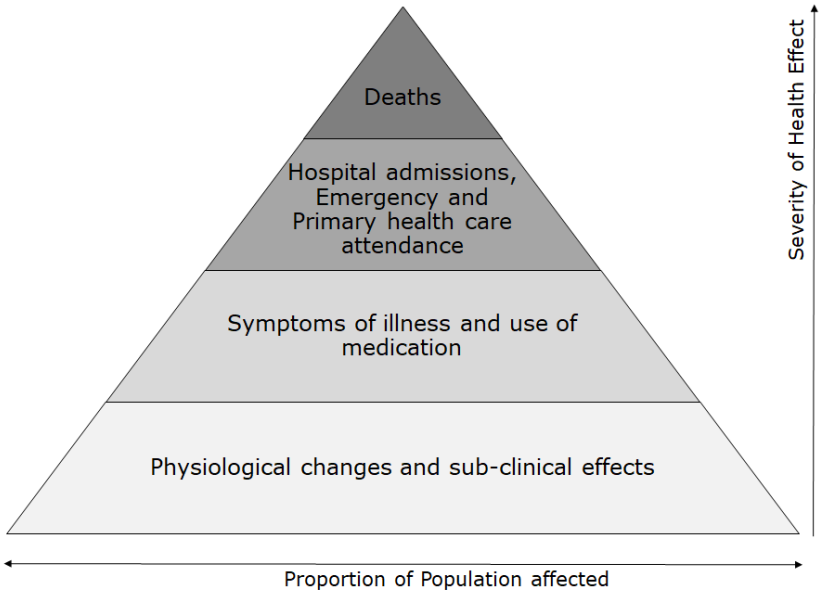

Figure 4 – Pyramid of the health effects associated with air pollution. Adapted from (61).

It is already known that PM exposure can affect multiple organs and systems (62). Besides the PM effects on CV system, which will be covered in the next section in more detail, there is already some consolidated scientific evidence on the association between exposure to PM and diseases of the respiratory system, namely asthma (63), chronic obstructive pulmonary disease (64) and lung cancer (65). PM is already classified as Group 1 carcinogen (induces carcinogenesis in human body) by the International Agency for Research on Cancer (66). Additionally, emerging evidence suggests that PM can also increases the incidence of a wide range of diseases, namely skin diseases like atopic dermatitis (67), diseases related with the central nervous system like depression and anxiety (68), dementia and Alzheimer's disease (69) and PM also seems to affect the human fertility (70) and birth outcomes (71).

PM exposure is near ubiquitous, affecting everyone, but those at greatest risk are children, people aged 65 and over, people with pre-existing comorbidities namely CV and respiratory diseases, people who work outdoors and people of lower socio-economic status because they are more likely to live in areas with worst air quality (72).

2.2.1. Cardiovascular related effects

Cardiovascular diseases (CVD) are a vast group of cardiac and vascular diseases, namely ischemic heart disease, stroke, heart failure, peripheral arterial disease and hypertension that continue to be the leading cause of morbidity and mortality worldwide and also in Portugal (73). In 2017, according to the GBD study, CVD caused an estimated 17.8 million deaths worldwide, corresponding to 330 million years of life lost and another 35.6 million years lived with disability (74,75). In Portugal, CVD is also the main cause of morbidity and mortality, having led to 32 336 deaths (29.4 % of all deaths) in 2017 (73). Moreover, it was estimated that, in 2015, about 17% of the Portuguese resident population aged between 40 and 65 years old were at high or very high risk of having a fatal CV event in the following 10 years (76).

PM is now a well-established risk factor to develop CVD, with more than two thirds of the mortality attributed to PM arising from CV causes, mainly cerebrovascular and ischaemic heart diseases (6,59). Consistent scientific evidence is available regarding the link between PM exposure and both fatal and non-fatal CVD, including stroke (77), heart failure (10), myocardial infarction (78), cardiac arrhythmia (79) and venous thromboembolism (80).

The pathophysiologic mechanisms linking the occurrence of CV events due to PM exposure are still an area of intensive research and scientific debate (81–83). Evidence from controlled exposure studies, animal models and in vitro studies suggests at least three probable mechanistic pathways linking inhaled PM to CV morbidity and mortality, as described in **Figure 5** (6,57). One of them is the direct PM translocation or the PM constituents into the blood circulation that can directly impair CV function and reach different organ systems (6,84).

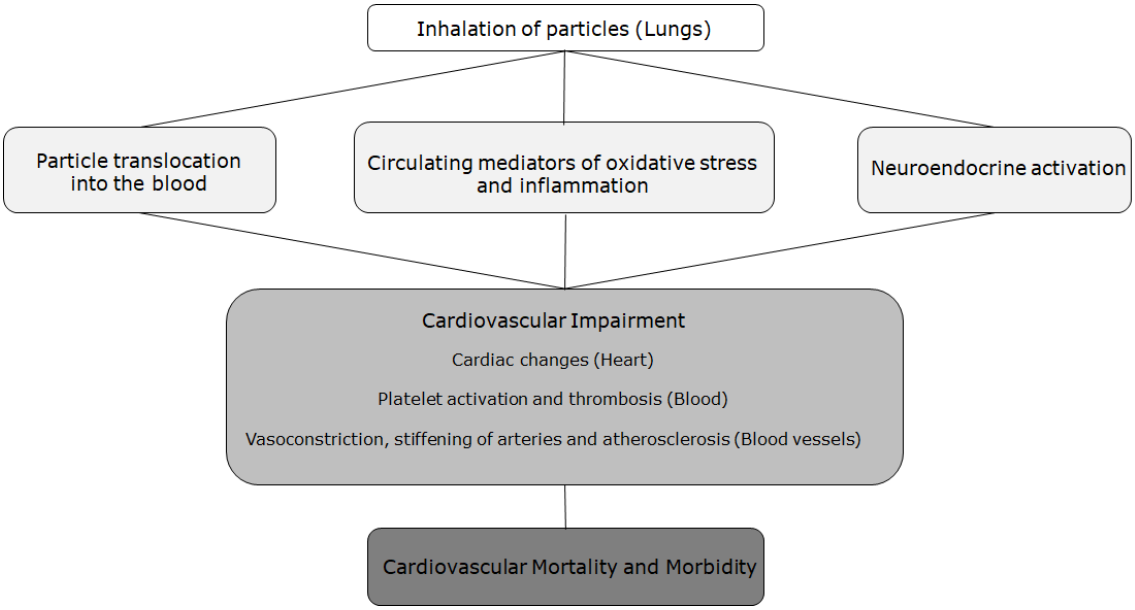

Figure 5 – Potential biological mechanisms linking inhaled PM to cardiovascular morbidity and mortality. Adapted from (6).

Another possible mechanism involved in the PM cardiovascular effects is related to lung oxidative stress and inflammation, leading to the passage of oxidative stress and inflammatory mediators, namely cytokines and other additional unidentified mediators, from the lung into the circulation (6,85,86). Finally, the neuroendocrine activation triggered by activation of alveoli sensory receptors activation is also currently considered as a potential mechanism linking inhaled PM to CV morbidity and mortality as it disrupt the balance of the autonomic nervous system (6). Additionally, some studies suggest that PM could also infiltrate in the central nervous system via translocation along the olfactory nerve into the olfactory bulb, exerting direct effects on this system (87,88). It is important to mention that these three mechanisms are not mutually exclusive and their activation will vary according to exposure duration, PM dose, constituents and size (89).

In addition to the direct CV impairment caused by PM exposure that will be achieved mainly by the three mechanisms previously described, nowadays it is also discussed the possibility of PM acting at the level of other tissues and organs. Direct action of PM on adipose tissue, liver, kidney and bone marrow, could promote the occurrence of CV risk conditions such as dyslipidaemia, hyperglycaemia, hypertension and exacerbated inflammatory response that will also contribute to CV mortality and morbidity (89). Considering the present uncertainty surrounding this hypothesis, there is a need for clarification of how PM can act on these subclinical and clinical CV risk conditions because evidence on this issue is still very limited and clarification could provide valuable insight into the mechanistic pathways through which PM exposure contributes to CVD development.

In this thesis, to study the association between PM and the subclinical and clinical CV risk conditions already mentioned, biomarkers of these conditions will be analysed. A biomarker could be defined as a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention (90). In this sense, blood lipid levels, routinely used as biomarkers of dyslipidaemia conditions, HbA1C, a more recent used biomarker of diabetes, blood pressure values, a classic biomarker of hypertension, and blood count parameters, important biomarkers for various physiological responses namely inflammatory response, are all also important biomarkers of CV risk. Detailed background information on the association between PM and each of these CV risk biomarkers will be presented in the next subsections.

2.2.1.1. Blood lipid levels and dyslipidaemia

Dyslipidaemia is characterized by any or a combination of raised LDL-C, TC, TG and low HDL-C. These lipid abnormalities are well-recognised CV risk factors, associated with an increased risk of CV events, namely ischaemic heart disease and stroke (91). Overall, raised TC is estimated to cause 2.6 million deaths and 29.7 million DALYs (92). In 2008, the global prevalence of raised TC among adults was 39% (93). In Portugal, according to the data from the first National Health Examination Survey (INSEF), in 2015, about 52% of the Portuguese population aged between 25 and 74 years old had TC values equal or higher than the recommended value of 190 mg/dL (94).

In the past years, some epidemiological studies have explored the association between PM exposure and blood lipid levels or dyslipidaemia but the results have not been consistent,

and some studies failed to demonstrate the deleterious effect of AAP on the lipid profile parameters. In fact, while some studies detected significant associations between $PM_{2.5}$ and PM_{10} and at least one of the dyslipidaemia biomarkers (95–98) others failed to detect these associations (99–101). For example, while Shanley and his colleagues (97), in the United States of America, found a 1.4% (95%CI: 1.2%; 1.7%) TC increase per each 11.1µg/m^3 PM_{10} increment, Yang and his colleagues (95) were not able to detect the same association in China (-0.2% TC increase per each per each 10 µg/m^3 PM_{10} increment, 95%CI: -0.5%; 0.1%). Furthermore, a recent study suggest that among the several CV risk factors, dyslipidaemias may be the most sensitive to AAP exposure (102) but more studies are needed to establish the relationship between PM and blood lipid levels. Given the considerable amount of epidemiological evidence recently published on the association between AAP and biomarkers of dyslipidaemia, It is considered important to perform a systematic review of the scientific literature, and, if possible, a meta-analysis of available data in order to clarify whether all the current epidemiological evidence support or not the existence of an association between AAP and changes in blood lipid levels.

Even though epidemiologic evidence regarding the association between exposure to PM and blood lipid values is still not clear, some physiological mechanisms have been proposed to support this association based on the evidence from experimental animal studies as presented in **Figure 6**. Thus, the three main potential mechanisms previously described in the section of the PM cardiovascular effects can also trigger physiological responses associated with metabolism of both lipids and glucose. In fact, PM can activate the innate immune system leading to the production of inflammatory cytokines and oxidative compounds which will pass into the circulation and originate a systematic inflammatory state affecting organs involved on lipid and glucose metabolism, like liver and adipose tissue (103). Moreover, PM exposure seems to increase macrophages in visceral adipose tissue, interfering with the adipokines regulation (104,105). It also promotes lipogenesis and decrease lipolysis in both adipose tissue and liver (106,107). There is already some evidence regarding the PM effect on glucose homeostasis at liver and muscle levels (108) and finally, some studies also support PM action on hypothalamic inflammation contributing to lipid and glucose metabolic dysregulation (88). All these physiological mechanisms can contribute to increase in glucose, free fatty acids and insulin resistance, favourable to the development of dyslipidaemia and, or, a diabetic condition (109).

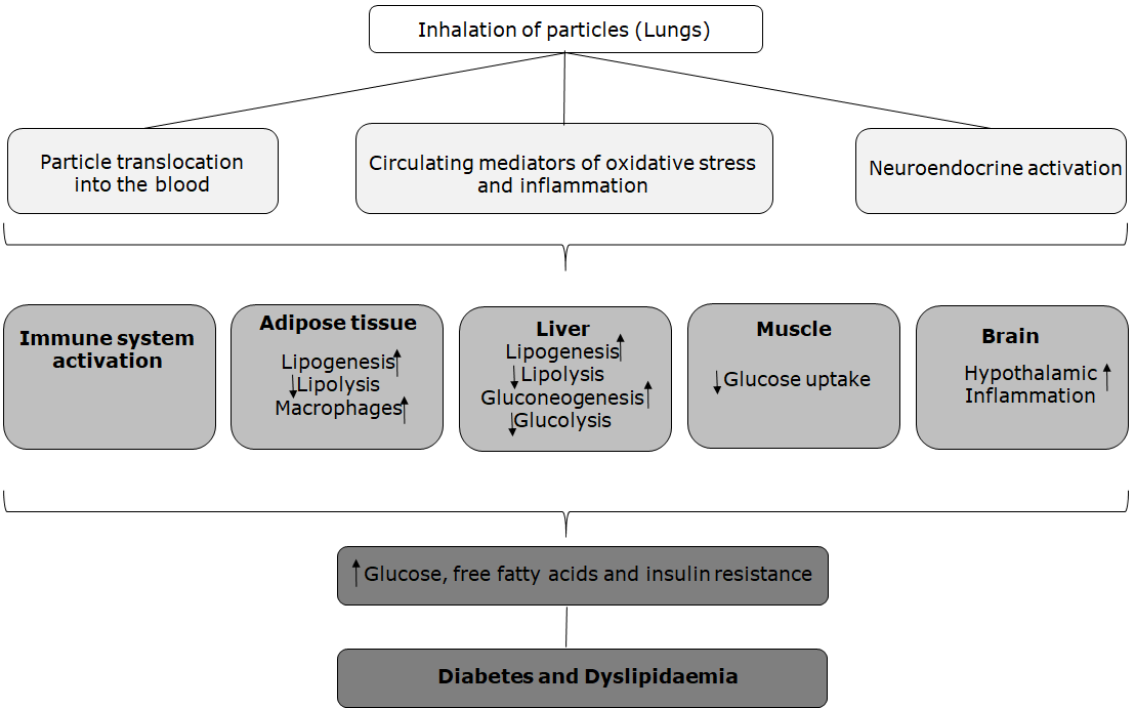

Figure 6 – Potential mechanisms whereby PM exposures can increase blood lipid and glucose levels. Adapted from (109).

2.2.1.2. Blood glucose levels and diabetes

Diabetes mellitus (DM) affected approximately 451 million people worldwide, in 2017, corresponding to a prevalence of 8.4%, and is expected to rise to 9.9% in 2045 (110). Moreover, DM is associated with a 2-fold excess risk of vascular diseases (coronary heart disease, ischaemic stroke, and vascular deaths) independent of other CV risk factors (111). In Portugal, according to the INSEF data, in 2015, the prevalence of diabetes (9.9%, 95%CI: 8.4; 11.5), among persons aged 25 to 74 years, remains higher than the global estimates (112).

Type 2 diabetes mellitus (T2DM), the most common type of Diabetes Mellitus, is a complex disorder, characterized by chronic hyperglycaemia caused by impaired insulin secretion (beta cell dysfunction) and, or, impaired insulin action (insulin resistance) (113). The three main ways to glucose estimation, used as T2DM diagnosis criteria, are fasting plasma glucose (≥ 126 mg/dl), 2 hour glucose in venous plasma (≥ 200 mg/dl) and HbA1c (≥ 6.5 %). HbA1c, considered a biomarker of long-term glucose control, has become one of the strongest biomarkers of diabetes and have several advantages when compared to the other methods namely fasting not required, greater pre-analytical stability and less day-to-day perturbation during periods of stress and illness (114).

In addition to genetic and behavioural factors, environmental factors, namely exposure to PM, have also been suggested as contributing factors to T2DM incidence and prevalence (115). In fact, the link between AAP exposure and T2DM have only recently been investigated but there are already some compiled evidence on this issue, including 6 meta-analysis studies (16,116–120).

In 2014, Janghorbani and his colleagues (118) found that PM exposure was associated with a slight increase in the risk of diabetes, and reported a pooled relative risk (RR) of 1.008 (95%CI: 1.003, 1.012) per each 10 μg/m^3 PM_{10} increase and a RR of 1.02 (95%CI: 0.97, 1.08) per each 10 μg/m^3 $PM_{2.5}$ increase. In the same year, Balti and his colleagues (119), despite using some different criteria to pool studies estimates, found a higher risk of diabetes for $PM_{2.5}$ (RR of 1.11 (95%CI: 1.03, 1.20) per each 10 μg/m^3 increase). In 2015, another meta-analysis performed with studies from only Europe and North America reported a RR of 1.10 (95%CI: 1.02, 1.18) per each 10 μg/m^3 $PM_{2.5}$ increase (117). However, all these 3 meta-analysis included a few number of heterogeneous studies (4, 5 and 3 studies, respectively) being inconclusive to draw a robust conclusion about the association between PM exposure and T2DM incidence.

In 2019, Liu and his colleagues updated pooled estimates between long-term PM exposure and the prevalence and incidence of T2DM polling estimates from 30 different studies. They found that higher levels of both $PM_{2.5}$ and PM_{10} were associated with a higher prevalence of T2DM (OR= 1.09 (95%CI: 1.05; 1.13) per 10mg/m^3 $PM_{2.5}$ increase and OR: 1.12 (95%CI: 1.06; 1.19) per 10mg/m^3 PM_{10} increase) but only higher levels of $PM_{2.5}$ was associated with a higher incidence of T2DM (HR= 1.10 (95%CI: 1.04; 1.16) per 10mg/m^3 $PM_{2.5}$ increase) (116). In 2020, Yang and his colleagues tried to perform a more inclusive meta-analysis and similar results were obtained (16).

Only one recent meta-analysis pooled the estimates regarding the association between PM and fasting blood glucose levels. Authors found that fasting blood glucose increased 0.10 mmol/L (95%CI: 0.02, 0.17) per each 10 μg/m^3 PM_{10} increment and 0.23 mmol/L (95%CI: 0.01, 0.45) per each 10 μg/m^3 $PM_{2.5}$ increment, considering a long-term period of exposure (1-year). Additionally, in a short-term period of exposure (less than 28 days), they found that fasting blood glucose increased 0.02 mmol/L (95% CI: −0.01, 0.04) per 10 μg/m^3 PM_{10} increment and 0.08 mmol/L (95%CI: 0.04, 0.11) per 10 μg/m^3 $PM_{2.5}$ increment (120).

Until now, to our knowledge, only a few number of recent studies included the association between PM and Hba1c levels and the majority of them suggest that higher PM exposures are associated with increased HbA1c levels (121–123). However, more studies are needed as there are also some studies not supporting this association (124). For example, while Elbarbary and her colleagues (125), in China, found a 1.8% increase of HbA1C levels per each 10 μg/m^3 PM increment (95%CI: 1.3%;2.3%), Wolf and her colleagues (124) were not able to detect the same association in Germany (0.5% HbA1C increase per each 7.9 μg/m_3 PM increment, 95%CI: -0.5%; 0.1%). Potential mechanisms whereby PM exposures can increase blood glucose levels and also HbA1c levels, are common with those described for the blood lipids in **Figure 6** of the previous subsection.

2.2.1.3. Blood pressure values and hypertension

High blood pressure (BP) or hypertension remains a major modifiable CV risk factor, with a strong correlation between BP and the risk of a CV event. High BP is estimated to be responsible for at least 45% of all deaths due to heart disease and 51% of all deaths due to stroke (126,127). Moreover, approximately 31% of adults aged over 25 years worldwide had hypertension (128). In Portugal, according to INSEF data, the overall hypertension prevalence was 36.0%, in 2015, among persons aged 25 to 74 years of age (129).

According to the WHO definition (130), hypertension is diagnosed if, when it is measured on two different days, the SBP readings on both days is ≥140 mmHg and/or the DBP readings on both days is ≥90 mmHg. Despite being essential in patient assessment and treatment, this definition is arbitrary as there is not a specific level of BP where CV and other complications start to occur (131).

The causes of hypertension are complex and are related to genetic, behavioural and also environmental factors, including also PM exposure. Over the last decades, multiple epidemiologic studies have been assessed the association between PM and BP values or hypertension. To our knowledge, until now, four meta-analysis have been conducted to pool the evidence on both long and short-term effect of AAP, including PM, on BP values or hypertension (17,132–134).

In 2014, Liang and his colleagues (133), performed a systematic review and meta-analysis specifically concerning the effect of exposure to $PM_{2.5}$ on blood pressure. They found an elevation of 1.39 mmHg (95%CI: 0.87; 1.91) and an elevation of 0.90 mmHg, (95%CI:

0.49; 1.30), per each 10 μg/m^3 increase of $PM_{2.5}$ for SBP and DBP, respectively. Additionally, they found that this association was stronger when considering a long-term period of exposure (previous 1 year or 30 days) than when considering a short-term period of exposure (less than 9 days).

Two years later, Zhang and their colleagues (134), conducted a similar analysis but included both $PM_{2.5}$ and PM_{10}. They found a significant association between PM_{10} levels and higher SBP and DBP values (a SBP elevation of 0.27 mmHg, 95%CI (0.07-0.48) and a DBP elevation of 0.22 mmHg, 95%CI (0.06-0.37) per each 10 μg/m^3 increase of PM_{10}). These positive associations between BP and PM_{10} were identified on both long-term (≥7 days) and short-term (<7 days) periods of exposure. Additionally, regarding the $PM_{2.5}$, a significant association was found only for SBP (an elevation of 0.50 mmHg, 95%CI (0.03-0.96) per each 10 μg/m^3 increase of $PM_{2.5}$) for a long-term period of exposure. On the same year, Cai and her colleagues (132) found that hypertension was significantly associated with short-term exposure (<7 days) to $PM_{2.5}$ (OR=1.07 (95%CI: 1.00–1.14) each 10 μg/m^3 increment) and PM_{10} (OR=1.02 (95%CI: 1.02–1.03) in each 10 μg/m^3 increment). Long-term (< 1 year) exposure to PM_{10} had also a significant association with hypertension (OR=1.05, 95%CI: 1.04–1.07, per each 10 μg/m^3 increment).

The most recent systematic review and meta-analysis that pool studies published before May 2017, supports a positive association between PM exposure with increased BP and hypertension. Regarding PM_{10}, a short-term exposure association with hypertension was found (OR: 1.06; 95 CI: 1.02; 1.10 per each 10 μg/m^3 increment). Additionally, a DBP elevation of 0.86 mmHg, 95%CI (0.37; 1.35) and 0.15 mmHg, 95%CI (0.01-0.29) per each 10 μg/m^3 increase of PM_{10} in long and short-term periods of exposure, respectively, were also found (17).

Despite the low robustness of some of these meta-estimates and some inconsistences in sensitivity analysis, at this moment, available evidence suggests us an association between PM exposure and raised blood pressure. PM exposure can raise blood pressure (**Figure 7**) by several potential biological mechanisms. In fact, the 3 main potential mechanisms previously described in the PM cardiovascular effects section can trigger some physiological responses associated with BP elevation such as vascular dysfunction and aortic stiffness (58), sodium retention at kidney level (135) and chronic kidney disease (136). With the exception of this last physiological response associated with BP elevation that will occur mainly in a long-term PM exposure scenario and will be irreversible, the remaining responses will occur in a few hours

to days after exposure and they are reversible. Consequently, reducing PM exposure will results in a decrease in BP within a few hours or days (137).

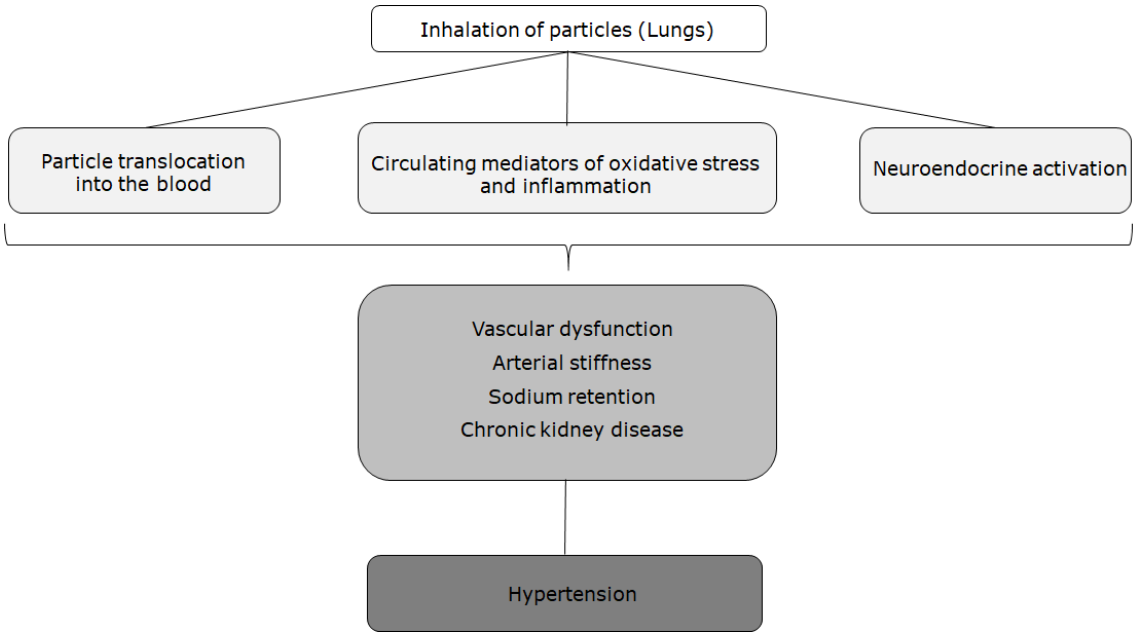

Figure 7 – Potential mechanisms whereby particulate matter exposures can increase blood pressure. Adapted from (137).

Regarding temporal relationships between PM exposure and BP changes, according to the perspective of Newman and his colleagues, prior studies may have reported inaccurate associations due to erroneous assumptions about the temporal relationship between exposure to PM and the outcome of BP changes (137). Despite the exposure window of PM exposure that is causally linked to BP levels is not well established, evidence supports that it is about a few days given the rapid occurrence of the physiological responses whereby PM influences on BP. Consequently, it is expected that annual PM averages will be biologically related to BP only if they are strongly correlated with those during the prior few days before BP measurement (137).

2.2.1.4. Blood count parameters and Inflammation

The blood count includes a wide range of parameters related to blood constituents that can be used as biomarkers of different diseases and subclinical conditions, namely inflammation state. White blood cells (WBC) count are an important and commonly used biomarker of inflammation as it is an inexpensive biomarker and various studies have supported it association with increased CVD risk (138,139). Other blood constituents, namely red blood cell (RBC) related parameters and platelet count, are less frequently used as biomarkers of inflammation, but all of them are involved in the pathogenesis of atherosclerosis and atherothrombosis, and consequently can be considered biomarkers of CV risk (140). For example, RDW, a measure of the size variability of the RBC that is often used for anaemia diagnosis, has recently been considered an important biomarkers of cardiovascular risk (141,142).

Some epidemiologic studies already found associations between both short and long-term PM exposure and blood count parameters, namely WBC (143,144), RBC (125,145) or platelets count (146,147). However, studies on this topic are still scarce and results remain controversial as some studies failed to detect the PM effect on these inflammation biomarkers (148,149). For example, Lee and her colleagues (144), in 2018, found that increased WBC counts were associated with the long-term (1-year average) PM_{10} exposure (0.76% WBC increase per each 4.4 $\mu g/m^3$ PM_{10} increment, 95%CI: 0.42%; 1.10%) but Gondalia and his colleagues (143), in 2020, failed to find the same association in a 1-month exposure period.

Regarding the potential biological mechanisms supporting the link between PM exposure and blood count parameters, there is already some evidence showing that exposure to PM, also through the three main mechanisms previously reported will directly or indirectly stimulate the bone marrow to produce WBC and platelets. These blood count parameters will contribute to the systemic inflammatory state contributing to destabilization of atherosclerotic plaques making them more vulnerable to rupture and thrombosis (86,150,151). Additionally, it seems that PM exposure could also interfere with RBC production and maturation, through the erythropoietin deregulation, leading to a lowering of the RBC cells production and suppressing the RBC maturation (86,140,152).

3. Research hypothesis and Objectives

The overall aim of this research is to improve our knowledge on the relationship between exposure to particulate matter and cardiovascular related biomarkers that can potentially trigger fatal and non-fatal CV diseases.

Accordingly, this thesis investigates the following main hypothesis: exposure to ambient particulate matter (APM) was associated with changes in the values of cardiovascular risk biomarkers in the Portuguese resident population, in 2015.

The main objectives of this work are the following:

(1) To systematically review and meta-analyse published studies on the association between AAP and biomarkers of dyslipidaemia (TC, HDL-C, LDL-C, TG), summarising the current state of knowledge prior to designing the plan of analysis on this topic (**Article 1**);

(2) To assess the association between exposure to PM_{10} and biomarkers of dyslipidaemia (CT, HDL-C, LDL-C, TG) (**Article 2**);

(3) To assess the association between exposure to PM_{10} and a biomarker of diabetes (HbA1c) (**Article 2**);

(4) To assess the association between exposure to PM_{10} and blood count parameters with potential to mediate a cardiovascular event (WBC, PLAT, RBC, RDW and HEMOG) (**Article 3**);

(5) To assess the association between exposure to PM_{10} and blood pressure values (**Article 4**);

(6) To assess the potential modifier and mediation effect of abdominal obesity on the association between exposure to PM_{10} and biomarkers of dyslipidaemia, diabetes and blood pressure (**Articles 2** and **4**);

(7) To compare the usage of two different indirect methods to assess the individual-level exposure to PM_{10} (**Articles 2, 3** and **4**).

4. Methodology

This section describes some general methodological aspects, common to the original scientific articles, which are the core of this thesis work. Further methodological specificities on each of the original articles, namely regarding the systematic review, the meta-analysis approach and the statistical analysis are described in detail in the methods sections of those original articles (Section 5).

4.1. Study design and population

With the exception of **Article 1**, which is a systematic review and meta-analysis, all the remain articles are cross-sectional studies that study the association between air quality data, collected through air quality monitoring network of the Environmental Portuguese Agency (APA), and data from a Health Examination Survey, performed in a Portuguese population-based probabilistic sample (First Portuguese Health Examination Survey – INSEF).

INSEF was a cross-sectional population-based study designed to be representative of the Portuguese population aged 25 to 74 years-old at regional and national levels which aimed to improve knowledge on the health status, health determinants and use of healthcare services of the Portuguese population. It was led by the Department of Epidemiology of the National Health Institute Doutor Ricardo Jorge (INSA) in partnership with the five Regional Health Administrations from mainland Portugal, the two Regional Health Secretariats from the Autonomous Regions of Azores and Madeira and the Norwegian Institute of Public Health (153). Data from this survey was collected between February and December 2015 and the target population consisted on non-institutionalized individuals aged between 25 and 74 years old, living in Portugal for more than 12 months, who were able to follow an interview conducted in Portuguese. The INSEF sample (n=4911, participation rate: 43.9%) was selected through a two-stage cluster probabilistic sampling, stratified by region and typology of urban area. Details about the sampling process and other INSEF procedures have already been described (153).

The INSEF survey was approved by the Ethics Committee of the Portuguese National Health Institute Doutor Ricardo Jorge (INSA), the National Data Protection Authority (Authorization nº 9348/2010) and by the regional Ethics Committees. During the fieldwork, each participant was given a brief description of the study objectives and had the opportunity to clarify any existing questions after which they signed a written informed consent form

agreeing to participate in the physical health examination, donate a blood sample for clinical tests and answer the health questionnaire through an interview. Additionally the informed consent form included no mandatory expressed permission to be re-contacted for re-examination and follow-up, to store the biological samples in the INSEF biospecimen collection at INSA, to link the survey data to other databases and to send the participants' blood tests results to their personal general practitioner. Finally, the ethics Committee of INSA also approved the study protocol of this thesis (Annex I).

For this thesis, the analysis was restricted to data from the subsample of INSEF participants from mainland Portugal (n=3467) which have consented to link their data from the survey with other databases. Additionally, only participants with available data on zip code address number, living within a 30-km radius of at least one air quality monitoring station with available PM_{10} concentration values and available data on the studied biomarkers were considered. Thus, considering those inclusion criteria a total of 2390, 2011 and 2271 participants were included, respectively, in **Articles 2** (Biomarkers of dyslipidaemia and diabetes), **3** (Biomarkers of inflammation) and **4** (Biomarkers of hypertension).

4.2. Health Data

During the INSEF fieldwork, data collection was conducted at public primary health care level facilities by trained health professionals. It included core physical measurements (blood pressure, height, weight and waist and hip circumference), a blood collection to perform blood tests (TC, HDL-C, LDL-C, TG, HbA1c and blood count) and a computer assisted personal interview (CAPI), according to the European Health Examination Survey (EHES) procedures (154).

Blood pressure was measured using an automated blood pressure measurement device (Omron M6). Cuffs were selected according to the individual's arm size (M for arm conference ≤32 cm; L for arm conference >32 cm). All participants rested for 5 min in a sitting position before the measurements. Three consecutive measurements were performed on the right arm at 1-min intervals.

Table 4 – Self-reported variables from INSEF data that were used in this thesis.

Variables name (Definition)	Categories	Article		
		2	3	4
Sociodemographic				
Sex	Male/ Female	x	x	x
Age group	25-49 years old 50-74 years old	x	x	x
Level of education (Highest level of education completed according to ISCED 2011 [1])	Low: levels 0-2 of the ISCED 2011 Medium: levels 3-4 of the ISCED 2011 High: levels 5-8 of the ISCED 2011	x	x	x
Occupation (Present or last job ISCO-08 [2])	White-collar occupation: Managers, Professionals, Technicians & Associate Professional, Clerical Support Workers and Services & Sales Workers Blue-collar occupation: Skilled Agricultural Workers, Craft & Related trades Workers, Plant & Machine Operators and Elementary occupations	x	x	x
Lifestyles				
Smokers	Yes: current daily and occasional smokers No: No smokers	x	x	x
Excessive alcohol consumption (Frequency of alcohol consumption during the past 7 days)	Yes: 3 or more days/week of consumption of at least one of the following alcoholic beverages (wine, beer, brandy/bagasse, Port wine/Martini/liqueur, Whisky/Gin/Vodka) No: Less than 3 days/week of consumption of at least one of the following alcoholic beverages (wine, beer, brandy/bagasse, Port wine/Martini/liqueur, Whisky/Gin/Vodka)	x	x	x
Sedentary (Leisure time activities during the last 12 Months)	Yes: Reading, watching TV or other sedentary activities declared as the best description of the leisure time activities during the last 12 months No: Hard training more than once a Week or Jogging or heavy gardening at least 4 hours a week or Light activities at least 4 hours a week	x	x	x
Unhealthy diet (Frequency of vegetable and fruit consumption)	Yes: No consumption of fruit and vegetables at least once a day No: Consumption of fruit and vegetables less than once a day	x	x	x
Health Status				
Dyslipidaemia (Diagnosed by a medical doctor)	Yes/No	x		
Lipid-lowering medication	Yes/No	x		
Diabetes (Diagnosed by a medical doctor)	Yes/No	x		
Diabetes medication	Yes/No	x		
Anaemia (Diagnosed by a medical doctor)	Yes/No		x	
Anaemia medication	Yes/No		x	
Hypertension (Diagnosed by a medical doctor)	Yes/No			x
Hypertension medication	Yes/No			x

[1](155);[2](156).

HbA1c and blood count were measured in fresh non-fasting whole blood samples while blood lipids (TC, HDL-C, LDL-C and TG) were measured in fresh non-fasting serum samples. Laboratory determinations were performed in the facilities of the 12 regional collaborating laboratories that participated in the National Program for External Quality Assessment (PNAEQ) to assure comparability and reliability of blood tests results.

Sociodemographic (age, sex, educational level and occupation), lifestyle (smoking, excessive alcohol consumption, sedentary, unhealthy diet) and health status variables (diagnosed dyslipidaemia, diagnosed diabetes, diagnosed hypertension, diagnosed anaemia, lipid-lowering medication, diabetes medication, hypertension medication and anaemia medication usage) were obtained by self-report through the interview. All the variables included in the articles are described in **Table 4** and **Table 5**.

Table 5 – Direct-measured variables from INSEF data that were used in this thesis.

Variables name	**Definition/Categories**	Article		
		2	3	4
Abdominal Obesity [1]	**Yes**: Waist-to-height ratio (WHtR) ≥0.5 **No**: Waist-to-height ratio (WHtR) <0.5	x		
SBP	Systolic blood pressure (mmHg)			x
DBP	Diastolic blood pressure (mmHg)			x
TC	Total cholesterol serum level (mg/dL)	x		
HDL-C	High-density lipoprotein cholesterol serum level (mg/dL)	x		
LDL-C	Low-density lipoprotein cholesterol serum level (mg/dL)	x		
TG	Triglycerides serum level (mg/dL)	x		
HbA1C	Fraction of glycated haemoglobin (%)	x		
WBC	Blood concentration of white blood cells (10^3 units/µL)		x	
PLAT	Blood concentration of platelets (10^3 units/µL)		x	
RBC	Blood concentration of red blood cells (10^6units/µL)		x	
HEMOG	Blood concentration of Haemoglobin (g/dL)		x	
RDW	Red blood cell distribution width (%)		x	

[1] Height and Waist measurements were used to obtain the waist to height ratio: Waist (cm)/Height (cm). Abbreviations: TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TG, Triglycerides; HbA1c, Glycated haemoglobin; DBP, Diastolic blood pressure; SBP, Systolic blood pressure; WBC, White blood cells; PLAT, Platelets; RBC, Red blood cells; RDW, Red cell distribution width; HEMOG, Haemoglobin.

4.3. Exposure Assessment

4.3.1. Definition of the time windows of exposure

There is a wide variability on the time windows of exposures considered on previous published association studies of PM effects and CV related outcomes, ranging from hours, when assessing the short-term exposure, to several years, when assessing the long-term exposure, mainly because it is still not clear which time window of exposure is most relevant to explain the increased CV morbidity and mortality associated with PM exposure (157). This is a complex issue, being plausible to assume that, probably, depending on the leading biological mechanism that is activated by a certain PM exposure, which will be in turn dependent on the PM constituents and concentration in a given space, different time windows of exposure will be needed to ensure that a certain effect is observed.

In this thesis, it was considered the exposure period of 1-year before the INSEF examination day as being representative of the long-term PM_{10} exposure for all studied participants as they reported to live in the same place (same zip code number used to estimate the individual-allocated PM_{10} exposure) at least on that period. Longer exposure periods were not considered to avoid additional exposure misclassification as INSEF participants could live in other places before this period of time. On the other hand, an exposure period of 3-days before the INSEF examination day to represent the short-term PM_{10} exposure as the published scientific evidence suggests that the acute particulate matter effects on the occurrence of cardiovascular events are generally larger at very shorter periods of time, namely in few days (158). Additionally, the periods of 2-days and 5-days before the INSEF examination day were also considered and were presented in the produced original articles as a sensitivity analysis to the considered 3 days period of exposure.

In the cross-sectional studies included in this thesis, the chosen long and/or short time windows of exposure varied according to the biological mechanisms that were considered to be the main pathway by which PM impact on these outcomes. Thus, regarding the blood lipid and glucose biomarkers (**Article 2**), the chosen time windows of exposure was 1-year before the INSEF examination date in order to detect the long-term effect of PM as an endocrine disruptor at the adipose tissue level. In fact, some in vivo exposure studies demonstrate that PM exaggerates visceral adipose tissue (VAT), increasing adipokines secretion and deregulate glucose and lipid metabolism in several months after exposure (104,105,159).

Consequently, it was assumed that a long-term period of exposure will be the most adequate to study the association between PM_{10} exposure and these outcomes.

For the remaining analysed outcomes, namely blood pressure and blood count parameters, evidence from the experimental studies in animals and also in humans show that the effect will be relatively immediate (160,161). Consequently, for these outcomes the period of 3 days before INSEF examination was considered as being representative of the short-term PM_{10} exposure. Additionally, as the association between PM_{10} exposure and blood count biomarkers are still less studied, the long-term period of exposure (1-year) was also considered to study this association and both analysis were performed in **Article 3** (Section 5).

4.3.2. Individually-allocated PM_{10} obtained from the air monitoring stations

4.3.2.1. Data extraction

The Portuguese Environment Agency (APA) provides hourly observation data for different atmospheric pollutants, including PM_{10}, measured by 68 fixed air quality monitoring stations (64 in mainland Portugal) that are classified according to the type of environment (demographic area) they represent (rural, urban and suburban) and the influence in terms of dominant atmospheric emission sources (background, traffic and industrial). For this thesis, PM_{10} hourly values were downloaded from the QualAr database on 20th February 2018, through the APA website (https://qualar.apambiente.pt/), using the option “by year and pollutant”, as described in **Figure 8**.

Accordingly, the PM_{10} hourly values from all air quality monitoring stations with available measurements between 2014 and 2015 were downloaded to cover the previous year of exposure before the INSEF examination day to all participants (INSEF fieldwork occurred between February and December 2015).

After downloaded the PM_{10} hourly values, daily average PM_{10} concentrations were calculated, in all days between February 2014 and December 2015, using the 24-hour PM_{10} values from each station but only for those stations with a data collection efficiency of at least 75%. Stations with less than 75% of efficiency in a particular day were represented with a missing value regarding its daily average PM_{10} concentration in that day.

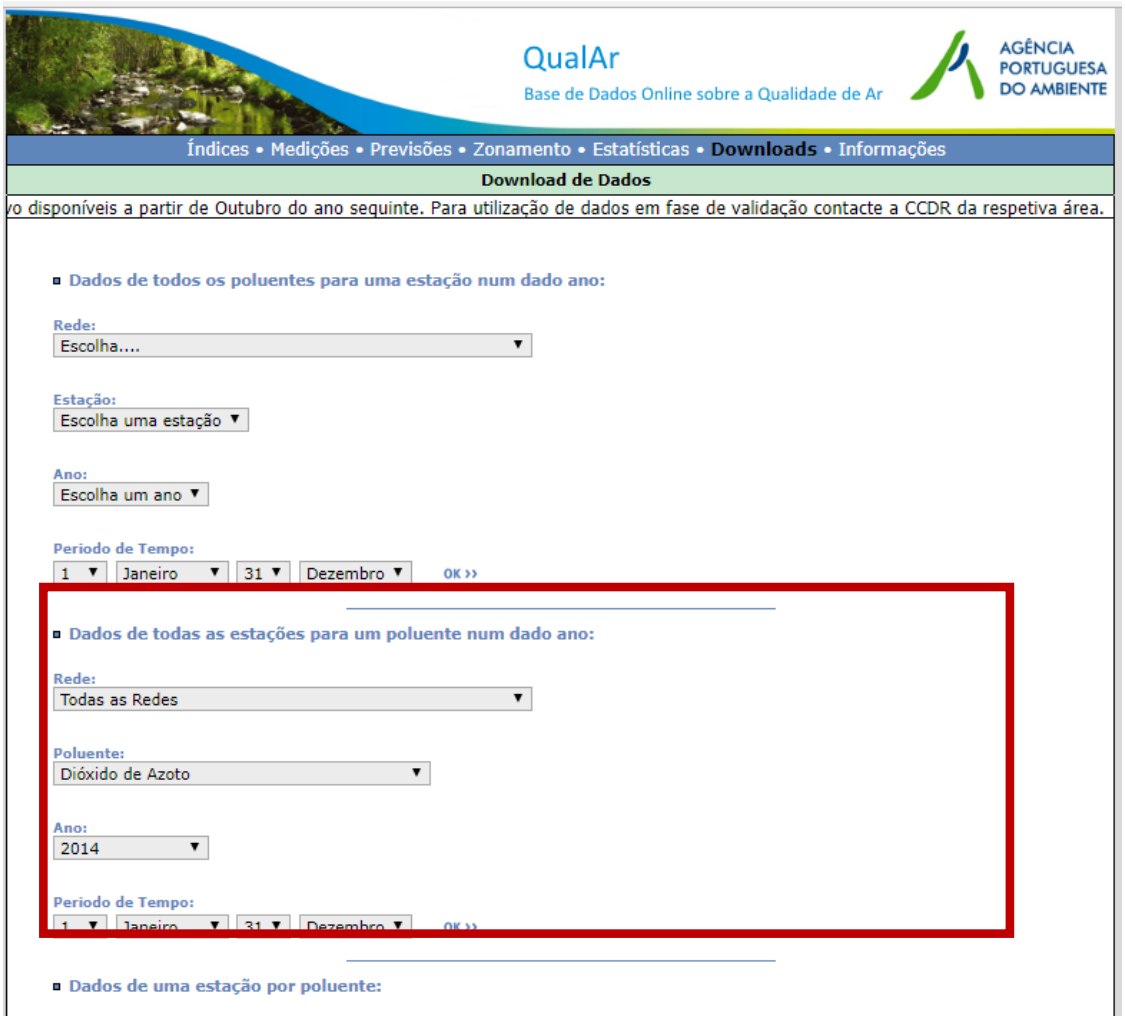

Figure 8 – Screen-shot of the APA webpage used to download PM_{10} data, on 20th February 2018.

The 1-year average PM_{10} concentration (long-term exposure measure) was computed for each station and for all INSEF fieldwork days, using the previous 365-daily average PM_{10} concentrations values. Once again, only stations with data collection efficiency of at least 75% were considered (with at least 75% of the 365-daily values available) and all 1-year averages PM_{10} concentrations computed using less than 75% of the previous daily values available were replaced by a missing value. Similarly, the 3-day average PM_{10} concentration (short-term exposure measure) was computed for each station and for all INSEF fieldwork days, using the previous 3-daily average PM_{10} concentrations values. 2 and 5-day averages of PM_{10} concentration were obtained in the same way. Daily averages of the PM_{10} concentrations were calculated for a particular day, only if the 24-hours averages on all of the previous 5 days were available.

In this way, databases with 1-year average PM_{10} concentrations and 2, 3 and 5-day average PM_{10} concentrations was obtained for all the stations and for all the INSEF fieldwork

days (**Table 6**). These databases were used to obtain the individually allocated PM_{10} values that will be described in the next subsection.

Table 6 – Database structure of the PM_{10} values used to obtain the individually allocated PM_{10} concentrations.

Date	**PM_{10}_Stationname1**		**…**	**PM_{10}_Stationname64**
2.2.2015	X (µg/m^3)[1]			
… **21.12.2015**				…

[1] x represent the 1-year average PM_{10} concentration (considering the previous 365-daily average PM_{10} concentrations) or the 2, 3 or 5-day averages PM_{10} concentration (considering the previous 2, 3 or 5-daily averages PM_{10} concentrations, respectively) on day 2.2.2015 regarding the air monitoring stationname1.

4.3.2.2. Linkage between air quality monitoring stations and INSEF participants

The localization of air monitoring stations (geographical coordinates made available by APA) and the participant's residences (zip code number of residence) were identified and managed by geographic information system (GIS) (ArcGIS version 10;.4 ESRI, Redlands, CA, USA). Distances (in kilometres) between stations and participant's residences were calculated and, for each participant, the 64 mainland stations were ranked according to its distance to each participant´s residence. Thus, for each INSEF participant the variables described in **Table 7** were obtained.

Table 7 – Database structure of the variables related to the georeferencing of INSEF participants and air monitoring stations.

Participant	**Date***	**Station1**	**Distance_Station1**	**…**	**Station64**	**Distance_Station64**
1		Station name	Km[1]	…		
… **3467….**		…				

[1] Km represent the distance between the participant`s residences (zip code number) and air monitoring station localization; * Date of INSEF participation.

This database was linked to the database represented in **Table 6**, using the variable date as the link variable. Thus, through the date, each participant was associated with the 1-year, 2, 3 and 5-day averages PM_{10} concentrations from all stations in the day of the INSEF participation (considering the previous 365, 2, 3 or 5-daily averages PM_{10} concentrations, respectively). Only averages of PM_{10} concentrations from background stations (n=39) were considered as it is expected that these stations monitor the background level of pollution in the environment far from traffic and industrial sources.

4.3.2.3. PM_{10} exposure individual attribution and database construction

After linkage of each the anonymous INSEF participants data to the 1-year, 2, 3 and 5-day averages PM_{10} concentrations from all background stations, and in order to reduce the exposure misclassification, only averages PM_{10} concentrations from stations within a 30-km radius from the participant's residence were considered, being the final individually allocated PM_{10} concentration weighted by the inverse of the squared distance between the residence and the various air quality monitoring stations, when more than one was available.

The usage of the 30 km radius criteria was based on previous published studies, whose criteria mostly ranged from 50 km (162) to 1 km (123), and also in order to maintain a reasonable sample size after sample restriction (more than 50%). This linkage strategy has been reported by previous authors (163,164). The 10 km radius and 20 km were also tested in the present thesis but taking into account that their application resulted in a large reduction of the sample size (around 50% and 30% of the total sample, respectively), the 30 km radius criterion was chosen. However, results using the 20-km radius for all performed analysis were also presented as a sensitive analysis to the criteria. The 10-km radius was not presented due to the huge sample size reduction that would compromise the precision of the estimates. **Table 8** describes individually-allocated PM_{10} exposure variables that were obtained.

Table 8 – Exposure variables related to the individually-allocated PM_{10} concentration obtained from the air monitoring stations according with distance to the residence of each individual.

Variables name	Definition	Article		
		2	3	4
PM_{10}_30km_1year	Individual allocated 1-year average PM_{10} concentration considering daily average values from air monitoring stations within 30km	x	x	
PM_{10}_30km_3days	Individual allocated 3-days average PM_{10} concentration considering daily average values from air monitoring stations within 30km		x	x
PM_{10}_30km_2days	Individual allocated 2-days average PM_{10} concentration considering daily average values from air monitoring stations within 30km		x	x
PM_{10}_30km_5days	Individual allocated 5-days average PM_{10} concentration considering daily average values from air monitoring stations within 30km		x	x
PM_{10}_20km_1year	Individual allocated 1-year average PM_{10} concentration considering daily average values from air monitoring stations within 20km	x	x	
PM_{10}_20km_3days	Individual allocated 3-days average PM_{10} concentration considering daily average values from air monitoring stations within 20km		x	x
PM_{10}_20km_2days	Individual allocated 2-days average PM_{10} concentration considering daily average values from air monitoring stations within 20km		x	x
PM_{10}_20km_5days	Individual allocated 5-days average PM_{10} concentration considering daily average values from air monitoring stations within 20km		x	x

4.3.3. Individually-allocated PM_{10} obtained from a mathematical Model

In addition to data from the fixed air monitoring stations that was used to assess the individually allocated PM_{10} exposure, data from a mathematical model was also used in this thesis, as another indirect method to assess PM_{10} exposure. As already mentioned in subsection 2.1.2, the application of mathematical models to estimate air pollutants concentration in a given space and time is usually computationally intensive and complex, requiring expertise in meteorology and climatology (51). Consequently, to obtain the individually allocated PM_{10} concentrations from mathematical modelling, a collaboration with a research group on Atmospheric Processes & Modelling (APM) from Universidade de Aveiro (CESAM & Department of Environment and Planning) was established.

In this away, atmospheric PM_{10} concentrations with spatial detail across Portugal were obtained by the application of an air quality modelling system – WRF-CAMx - composed by the Weather Research & Forecasting (WRF-ARW, version 3.7.1) model (WRF) (165) and the Comprehensive Air Quality Model with Extensions (CAMx, version 6.40) (166). The WRF model was developed by the National Center for Atmospheric Research (NCAR) and is a next generation mesoscale numerical weather prediction system designed for operational forecasting and atmospheric research needs. CAMx is a three-dimensional (3D) chemical transport model suited for the simulations of the emission, dispersion, chemical reactions, and removal of pollutants in the troposphere based on the integration of the continuity equation for each chemical species on a system of nested 3D grids. This air quality modelling system has been extensively applied for Portugal and worldwide (167–169).

CAMx aerosol scheme divides the size distribution into coarse and fine modes. Primary species are modelled as fine and, or, coarse particles, while all chemically-formed compounds are simulated as fine particles only. CAMx was applied to the whole years of 2014 and 2015 following a downscaling approach, on two nested domains, the first domain over Europe with 25×25 km^2 grid spacing and the domain of interest over Portugal with a dimension of 475 km by 625 km, gridded as 95 by 125 cells of 5×5 km^2 horizontal resolution (166).

Input data for WRF-CAMx simulations include ERA Interim reanalysis data from ECMWF (European Centre for Medium Range Weather Forecast) at 6 hours and 0.75 degrees temporal and spatial resolution respectively, to initialize and drive the WRF meteorological simulation. Other inputs comprise anthropogenic emissions of atmospheric pollutants and initial and boundary conditions for the CAMx European domain taken from the outputs of the global

chemical model MOZART (170) at every 6 hours. Anthropogenic emissions were derived from the European Monitoring and Evaluation Programme (EMEP) gridded European emission inventory based on Member States submissions for the year 2015, regulated under the Convention on Long-range Transboundary Air Pollution (CLRTAP). The EMEP inventory, with a horizontal resolution of 0.1 degrees (approximately 10 km), comprises annual emission totals by activity sector for gases and particulate species. For the Portuguese domain, the EMEP gridded emissions were spatially disaggregated to the 5 km horizontal resolution for the different activity sectors (e.g. industry, road traffic, residential combustion, among others) considering different proxies as land use, population distribution, industrial areas, road networks, etc. CAMx returns surface hourly average concentrations of simulated species by grid cell that were used to compute daily averages of PM_{10} for 2014 and 2015.

After obtaining the daily averages of PM_{10} for 2014 and 2015 through the WRF-CAMx in each grid cell of 5×5 km^2, participants health data, for whom it was possible to attribute individually allocated average PM_{10} concentration from air monitoring stations within 30km of their place of residence were linked to the correspondent grid cell and PM_{10} daily averages of each grid cell were considered as being representative of the individual exposure obtained by the mathematical model WRF-CAMx. As a result, daily averages of PM_{10} concentration obtained from the WRF-CAMx model in the years 2014 and 2015 were linked to each participant. After that, the preceding 365-daily average PM_{10} concentrations at the INSEF examination day were considered to obtain the individual allocated 1-year average PM_{10} concentrations for each participant. The preceding 24-h average on the 3 days before the INSEF examination day was considered to obtain the individual allocated 3-day average PM_{10} concentrations for each participant. The obtained variables are described in **Table 9.**

Table 9 – Description of the variables derived from mathematical modelling used in this thesis.

Variables name	**Definition**	Article		
		2	3	4
PM_{10}_modelled_1year	Individual allocated 1-year average PM_{10} concentrations considering values from a mathematical model	x	x	
PM_{10}_ modelled _3days	Individual allocated 3-days average PM_{10} concentrations considering values from a mathematical model		x	x

4.3.4. Individually-allocated ambient temperature

Temperature is an important confounding variable in the association between PM_{10} exposure and biomarkers of cardiovascular risk as it is strongly related with the exposure (171) and also with the analysed outcomes, namely with lipid levels (172) and blood pressure (173). Consequently, individually allocated average temperatures were also obtained for each INSEF participant in a similar away as done for the individual allocated PM_{10} concentrations.

Daily temperatures were obtained using data from the National Oceanic and Atmospheric Administration (NOAA) database (www.ncdc.noaa.gov). Data from a total of 24 stations were download and georreferenciated according to their localization. Each participant was associated to the closest temperature monitoring station and the calculations of the 1-year, 2, 3 and 5 days were performed in a similar way as previously described for the individually allocated PM_{10} concentrations. The obtained variables are described in **Table 10.**

Table 10 – Description of the variables temperature-related used in this thesis.

Variables name	**Definition**	Article		
		2	3	4
Temp_1year	Individually allocated 1-year average temperature (ºC)	x	x	x
Temp_3days	Individually allocated 3-days average temperature (ºC)		x	x
Temp_2days	Individually allocated 2-days average temperature (ºC)		x	x
Temp_5days	Individually allocated 5-days average temperature (ºC)		x	x

4.4. Statistical analysis

Statistical analysis carried out was well described in each of the original articles presented in the results section of the thesis, namely the construction of the directed acyclic graphs (DAG), the application of the generalized linear regression models and the sensitivity analysis. Consequently, in this subsection, it will only be described the components of the statistical analysis that are not detailed or are missing in the various articles, namely mediation and effect modification analysis.

Mediation and effect modification analysis

To assess the potential mediation effect of abdominal obesity (AO) on the association between exposure to PM_{10} and biomarkers of dyslipidaemia, diabetes and blood pressure, a mediation analysis was performed that assume the presence of statistically significant associations between the exposure and the mediator, the mediator and the outcome and between the exposure and the outcome (174–176).(**Figure 9**).

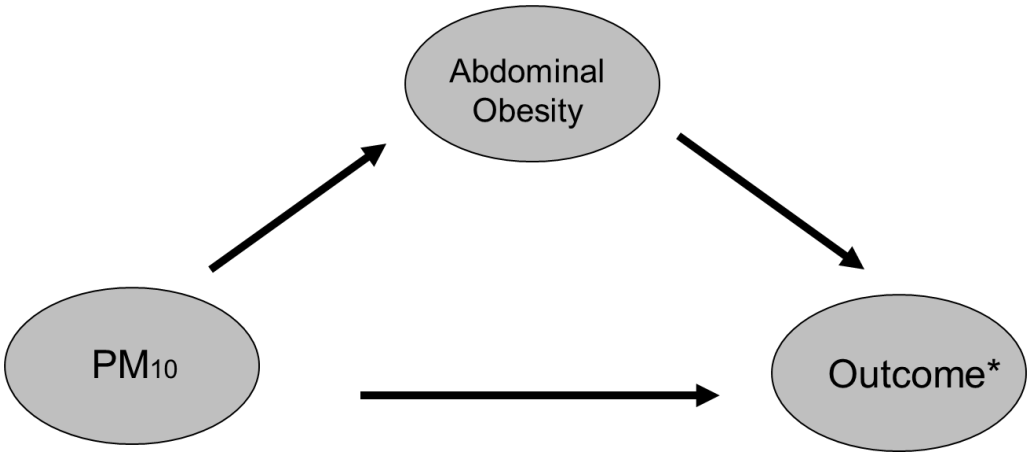

Figure 9 – Schematic representation of the potential mediation effect of Abdominal Obesity on the association between exposure to PM_{10} and biomarkers of dyslipidaemia, diabetes and blood pressure. *Total cholesterol, High-density lipoprotein cholesterol, Low-density lipoprotein cholesterol, Triglycerides, Glycated haemoglobin, Diastolic blood pressure or systolic blood pressure.

After assessing the existence of these associations by regression models analysis, the following approach was considered: (1) fit a model to estimate the association between PM_{10} and each outcome (TG, TC, HDL-C, LDL-C, HbA1C, DBP, SBP) without controlling for

potential mediator AO (Model1); (2) fit a model to examine the association between PM_{10} and the mediator AO (Model 2); (3) fit a model to estimate the association between PM_{10} and each outcome (TG, TC, HDL-C, LDL-C, HbA1C, DBP, SBP) after controlling for the potential mediator AO (Model 3).

Model 1: $outcome = \beta_{PM10(1)} PM_{10} + \beta\ confounders$

Model 2: $AO = \beta_{PM10(2)} PM_{10} + \beta\ confounders$

Model 3: $outcome = \beta_{PM10(3)} PM_{10} + \beta_{AO(3)} + \beta\ confounders$

The parameter estimates and standard errors from these models were used to estimate the Sobel test statistic value (Z - score), the percentage of the total effect that is mediated and the ratio of indirect to direct effects according to the formulas:

$$\text{Sobel test statistics} = \frac{\beta_{PM10(2)}\ \beta_{AO(3)}}{\sqrt{\left(\beta^2_{PM10(2)} SE^2_{\beta_{AO(3)}}\right) + \left(\beta^2_{AO(3)} SE^2_{\beta_{PM10(2)}}\right)}}$$

$$\text{Percent of the total effect that is mediated} = \frac{\beta_{PM10(2)}\ \beta_{AO(3)}}{(\beta_{PM10(2)}\ \beta_{AO(3)}) + (\beta_{PM10(1)} - (\beta_{PM10(2)}\ \beta_{AO(3)}))}$$

$$\text{Ratio of the indirect to the direct effect} = \frac{\beta_{PM10(2)}\ \beta_{AO(3)}}{(\beta_{PM10(1)} - (\beta_{PM10(2)}\ \beta_{AO(3)}))}$$

To assess the potential modifier effect of abdominal obesity, an interaction term (PM_{10}*AO) was introduced in the final model of each outcome (177). If the *p-value* were statistically significant ($p<0.05$), a stratified analysis according to the abdominal obesity status was performed.

5. Results

5.1. Association between ambient particulate matter and blood lipids and glucose levels

5.1.1. Article 1: Ambient air pollution and lipid profile: Systematic review and meta-analysis

Vânia Gaio [a,b], Rita Roquette [a,c,] Carlos Matias Dias [a,b,] Baltazar Nunes [a,b]

[a] Departamento de Epidemiologia, Instituto Nacional de Saúde Doutor Ricardo Jorge IP (INSA, IP), Lisboa, Portugal;

[b] Centro de Investigação em Saúde Pública, Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Lisboa, Portugal;

[c] Nova IMS Information Management School, Universidade Nova de Lisboa, Lisboa, Portugal.

Abstract

Ambient air pollution (AAP) is recognized a cardiovascular risk factor and lipid profile dysregulation seems to be one of the potential mediators involved. However, results from epidemiologic research on the association between exposure to AAP and altered lipid profile have been inconsistent. This study aims to systematically review and meta-analyse epidemiologic evidence on the association between exposure to ambient air pollutants (particulate matter, nitrogen oxides, sulphur dioxide, ozone, carbon monoxide, back carbon) and lipid profile parameters (Total cholesterol; High-Density Lipoprotein Cholesterol; Low-Density Lipoprotein Cholesterol; TG-Triglycerides) or dyslipidaemia.

Systematic electronic literature search was performed in PubMed, Web of Science and Scopus databases (last search on 24th May 2018) using keywords related to the exposure (ambient air pollutants) and to the outcomes (lipid profile parameters/dyslipidaemia). Qualitative and quantitative information of the studies were extracted and fixed or random-effects models were used to obtain a pooled effect estimate per each pollutant/outcome combination.

22 studies were qualitatively analysed and, from those, 3 studies were quantitatively analysed. Particulate matters were the most studied pollutants and a considerable heterogeneity in air pollution assessment methods and outcomes definitions was detected. Age, obesity related measures, tobacco consumption, sex and socioeconomic factors were the most frequent considered variables for confounding adjustment in the models. In a long-term exposure scenario, we found a 3.14% (1.36%-4.95%) increase in TG levels per 10µg/m^3 PM_{10} increment and a 4.24% (1.37%-7.19%) increase in TG levels per 10µg/m^3 NO_2 increment. No significant associations were detected for the remaining pollutant/outcome combinations.

Despite the few studies included in the meta-analysis, our study suggests some epidemiologic evidence supporting the association between PM_{10} and NO_2 exposures and increased TG levels. Due to the very low level of evidence, more studies are needed to clarify the role of lipid profile dysregulation as a mediator on the AAP adverse cardiovascular effects.

Keywords: Ambient air pollution; Lipid Profile; systematic review, meta-analysis

Introduction

Ambient air pollution (AAP) is a major global environmental problem posing numerous management challenges. In the last decades, increasing industrialization and urbanization has resulted in higher levels of air pollution worldwide making air quality management a priority due to its influence on human health. Nevertheless, a large proportion of the population is still exposed to air pollutants levels above recommended standards despite air quality policies implemented in many countries and according to the World Health Organization (WHO), in 2016, 91% of the world's population was living in places where the WHO air quality guidelines levels were not met (178).

Although it may seem intuitive that exposure to air pollutants affects mostly the respiratory system, there is evidence that the majority of its adverse effects are on the cardiovascular system (179–181). Worldwide, 3.7 million deaths were attributable to AAP in 2012, with ischaemic heart disease and stroke accounting for 80% of premature deaths, followed by lung diseases and lung cancer (182).

The pathophysiologic mechanisms linking AAP and cardiovascular events are still an area of research and scientific debate. However, lipid metabolism alteration through oxidative stress and subsequent systemic inflammatory response seems to be one of the potential mediators of the adverse cardiovascular effects of air pollution (57,183,184). It is plausible that lipid profile parameters, namely Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C) and Triglycerides (TG) levels are affected by exposure to air pollutants which will, in turn, contribute to trigger cardiovascular events.

In the past years, some epidemiological studies explored the association between AAP exposure and lipid profile parameters or dyslipidaemia (95,97,98,123,185–187) but the results are not consistent and some studies failed to demonstrate the deleterious effect of AAP on the lipid profile parameters, namely on TC and HDL (188). Moreover, a recent study suggest that among the several cardiometabolic risk factors, dyslipidaemias may be the most sensitive to air pollution exposure (102).

Until now, to the best of our knowledge, no systematic review exploring the link between exposure to AAP and lipid profile parameters or dyslipidaemia has been published. This study aimed to systematically review and meta-analyse the association between exposure to ambient air pollutants (particulate matter – PM_{10}, $PM_{2.5}$ and ultrafine particles, nitrogen oxides-NOx, nitrogen dioxide – NO_2, sulphur dioxide – SO_2, ozone – O_3, carbon monoxide – CO, Black carbon– BC) and levels of blood lipid parameters (TC, HDL-C, LDL-C and TG) or dyslipidaemia conditions. Our hypothesis is that an increase in levels of ambient air pollutants is associated with an increase in levels of TC, LDL-C and TG, a decrease in levels of HDL-C and an increase in the prevalence of dyslipidaemia.

Methods

Search Strategy

The systematic review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines (189). Full search criteria are presented in Table S1 (Supplementary material). In short, three bibliographic databases (PubMed, Web of Science and Scopus) were considered to the literature search using the combinations of the keywords related to the exposure (air pollution, air pollutant*, particulate matter, PM_{10}, $PM_{2.5}$, ultrafine particles, ozone, sulphur dioxide, nitrogen oxides, carbon

monoxide, black carbon) and the outcomes (lipid profile, high density lipoprotein cholesterol, HDL, low density lipoprotein cholesterol, LDL, cholesterol, triglycerides, dyslipid*, hypercholesterol*, hypertriglycerid*). Moreover, the reference lists of all included studies as well as their citation lists were also screened. All observational studies (cohort, cross sectional and case-control studies) published until the date of the search (24th May 2018) were considered. Two of the authors (VG and RR) independently searched the articles and downloaded them into EndNote Web® reference management software to remove duplicated references. The title and abstracts of the remaining articles were screened for eligibility and the full texts of the potential eligible articles were further analysed. In case of disagreement between the two authors, a third author (BN) resolved any discrepancies.

Eligibility criteria

Studies were included if they met all of the following criteria: (a) Considering at least one of the following air pollutants exposure: PM_{10}, $PM_{2.5}$, ultrafine particles, NO_X NO_2, SO_2, O_3, CO; BC (b) Reporting the association of exposure to the air pollutants with at least one lipid parameter values (TC, HDL-C, LDL-C, TG) or with any dyslipidaemia condition related with these lipid parameters (high TC; low HDL-C, high LDL-C, high TG); (c) reporting a quantitative measure of the association.

Due to the wide range of definitions and cut-offs used to define dyslipidaemia conditions (190–192) no specific definition was assumed as an inclusion criteria and all of the studies reporting any type of dyslipidaemia (high TC; low HDL-C, high LDL-C, high TG or dyslipidaemia diagnosed by a physician) were considered. Only original research published in English as a full publication in a peer reviewed journal were included.

Data Extraction and synthesis

The following data were extracted into the final list of studies for qualitative synthesis: author, publication year, country, study period, study population, study design, sample size, type of pollutant, exposure period, exposure assessment method, type of outcome, fasting state of participants for blood collection, association assessment method, effect estimate definition, effect estimate (EE) values and their 95% confidence intervals (95% CI) and confounding adjustment variables.

Only single-pollutant model estimates were extracted even when both single pollutant and multi-pollutant models were available. Effect estimates from the fully adjusted models were those extracted and extra estimates from sensitivity analysis were not considered.

Exposure times up to 30 days were considered as short-term exposure and exposure times more than 30 days were considered as long-term exposure (193). In the case of articles reporting multiple lags estimates to assess short-term exposures, the lag estimate considered in this review was chosen based on the following priority order criteria already considered in previous systematic reviews by the following: (1) the lag that the author focused on or stated as a priority; (2) the lag that showed the highest significance level (positive or negative); or (3) the lag with the largest effect estimate (positive or negative) (17,194). The same priority order criteria were applied when articles presented more than one effect estimate to assess long-term exposure: (1) the time period that the author focused on or stated as a priority; (2) the time period that showed the highest significance level (positive or negative); or (3) the time period with the largest effect estimate (positive or negative).

Data extraction was performed independently by two of the authors and disagreements were resolved by a third one. In the case of studies with missing information it was tried to contact their authors by email.

Quality assessment

The quality of each study was independently assessed by two investigators (VG and RR) using the Joanna Briggs Institute (JBI) checklists for cohort studies and cross-sectional studies (195). In the case of the cohort studies, their quality was graded as poor (0–4), intermediate (5–8) or high (9–11). Similarly, in the case of the cross sectional studies, their quality was graded as poor (0–3), intermediate (4–6) or high (7–8) according to the JBI checklist items. Any disagreement was resolved by consensus after discussion between the two investigators. Moreover, the approach of GRADE to rating quality of evidence was used to assess the quality of evidence from the meta-analysis. Accordingly, the level of evidence was downgraded according to the risk of bias of the included studies, inconsistency, indirectness, imprecision and publication bias (196). Because all meta-analysis were based on observational studies, the produced evidence was immediately classified as “low level evidence” and there were no reasons to upgrade the level of evidence.

Statistical Analysis

Beyond the PRISMA guidelines (189), meta-analysis of selected studies followed the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (197). Meta-analysis were only performed if two or more studies were comparable in terms of study population group, pollutant, outcome, exposure period and effect estimate.

Effect estimates (EE) of each air pollutant concentrations expressed as parts per billion (ppb) were converted to $\mu g/m^3$ making the following assumptions: 1 ppb= 1.96 $\mu g/m^3$ for O_3; 1 ppb= 1.88 $\mu g/m^3$ for NO_2; 1 ppb= 2.62 $\mu g/m^3$ for SO_2; 1 ppb= 1.15 $\mu g/m^3$ for CO (198).

According to the Cochrane handbook for systematic reviews, it is recommended that meta-analysis of EE coming from skewed data on a log scale be done on the scale of the log-transformed data (199). Thus, EE from each study on a log scale (presented as % change in the outcome per $\mu g/m^3$ of pollutant) were back-transformed to beta coefficients (ß) assuming the following formula: ß=ln[(EE/100)+1] (200). This formula was applied to the EE and to both limits of the respective 95% CI values in order to obtain the standard error of ß estimate. Obtained ß estimate values were converted for a fixed increment in pollutant concentration (per 10 $\mu g/m^3$ of pollutant) and these estimates, and corresponding 95%CI, were used in the meta-analysis.

The ß estimate standard error (SE) from the transformed 95%CI limits values of EE was obtained from the formula SE= (ßUL – ßLL)/3.92 where ßUL=ln[(EE_UL/100)+1], ßLL=ln[(EE_LL/100)+1] and EE_UL and EE_LL are respectively the upper (UL) and lower (LL) 95% confidence limits of the effect estimates (EE) (199). Finally, the pooling estimate and 95% confidence limits estimates were back-transformed (EE=100x [exp (ß)-1]) and presented in the forest plots as % change in the outcome per $\mu g/m^3$ of pollutant.

The heterogeneity of the included studies was evaluated by using the Cochran´s Q test and I2 statistic. Regarding the Cochran´s Q test, if the p-value was <0.05, a random-effects model was assumed to calculate the Pooled effect estimate (PEE). Otherwise, a fixed-effect model was considered. Concerning the I2 statistic, if the value was >50%, we considered that there is a statistically significant heterogeneity (201). Additionally, The Egger's test for asymmetry was used to assess the publication bias (202).

Meta-analysis were performed using the package "meta" (203) of the R software (version 3.4.3, R Development Core Team 2017) assuming 0.05 level of significance, and forest plots were constructed using the package "forestplot" (204) of the same software.

Results

From the 1505 records identified through database searching and after duplicates were removed, the title/abstract of 1123 records were screened. From those, 41 were full-text assessed for eligibility and 1 more article were selected from their reference lists. A total of 22 articles met the inclusion criteria and all of them were considered to have moderate/high quality based on the JBI checklists for cohort studies and cross-sectional studies (Tables S2 and S3, Supplemental material) and were included in the qualitative synthesis (95,96,186–188,205–211,97,212–214,98–101,123,162,185). Finally, 3 articles (95,97,98) covering the same population group (Adults, both sexes), pollutant (PM_{10} and NO_2), outcomes (TC, HDL-C, LDL-C and TG), period of exposure (long-term exposure) and effect estimate definition (% changes in the outcome per $\mu g/m^3$ of pollutant) were quantitative analysed (**Figure 10**).

Qualitative description of the articles

The publication year of the articles range from 2007 to 2019 and more than half were published in the last 3 years. Studies were mostly conducted in the USA (n=8), the data collection period range from 1988 to 2016 and the majority were cross-sectional studies (n=14). Adults of the general population were the most frequently analysed population group (n=11) although ages considered to define adults varied in the different studies, ranging from 16 to 90 years old. Moreover, a considerably proportion of the studies explored the ambient AAP effects among specific sub population groups, namely the elderly (n=3), midlife women in menopausal transition (n=1), children or adolescents (n=2) and patients with chronic diseases (n=6). Long-term exposures to $PM_{2.5}$, PM_{10} or NO_2 were the most frequently studied exposure conditions.

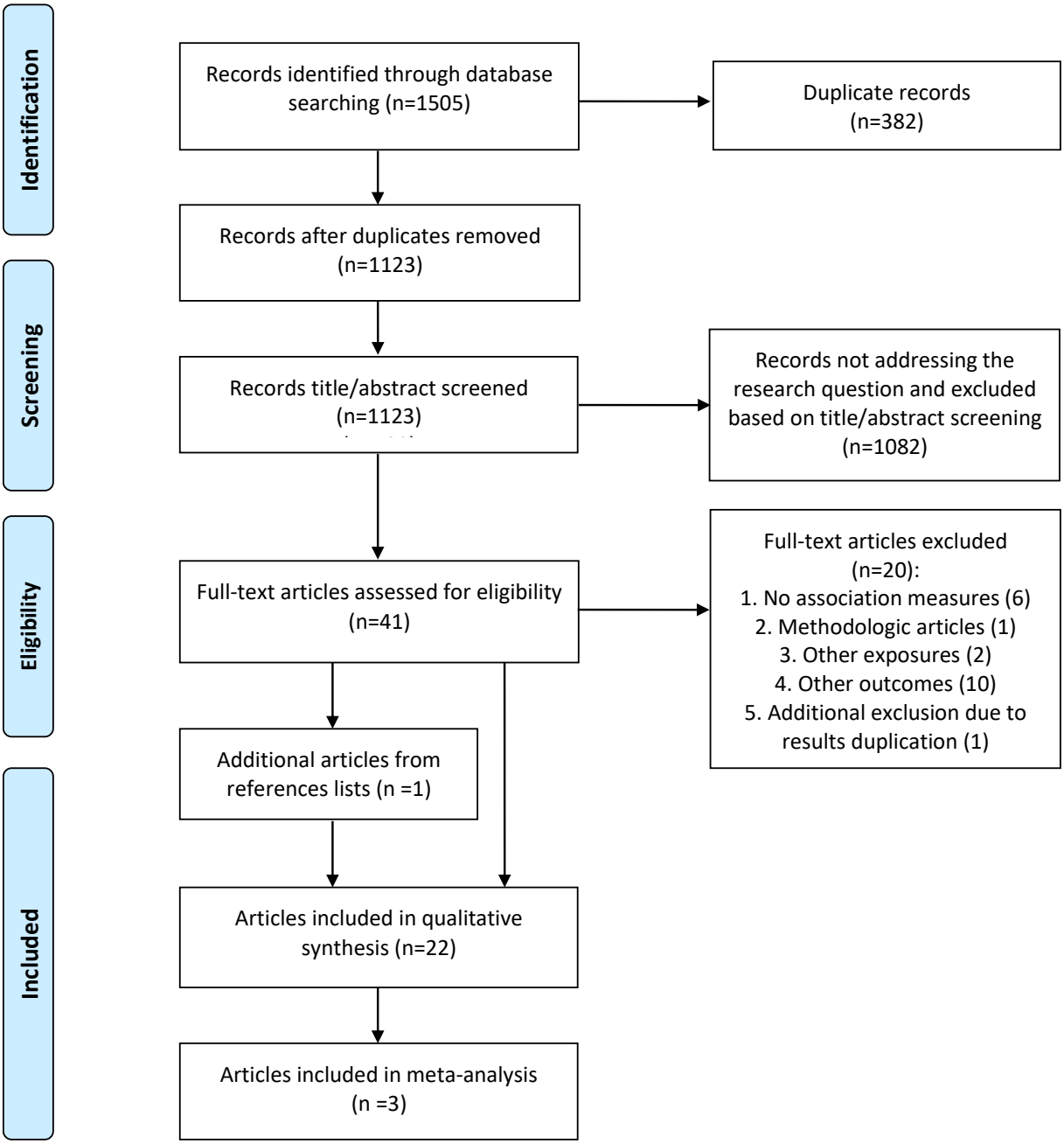

Figure 10 – Flow diagram of study selection process. Adapted from (189)

Table 11 – General characteristics of the studies included in the qualitative synthesis.

Author, year	Country	Study period	Study Population	Study Design	Sample size	Pollutants	Exposure Duration	Outcomes
Yeatts et al. 2007 (185)	USA	2003-2004	Patient Adults, 21-50y	Cohort	12	$PM_{2.5}$	Short-term	TC, TG
Chuang et al. 2010 (186)	Taiwan	2002	Adults, 16-90y	Cross-sectional	7578	PM_{10}, NO_2, SO_2, O_3, CO	Short-term	HDL-C, LDL-C, TG
Chuang et al. 2011 (205)	Taiwan	2000	Elderly, 54-90y	Cross-sectional	1023*	$PM_{2.5}$, PM_{10}, NO_2, SO_2, O_3, CO	Long-term	TC, HDL-C, TG
Sorensen et al. 2015 (206)	Denmark	1993-1997	Adults, 50-64y	Cross-sectional	36039	$PM_{2.5}$, NO_2	Long-term	TC
Eze et al. 2015 (99)	Switzerland	2001-2002	Adults, 29–73y	Cross-sectional	3684	PM_{10}, NO_2	Long-term	Dyslipidaemia[1]
Bind et al. 2016 (187)	USA	1995-2013	Elderly Men, 49-100y	Cohort	1112	$PM_{2.5}$	Short-term	HDL-C, LDL-C, TG
Sade et al. 2016 (123)	Israel	2003-2012	Patient Adults, >18y	Cohort	73117*	$PM_{2.5}$, PM_{10}	Short/Long-term	HDL-C, TG, LDL-C
Chen et al. 2016 (162)	USA	2002-2008	Patient Adults, 34.5±8.1y	Cross-sectional	1023*	$PM_{2.5}$, NO_2, O_3	Short/Long-term	TC, HDL-C, LDL-C, TG
Shanley et al. 2016 (97)	USA	1988-1994	Adults, 17-90y	Cross-sectional	11623*	PM_{10}	Long-term	TC, HDL-C, TG, LDL-C
Wallwork et al. 2017 (207)	USA	2000-2011	Elderly men, 70.4±6.9y	Cohort	326*	$PM_{2.5}$	Long-term	Dyslipidaemia[2]
Bell et al. 2017 (100)	USA	2000-2002	Adults, 45-84y	Cross-sectional	6654	$PM_{2.5}$	Short/Long-term	HDL-C, BC
Cai et al. 2017 (98)	Norway Netherlands	2006-2013	Adults, 47.6±13.7y	Cross-sectional	114082*	PM_{10}, NO_2	Long-term	TC, HDL-C, TG
Poursafa et al. 2017 (208)	Iran	2014-2016	Children and Adolescents, 6-18y	Cross-sectional	186	$PM_{2.5}$	Long-term	TC, HDL-C, LDL-C, TG
Fioravanti et al. 2018 (188)	Italy	2011-2012	Children, 8y	Cross-sectional	410	$PM_{2.5}$, PM_{10}, NO_2	Long-term	TC, HDL-C
Wang et al. 2018 (210)	China	2011-2015	Patient Adults, 52.2% ≥ 60y	Cross-sectional	3912	PM_{10},NO_2, SO_2	Short-term	TC, HDL-C, TG, LDL-C
Yang et al. 2018** (95)	China	2009	Adults,18-74y	Cross-sectional	15477	$PM_{2.5}$, PM_{10}, NO_2,SO_2 O_3	Long-term	TC, HDL-C, TG, LDL-C Dyslipidaemia[3]
Ghosh et al. 2018 (211)	USA	2005-2014	Patient Adolescents, 14-17y	Cohort	75	$PM_{2.5}$, NO_2, O_3	Long-term	HDL-C, TG
Lee et al. 2019 (96)	South Korea	2009-2013	Adults, 55.1±7.1y	Cohort	87417*	$PM_{2.5}$	Long-term	Dyslipidaemia[4]
Li et al. 2019 (101)	China	2014-2016	Adults, 23.3±5.4 y	Cohort	73	$PM_{2.5}$, NO_2,SO_2, CO, BC, PNC	Short-term	HDL-C
McGuinn et al. 2019 (212)	USA	2001-2010	Patients, 60.8±12.1 y	Cross-sectional	6587	$PM_{2.5}$	Long-term	TC, HDL-C, LDL-C, TG
Shin et al. 2019 (213)	South Korea	2012	Adults, 47.8±0.06 y	Cross-sectional	100867	PM_{10}, NO_2, SO_2, O_3, CO	Long-term	Dyslipidaemia[5]
Wu et al. 2019 (214)	USA	1999-2005	Midlife women in menopausal transition, 49±3y	Cohort	2289*	$PM_{2.5}$	Short/Long-term	TC, HDL-C, LDL-C, TG

*Sample size varies according to the analysed outcome. **Another study by Yang et al. (2019) was also found but it report duplicate results regarding the outcomes High TG and High LDL-C. Consequently, we opted to report only results from Yang et al. 2018 because they were presented adjusted for more confounding variables.

[1] Dyslipidaemia was defined according to 3 different definitions: High TG: TG ≥ 150mg/dL or medication; Low HDL-C: HDL-C <35 mg/dL for men and HDL-C <39 mg/dL for women (WHO definition); and Low HDL-C: HDL-C <40 mg/dL for men and HDL-C <50 mg/dL for women or medication (IDF/ATP definition).

[2] Dyslipidaemia was defined according to 2 different definitions: High TG: TG ≥ 150mg/dL or medication; and Low HDL-C: HDL-C <40 mg/dL or medication.

[3] Dyslipidaemia was defined according to 4 different definitions: High TG: TG ≥ 200mg/Dl; High TC: TC ≥240mg/Dl; High LDL-C: LDL-C (≥160mg/dL); and High HDL: HDL ≤40 mg/dL.

[4] Dyslipidaemia was defined according to 2 different definitions: High TG: TG ≥ 150mg/dL; and Low HDL-C: HDL-C <40 mg/dL for men and HDL-C <50 mg/dL for women.

[5] Dyslipidaemia diagnosed by a physician (auto reported information). **Abbreviations:** y-years; PM - particulate matter; PNC-Ultrafine Particulate number concentration; NO_2–nitrogen dioxide; SO_2–sulphur dioxide; O_3–ozone; CO – carbon monoxide; TC – Total cholesterol, HDL-C - High Density Lipoprotein Cholesterol, LDL-C - Low Density Lipoprotein Cholesterol; TG – Triglycerides.

Regarding the outcomes, continuous variables of lipid profile parameters were more frequently considered than the dichotomous variables regarding the dyslipidaemia condition (Yes/No). Five of the reviewed studies aimed at the association between air pollution and dyslipidaemia but they used discrepant definitions of the disease and thus were not comparable (**Table 11**).

While some studies had direct measurements of exposure from AAP monitoring stations (n=10), other considered more complex methods namely spatiotemporal hybrid models based on satellite information (n=12) (**Table 12**). Most studies collected blood in fasting state (n=13) but a considerable number of studies (n=6) did not report this information. Generalized Linear models (GLM) were the most frequently used method to assess the association between pollutant exposure and lipid profile parameters (n=9). The most common way to express the results was change in outcome (%, 95%CI) per IQR increase in pollutants (**Table 12**).

Age, obesity related measures, tobacco consumption, sex and socioeconomic factors were the 5-most frequent considered variables for confounding adjustment in the models (**Figure 11**). Additional information about the characteristics of the 16 studies included in qualitative analysis is available in Excel Table S1.[1]

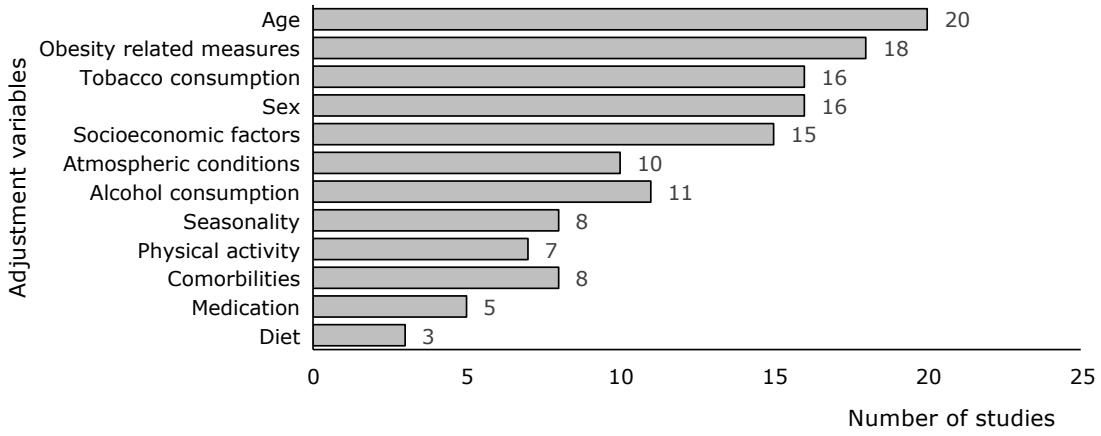

Figure 11 – Distribution of studies according to the 12 most frequently considered adjustment variables (Obesity related measures includes Body mass index, waist circumference measures or percent body fat; Socioeconomic factors includes employment status, family income, neighbourhood socio-economic index, occupation, gross domestic product, poverty-income ratio, occupation, socioeconomic status, education level, educational attainment, school attendance or Area level of Socioeconomic position).

[1] Due to its very large size, Excel Table S1 is not presented in this thesis. It is available online at https://doi.org/10.1016/j.envpol.2019.113036.

Table 12 – Description of Methods of Exposure Assessment, Fasting State of Participants, Methods of Association Assessment and Effect Estimate Definition of the studies included in the qualitative synthesis.

Author, year	Method of Exposure Assessment	Fasting State of Participants	Method of Association Assessment	Effect Estimate Definition
Yeatts et al. 2007(185)	Direct measurement on the local	Not reported	Mixed models	Change in outcomes (%, 95%CI) per 1 μg/m³ increase in pollutant
Chuang et al. 2010 (186)	Direct measurement from the nearest station within 10km	Yes	GAM	Change in outcome (mg/dL,95%CI) per IQR (μg/m³ to PM_{10} and ppb to O_3) increase in pollutants
Chuang et al. 2011 (205)	Direct measurement from the nearest station within 10km	Yes	GAM	Change in outcome (mg/dL,95%CI) per IQR (μg/m³ to PM_{10} and ppb to O_3) increase in pollutants
Sorensen et al. 2015 (206)	NO_2: Dispersion model; $PM_{2.5}$: LUR model	No	GLM	Change in outcome (mg/dL,95%CI) per IQR (μg/m³) increase in pollutant
Eze et al. 2015 (99)	PM_{10}: Dispersion models; NO_2: hybrid model incorporating LUR	Yes	Mixed logistic regression models	OR per 10 increase in pollutants
Bind et al. 2016 (187)	Direct measurement from a single monitor station	Not reported	Quantile regressions for longitudinal data	Differences in a given percentile of the outcome (mg/dL) per IQR (μg/m³) increase in pollutant
Sade et al. 2016 (123)	Spatiotemporal hybrid model (satellite and LUR)	Yes	Mixed models	Change in outcome (%,95%CI) per IQR (μg/m³) increase in pollutant
Chen et al. 2016 (162)	Spatial interpolation (maximum radius of 50Km)	Yes	Variance component models	TC, HDL-C, LDL-C: Changes in the outcome (mg/dL) per 1-SD change in pollutants. TG: % change (%,95%CI) in the outcome per with 1-SD change of pollutants
Shanley et al. 2016 (97)	Direct measurement from the nearest station within 20 miles	Yes/No	GLM	Change in outcome (%,95%CI) per IQR (μg/m³) increase in pollutant
Wallwork et al. 2017 (207)	Spatiotemporal hybrid model (satellite and LUR)	Yes	Cox proportional hazards models	Hazard ratio
Bell et al. 2017 (100)	Hierarchical spatiotemporal models	Yes	GLM	Change in outcome (mg/dL) per 5 μg/m³ increase in pollutant
Cai et al. 2017 (98)	LUR models	Yes/No	GLM	Change in outcome (%,95%CI) per IQR (μg/m³) increase in pollutant
Poursafa et al. 2017 (208)	Direct measurement from 6 monitor stations	Yes	GLM	Correlation coefficient between outcome and pollutant levels
Fioravanti et al. 2018 (188)	LUR models	Not reported	GLM	Change in outcomes (mg/dL, 95%CI) per 10μg/m³ increase in NO_2 and PM_{10} or 5μg/m^3 increase in $PM_{2.5}$
Wang et al. 2018 (210)	Direct measurement from the monitoring stations	Not reported	GLM	Changes in outcome (mg/dL,95%CI) per 10 μg/m^3 increment in pollutant
Yang et al. 2018** (95)	$PM_{2.5}$: Spatial model; PM_{10}, NO_2, SO_2, O_3: Direct measurement from 11 monitoring stations	Yes	2 level logistic regression and GLM	Change in outcomes (%, 95%CI) per 10-μg/m³ increase of pollutants and OR
Ghosh et al. 2018 (211)	Spatial interpolation by inverse distance-squared weighting (stations within 50Km)	Not reported	Multilevel linear spline model	Change in outcomes (mg/dL, 95%CI) per tertile of pollutant exposure (regression coefficients)
Lee et al. 2019 (96)	Community Multiscale Air Quality Model	Yes	Cox models	Hazard Ratio (95% CI)
Li et al. 2019 (101)	Direct measurements from monitoring stations	Yes	Generalized estimating equation models	Change in outcome (mg/dL, 95%CI) per inter-quartile range (IQR) higher exposure.
McGuinn et al. 2019 (212)	Hybrid prediction model	Yes	GLM	Change in outcome (%, 95%CI) per 1μg/m³ higher average of $PM_{2.5}$
Shin et al. 2019 (213)	Direct measurements from monitoring stations	Not reported	Multiple logistic regression models	OddsRatio (OR)

Table 10 (Continued) - Description of Methods of Exposure Assessment, Fasting State of Participants, Methods of Association Assessment and Effect Estimate Definition of the studies included in the qualitative synthesis.

Author, year	Method of Exposure Assessment	Fasting State of Participants	Method of Association Assessment	Effect Estimate Definition
Wu et al. 2019(214)	Direct measurements from monitoring stations (stations within 20Km)	Yes	Mixed models	Change in outcomes (%, 95%CI) per inter-quartile range (IQR) higher exposure

*Another study by Yang et al. (2019) was also found but it report duplicate results regarding the outcomes High TG and High LDL-C. Consequently, we opted to report only results from Yang et al. 2018 because they were presented adjusted for more confounding variables. **Abbreviations:** LUR-Land Use regression; GLM -Generalized linear models; SD-standard deviation; GAM-generalized additive model; OR-OddsRatio; IQR-interquartile range.

Table 13 – Summary of the statistical significant associations found in the included studies, in a long-term exposure scenario.

Population	Study	Pollutant	Continuous Outcome (Lipid levels)				Categorical Outcome (Yes/No)				
			TC	HDL-C	LDL-C	TG	High TC	Low HDL-C	High LDL-C	High TG	Dyslipidaemia*
Adults	Bell et al. 2017 (100)	$PM_{2.5}$		NA							
		BC		**A**							
	Cai et al. 2017 (98)	PM_{10}	NA*	NA*		**A***					
		NO_2	NA*	**A***		**A***					
	Eze et al. 2015 (99)	PM_{10}						NA		NA	
		NO_2						NA		NA	
	Lee et al. 2019 (96)	$PM_{2.5}$						**A**		**A**	
	Shanley et al. 2016 (97)	PM_{10}	**A***	NA*	NA*	**A***					
	Sorensen et al. 2015 (206)	$PM_{2.5}$	**A**								
		NO_2	**A**								
	Yang et al. 2018 (95)	PM_1	**A**	**A**	**A**	NA	**A**	**A**	**A**	NA	
		$PM_{2.5}$	**A**	**A**	**A**	**A**	**A**	**A**	**A**	NA	
		PM_{10}	NA*	NA*	**A***	**A***	NA	NA	NA	**A**	
		NO_2	**A***	**A***	NA*	**A***	**A**	NA	NA	NA	
		SO_2	NA	NA	NA	**A**	NA	NA	NA	**A**	
		O_3	**A**	**A**	**A**	**A**	NA	NA	NA	**A**	
	Shin et al. 2019 (213)	PM_{10}									**A**
		NO_2									**A**
		SO_2									**A**
		CO									**A**
		O_3									**A**
Elderly	Chuang et al. 2011 (205)	$PM_{2.5}$	**A**	NA		NA					
		PM_{10}	**A**	NA		NA					
		NO_2	**A**	NA		NA					
		SO_2	NA	NA		NA					
		O_3	**A**	NA		NA					
		CO	NA	NA		NA					
	Wu et al. 2019(214)	$PM_{2.5}$	NA	**A**	NA	NA					
	Wallwork et al. 2017 (207)	$PM_{2.5}$						NA		NA	
Midlife women in menopausal transition	Wu et al. 2019(214)	$PM_{2.5}$	NA	**A**	NA	NA					
Children and adolescents	Fioravanti et al. 2018 (188)	$PM_{2.5}$	NA	NA							
		PM_{10}	NA	NA							
		NO_2	NA	NA							
		No_x	NA	NA							
	Poursafa et al. 2017 (208)	$PM_{2.5}$	NA	NA	**A**	**A**					

Table 11(Continued) – Summary of the statistical significant associations found in the included studies, in a long-term exposure scenario.

Population	Study	Pollutant	Continuous Outcome (Lipid levels)				Categorical Outcome (Yes/No)				
			TC	HDL-C	LDL-C	TG	High TC	Low HDL-C	High LDL-C	High TG	Dyslipidaemia*
Patients	Sade et al. 2016 (123)	$PM_{2.5}$	**A**	**A**	**A**	**A**					
		PM_{10}	**A**	**A**	**A**	**A**					
	Chen et al. 2016 (162)	$PM_{2.5}$	NA	NA	**A**	NA					
		NO_2	NA	**A**	NA	NA					
		O_3	NA	NA	NA	NA					
	Ghosh et al. 2018 (211)	$PM_{2.5}$		**A**		**A**					
		NO_2		**A**		**A**					
		O_3		NA		**A**					
	McGuinn et al. 2019 (212)	$PM_{2.5}$	**A**	**A**	**A**	**A**					

Abbreviations: PM - particulate matter; NO_2–nitrogen dioxide; NOx–nitrogen oxides; SO_2–sulphur dioxide; O_3–ozone; CO – carbon monoxide; BC-Black Carbon; TC – Total cholesterol; HDL-C - High Density Lipoprotein; Cholesterol, LDL-C - Low Density Lipoprotein Cholesterol; TG – Triglycerides; A - Statistical significant association; NA - Not statistical significant association;* Dyslipidaemia condition was defined through autorrepored information, considering dyslipidaemia diagnosed by a physician.

* The effect estimate correspondent to this association were included in the meta-analysis

Table 14 – Summary of the statistical significant associations found in the included studies, in a short-term exposure scenario.

Population	Authors	Pollutant	Continuous Outcome (Lipid levels)				Categorical Outcome (Yes/No)				
			TC	HDL-C	LDL-C	TG	High TC	Low HDL-C	High LDL-C	High TG	Dyslipidaemia*
Adults	Bell et al. 2017 (100)	$PM_{2.5}$		NA							
	Chuang et al. 2010 (186)	PM_{10}		**A**	NA	NA					
		NO_2		NA	NA	NA					
		O_3		NA	NA	NA					
		CO		NA	NA	NA					
	Eze et al. 2015 (99)	PM_{10}						NA		NA	
		NO_2						NA		NA	
	Li et al. 2019 (101)	$PM_{2.5}$		NA							
		PNC_{5-50}		NA							
		PNC_{50-100}		**A**							
		$PNC_{100-500}$		NA							
		NO_2		**A**							
		SO_2		**A**							
		BC		**A**							
		CO		NA							
Elderly	Bind et al. 2016 (187)	$PM_{2.5}$		**A**	**A**	**A**					
		BC		**A**	**A**	**A**					
	Wu et al. 2019(214)	$PM_{2.5}$	NA	NA	NA	NA					
	Wallwork et al. 2017 (207)	$PM_{2.5}$						NA		NA	
Midlife women in menopausal transition	Wu et al. 2019(214)	$PM_{2.5}$	NA	NA	NA	NA					
Patients	Sade et al. 2016 (123)	$PM_{2.5}$	NA	NA	**A**	NA					
		PM_{10}	NA	NA	**A**	NA					
	Chen et al. 2016 (162)	$PM_{2.5}$	**A**	NA	**A**	NA					
		NO_2	NA	NA	NA	NA					
		O_3	NA	NA	NA	NA					
	Wang et al. 2018 (210)	PM_{10}	**A**	**A**	**A**	NA					
		NO_2	NA	**A**	**A**	**A**					
		SO_2	**A**	NA	**A**	**A**					
	Yeatts et al. 2007(185)	$PM_{2.5}$	NA			NA					

Abbreviations: PM - particulate matter; PNC – ultrafine particulate number concentration; NO_2–nitrogen dioxide; NO_x–nitrogen oxides; SO_2–sulphur dioxide; O_3–ozone; CO – carbon monoxide; BC-Black Carbon; TC – Total cholesterol; HDL-C - High Density Lipoprotein; Cholesterol, LDL-C - Low Density Lipoprotein Cholesterol; TG – Triglycerides; A - Statistical significant association; NA - Not statistical significant association;*Dyslipidaemia condition was defined through autorrepored information, considering dyslipidaemia diagnosed by a physician.

In a long-term scenario, concerning studies about adults from the general population (95–100,206,213) we found that statistically significant associations were more frequently found regarding the parameters TG and TC (**Table 13**), but some studies also reported no significant statistically significant associations regarding these two lipid parameters (95,98). In the elderly, in midlife women in menopausal transition and in the children/adolescents groups (205,207,214), the majority of the tested associations between pollutants and lipid outcomes were not statistically significant (**Table 13**). Regarding the Patients group studies (123,127,162,211,212), a considerable number of the tested associations between pollutants and lipid outcomes were statistically significant (**Table 13**).

In a short-term scenario, concerning studies about adults from the general population (100,101,117,186), we found that statistically significant associations were only found concerning the HDL-C parameter (101,186). In the elderly, one study reported statistically significant associations between particulate matter and HDL-C, LDL-C and TG (**Table 14**). Regarding the Patients group studies (123,162,185,210), the majority of the tested associations between pollutants and lipid outcomes were not statistically significant (**Table 14**).

Quantitative analysis

From the 3 studies (95,97,98) selected for quantitative comparison, it was possible to extract data on 17 effect estimate values covering 7 different pollutant/outcome combinations. Each study present its estimates in different pollutant increments (**Table 15**). After conversion, these effect estimates were meta-analysed in seven different analyses regarding each pollutant/outcome combination (Figure 12). Accordingly, it showed that PM_{10} and NO_2 exposures were significantly associated with increased levels of TG, in a long-term exposure scenario. We found a 3.14% (1.36% to 4.95%) increase in TG levels per 10 µg/m3 PM_{10} increment and a 4.24% (1.37% to 7.19%) increase in TG levels per 10 µg/m^3 NO_2 increment. Concerning the publication bias assessment, in the case of the meta-analysis reporting results from 3 studies, we found no indication for such source of bias (PM_{10}/TC: p-value of Egger's test = 0.54; PM_{10}/HDL-C: p-value of Egger's test = 0.40; PM_{10}/TG: p-value of Egger's test = 0.63). In the case of the meta-analysis reporting results from 2 studies, publication bias was not assessed as there were inadequate numbers of included studies to apply the test. No significant associations were detected for the remaining pollutant/outcome combinations. Regarding the short-term exposure scenario, the number of effect estimates were insufficient

to be meta-analysed for each pollutant/outcome combination. Based on the GRADE approach, all the cumulative evidence from the meta-analysis were considered very low level evidence (Table S4, Supplemental material).

Table 15 – Effect estimate values collected from the 3 studies quantitatively analysed.

Pollutant/Outcome	Author	% change in outcome (95% CI) per Pollutant increment	% change in outcome (95% CI) per 10 µg/m^3 Pollutant increment
PM_{10}/TC	Cai et al., 2017 (98)	-0.1(-0.3 to 0.05) per 2 µg/m^3	-0.5 (-1.5 to 0.25)
	Shanley et al., 2016 (97)	1.43(1.21 to 1.66) per 11.1 µg/m^3	1.29 (1.09 to 1.50)
	Yang et al., 2018 (95)	-0.2(-0.5 to 0.1) per 10 µg/m^3	-0.2 (-0.5 to 0.1)
NO_2/TC	Cai et al., 2017 (98)	-0.1(-0.3 to 0.1) per 7.4 µg/m^3	-0.14 (-0.41 to 0.14)
	Yang et al., 2018 (95)	0.7(0 to 1.4) per 10 µg/m^3	0.7 (0 to 1.4)
PM_{10}/HDL	Cai et al., 2017 (98)	0.2(-0.1 to 0.4) per 2 µg/m^3	1.0 (-0.5 to 2.0)
	Shanley et al., 2016 (97)	0.18(-0.32 to 0.68) per 11.1 µg/m^3	0.16 (-0.29 to 0.61)
	Yang et al., 2018 (95)	-0.2(-0.7 to 0.2) per 10 µg/m^3	-0.2 (-0.7 to 0.2)
NO_2/HDL	Cai et al., 2017 (98)	0.5(0.3 to 0.8) per 7.4 µg/m^3	0.68 (0.41 to 1.08)
	Yang et al., 2018 (95)	-1.6(-2.3 to -1) per 10 µg/m^3	-1.6 (-2.3 to -1)
PM_{10}/LDL	Shanley et al., 2016 (97)	1.18(0.81 to 1.56) per 11.1 µg/m^3	1.06 (0.73 to 1.41)
	Yang et al., 2018 (95)	-0.9(-1.3 to 0.4) per 10 µg/m^3	-0.9 (-1.3 to 0.4)
PM_{10}/TG	Cai et al., 2017 (98)	1.9(1.5 to 2.4) per 2 µg/m^3	9.5 (7.5 to 12.0)
	Shanley et al., 2016 (97)	2.42(1.09 to 3.76) per 11.1 µg/m^3	2.18 (0.98 to 3.39)
	Yang et al., 2018 (95)	4.7(3.6 to 5.9) per 10 µg/m^3	4.7 (3.6 to 5.9)
NO_2/TG	Cai et al., 2017 (98)	2.2(1.6 to 2.7) per 7.4 µg/m^3	2.97 (2.16 to 3.65)
	Yang et al., 2018 (95)	6(3.5 to 8.6) per 10 µg/m^3	6 (3.5 to 8.6)

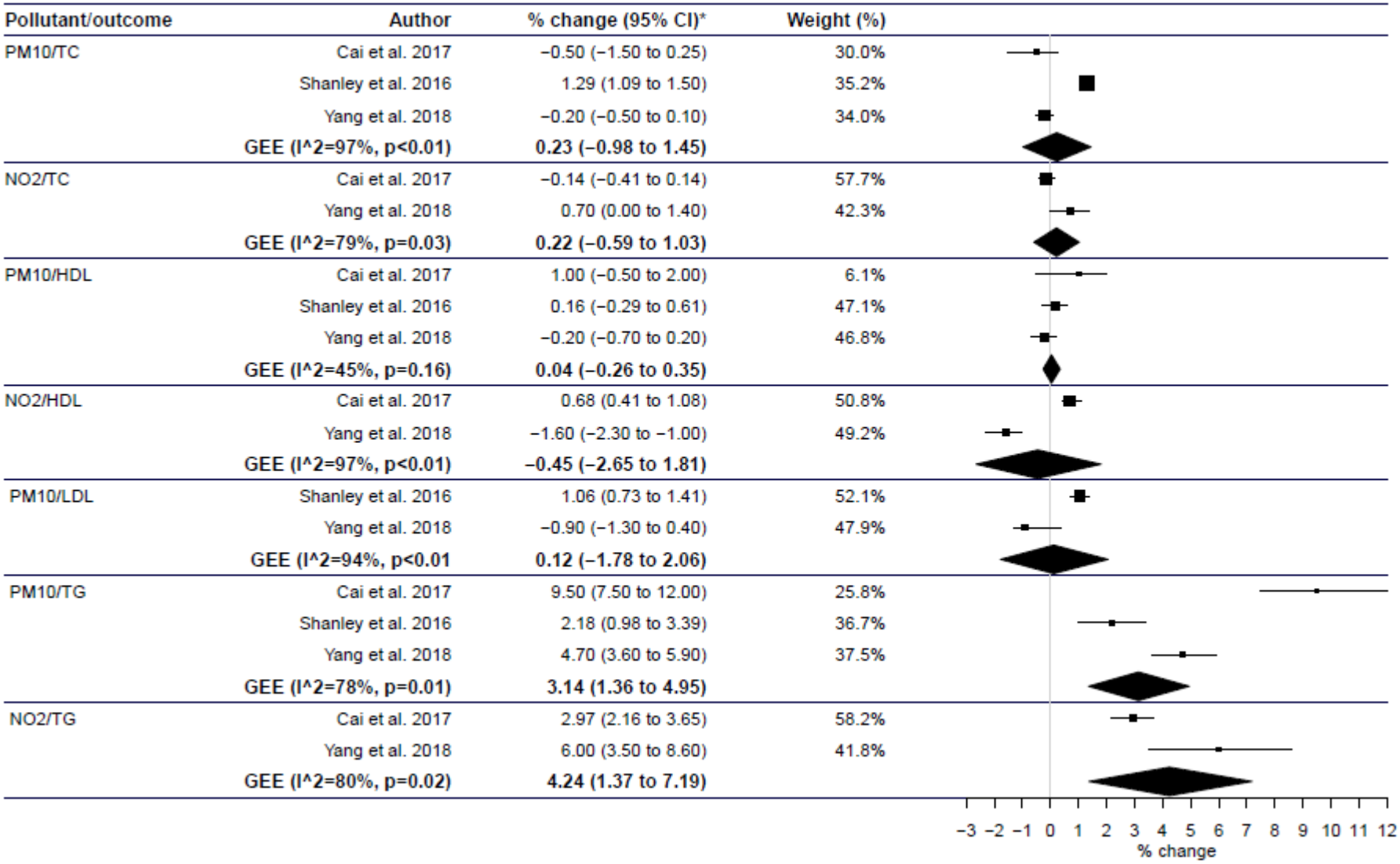

Figure 12 – Forestplots of the seven meta-analyses performed regarding each pollutant/outcome combination in a long-term exposure scenario (*percentage change in the outcome per 10 µg/m^3 pollutant increase. Abbreviations: GEE = Pooled effect estimate; I^2= measure of between-study heterogeneity; p= Cochran's Q test p-value).

Discussion

Despite the few studies included in the meta-analysis, our study suggests that, there is already some epidemiologic evidence supporting the association between PM_{10} and NO_2 exposures and TG levels in the adults from the general population. In a long-term exposure scenario, we found a 3.14% (1.36% to 4.95%) increase in TG levels per 10 $\mu g/m^3$ PM_{10} increment and a 4.24% (1.37% to 7.19%) increase in TG levels per 10 $\mu g/m^3$ NO_2 increment.

The biological mechanisms explaining the effect of AAP on lipid profile parameters are still not very clear but one hypothesis is that the oxidative stress and the systemic inflammation caused by AAP exposure could induce a dysfunction of the lipid metabolism (184). Another plausible mechanism is the DNA methylation of genes related to lipid metabolism caused by exposure to AAP (215). Nevertheless, to the best of our knowledge, there is no plausible biological mechanism that could explain a different deleterious effect of the AAP on TG levels compared to the other lipid profile parameters, as our results suggest. Consequently, we hypothesize that our meta-analysis in not correctly detecting the deleterious effect of ambient air pollutants on the other lipid profile parameters and this may be due to the bias introduced by the inclusion of lipid-lowering medicated participants in the included studies. In fact, lipid-lowering medication was not considered in the 3 studies quantitatively analysed, resulting in lower than the real values analysed in this study. Taking into account that statins are still used as the first line medication to lower lipid profile parameters levels and achieve substantial cholesterol reduction, evidence shows that reduction in TG levels are more modest when using statins (216,217). Thus we hypothesize that medicated participants who will have the values of the other lipid parameters controlled, will most likely continue to show uncontrolled TG values. This can explain the stronger associations between ambient air pollutants exposure and TG levels in the few included studies because the confounding effect of medication is lower or absent for this parameter. However, this hypothesis cannot be guaranteed because use of other drugs targeted at TG has become increasingly frequent to deal with high TG in addition to statins, especially in the most recent years (218) or the use of non-pharmacological therapeutics such as diet behaviour modifications and physical exercise practice that also will have impact on TG levels. Consequently, to validate this hypothesis, more information about the medicated participants of the various included studies would be needed.

Through our systematic review, we also verify that the most studied ambient air pollutant, among the 22 considered studies, was particulate matter (PM). This could be a consequence

of a publication bias, that was not possibly to evaluate conveniently due to the small number of included studies, but it remains unclear if some pollutants were considered and not reported in the articles. It is also plausible to assume that the deleterious effect of the gaseous pollutants, namely NO_2, SO_2, O_3 or CO, on lipid profile parameters are less explored and must be considered in future studies. In fact, there is more available evidence supporting the deleterious effect of the particulate matter on cardiovascular health (81,219). However, available evidence supports the importance of the gaseous pollutants on cardiovascular health (220), advising to explore its effects also on the lipid profile. Another plausible reason is that PMs data could be the most used due its greater spatial and temporal availability.

Additionally, we found a huge diversity of methods to assess the air pollutants exposure which make it difficult to compare published results. The traditional method of air pollution exposure assessment is through direct measurements obtained from fixed air quality network sites (186,205,208). This method is still a commonly used method but some studies reported in this systematic review already used more complex approaches namely spatiotemporal hybrid modelling (100,123,207). Although it is expected that there is a higher percentage of misclassification associated with traditional methods compared to more advanced methods (50), it was not possible to detect a clear pattern of influence of exposure misclassification on estimated values in our study. Regarding this issue, it is still important to mention that even these more sophisticated methods are problematic regarding their capacity to assess the real individual exposure and the use of new technology, namely GPS, smartphones or smaller pollution sensors are promising new methods to assess more real individualized exposures (56) and reduce exposure misclassification. So, more adequate approaches to quantify the individual air pollution exposure is warranted in order to standardize effect measures obtained from different epidemiological studies.

Another important consideration about our systematic review and meta-analysis is related to residual confounding. Although all studies reported effect estimates values based on models adjusted for multiple confounding variables, there is not consistency in the chosen confounding variables among the selected studies and important covariates were not considered in some studies. This could led to a considerable residual confounding related to the number and type of variables not included in the models. When we look at the 3 studies that we meta-analysed, all of them include age, sex, smoking status, individual socioeconomic factors as confounding variables, but important variables related to area-level socioeconomic status, for example, were only considered by two of them (95,97). To clarify the influence of

each confounding variable in the effect estimates values, more information would be necessary and, consequently, it is recommended to provide supplemental data regarding available data and the modelling process in futures studies.

The present meta-analysis includes a small number of studies found in the literature review, which does not allow sensitivity analyses to be performed and it would be important for validation of the meta-analysis results. Moreover, the between-study heterogeneity is very high for nearly all meta-analyses, which might have reduced the confidence of the cumulative evidence.

Effect estimates included in the present meta-analysis were based on a single-pollutant model and interactions between the ambient air pollutants were not evaluated. Despite multipollutant models being difficult to implement and validate, it is known that there are important interactions between the pollutants, namely the potential additive effects of multiple pollutants and they should be considered in future studies (221,222). Finally, the outcome assessment in the different studies was not uniform. The definitions of dyslipidaemia conditions varied in each study which compromises the comparison of the effect estimates of the different studies (95,96,99,207,213). Additionally, while some studies performed lipid profile parameters determination in a fasting condition, other considered the non-fasting state, which could be considered a source of bias. It is well known that while cholesterol levels only display modest postprandial variations, TG show significant postprandial elevations according to the diet content (223,224). However, in the study in which participants were all in a fasting state (95), the effect of PM_{10} and NO_2 exposures on TG levels remains statistically significant.

Conclusions and Recommendations

To our knowledge, this is the first study approaching the systematic review and meta - analysis of epidemiologic evidence on the association between AAP exposure and lipid profile parameters or dyslipidaemia conditions. Despite the very inclusive research strategy used, including no restrictions in the study design or population groups, we found only a few number of recently published studies on the association between air pollution and lipid profile. Nevertheless, we report results that could help to guide future research in this area. Moreover, given the high prevalence of dyslipidaemia (225) and the increasing AAP levels (59), we consider that our findings are of considerable and growing global public health importance. We suggest that if air pollution were continuously reduced, it could potentially reduce the incidence of dyslipidaemias and therefore the incidence of cardiovascular events.

As recommendations in the future studies about the association between exposure to ambient air pollutants and lipid profile parameters or dyslipidaemia conditions, we suggest further investigation of the gaseous pollutants exposure, that were less frequently included. We also recommend to explore the potential additive effects of multiple pollutants, as well as developing more robust spatiotemporal models to assess air pollution exposure more precisely.

Acknowledgments

The authors are grateful to all the professionals and participants involved in the INSEF. INSEF was developed as part of the Pre-defined project financed under the Public Health Initiatives Program, ‘‘Improvement of epidemiological health information to support public health decision and management in Portugal. Towards reduced inequalities, improved health, and bilateral cooperation’’, with a 1.500.000€ Grant from Iceland, Liechtenstein and Norway from EEA Grants and the Portuguese Government. The present study was also funded by the Portuguese Foundation for Science and Technology (FCT) (PhD Scholarship Reference: SFRH/BD/129426/2017).

Competing Financial Interests

The authors declare they have no actual or potential competing financial interests.

5.1.2. Article 2: Air pollution exposure interacts with abdominal obesity to increase blood triglycerides: a cross-sectional linkage study

Vânia Gaio [*1,2], Rita Roquette [1,3], Alexandra Monteiro [4], Joana Ferreira [4], Diogo Lopes, Carlos Matias Dias [1,2], Baltazar Nunes [1,2]

[1] Department of Epidemiology, Instituto Nacional de Saúde Doutor Ricardo Jorge IP (INSA, IP), Lisboa, Portugal;

[2] NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisboa, Portugal;

[3] Nova IMS Information Management School, Universidade NOVA de Lisboa, Lisboa, Portugal.
[4] CESAM & Department of Environment and Planning, Universidade de Aveiro, Aveiro, Portugal

Abstract

Background: Blood lipids and glucose levels dysregulation represent potential mechanisms intermediating the adverse cardiovascular effects of ambient particulate matter (PM) exposure. This study aims to estimate the effect of long-term PM_{10} exposure on blood lipids and glucose levels and to assess the potential mediation and/or modification action of abdominal obesity (Waist-to-height ratio).

Methods: Our study was based on 2390 participants of the 1st Portuguese Health Examination Survey (INSEF, 2015) with available data on blood lipids and glucose parameters and living within a 30km radius of an air quality monitoring station with available PM_{10} measurements. PM_{10} concentrations were acquired from the air quality-monitoring network of the Portuguese Environment Agency. Generalized linear models were used to assess the effect of 1-year PM_{10} exposure on blood lipids and glucose levels. An interaction term was introduced in the models to test the modification action of abdominal obesity.

Results: We found an association between PM_{10} and non-fasting blood triglycerides after adjustment for age, sex, education, occupation, lifestyles related variables and temperature but only in participants with abdominal obesity. Per each 1µg/m^3PM_{10} increment, there was a

1.84% (95%CI:0.02-3.69) increase in triglycerides. For the remaining blood lipid and glucose parameters, no associations were found.

Conclusions: Our study demonstrates that even at low levels of exposure, long-term PM_{10} exposure interacts with abdominal obesity to increase blood triglycerides. Our findings suggest that reducing both abdominal obesity prevalence and PM_{10} below current standards would result in additional health benefits for the population.

Keywords: Air pollution, Particulate matter, Blood lipid levels, Blood glucose levels, Triglycerides

Introduction

Ambient particulate matter (PM) exposure is a major global environmental problem and is a recognized factor to develop cardiovascular diseases, the leading cause of death globally (226,227). Blood glucose and lipids levels dysregulation, represent potential mechanisms intermediating the cardiovascular adverse effect of the PM exposure. Some epidemiologic studies assessed the association between air pollutants exposure and uncontrolled blood glucose and lipid levels (228,229). However, evidence on this association is still inconsistent (16,230). In the particular case of blood lipid levels, a recent published systematic review and meta-analysis suggests already some epidemiologic evidence supporting the association between PM_{10} (particles with an aerodynamic equivalent diameter ≤10μm) and blood levels of triglycerides (TG) but only three studies were meta-analysed and more epidemiologic studies are essential to clarify the strength of this association (230).

PM has been suggested as acting as an environmental endocrine disruptor and one potential biological mechanism explaining its deleterious effect on the blood glucose and lipid levels is through adipokines dysregulation at the adipose tissue level (159,231). Actually, some *in vivo* exposure studies, in animal models, demonstrate that PM exaggerates visceral adipose tissue (VAT) and increase adipokines secretion (104,105). Subsequently, a wide range of physiological mechanisms are induced, including insulin resistance and consequently blood glucose levels increase and also uncontrolled lipolysis, leading to inflated fatty acids delivery to the liver, which will in turn act as substrate to promote lipid synthesis and raised blood lipids levels, mainly TG levels (232). Therefore, we hypothesize that VAT could be a mediator of

PMs effect on blood glucose and lipid levels because PM will exaggerates VAT and then the subsequent mechanisms will be activated up to dysregulated glucose and lipid metabolism. On the other hand, VAT could also be considered a modifier of the PM effect on blood glucose and lipid levels because the pre-existing VAT could interact with PM to induce adipokines secretion and subsequent uncontrolled blood lipids and glucose levels.

Taking into account the hypothesis previously described, the present study aims (1) to estimate the effect of PM_{10} exposure on blood lipid and glucose levels (TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; TG, Triglycerides; HbA1c, Glycated haemoglobin) in the adult Portuguese mainland population and (2) to assess the potential mediation and/or modification action of abdominal obesity (as a proxy of VAT) on this effect.

Methods

Study population

This study was conducted using data from the 1st Portuguese National Health Examination Survey (INSEF), collected between February and December 2015. This survey was described in more detail by Nunes and his colleagues (153). This analysis was restricted to the subsample of INSEF participants from mainland Portugal (n=3467) with participants consent to link data, available data on zip code number, living within a 30-km radius of an air quality monitoring station with available PM_{10} concentration values and available data on blood lipids or glucose parameters (n=2390)(**Figure 13**).

The INSEF survey received approval from the Ethics Committee of the Portuguese National Health Institute Doutor Ricardo Jorge, the National Data Protection Authority (Authorization nº 9348/2010) and from the regional Ethics Committees.

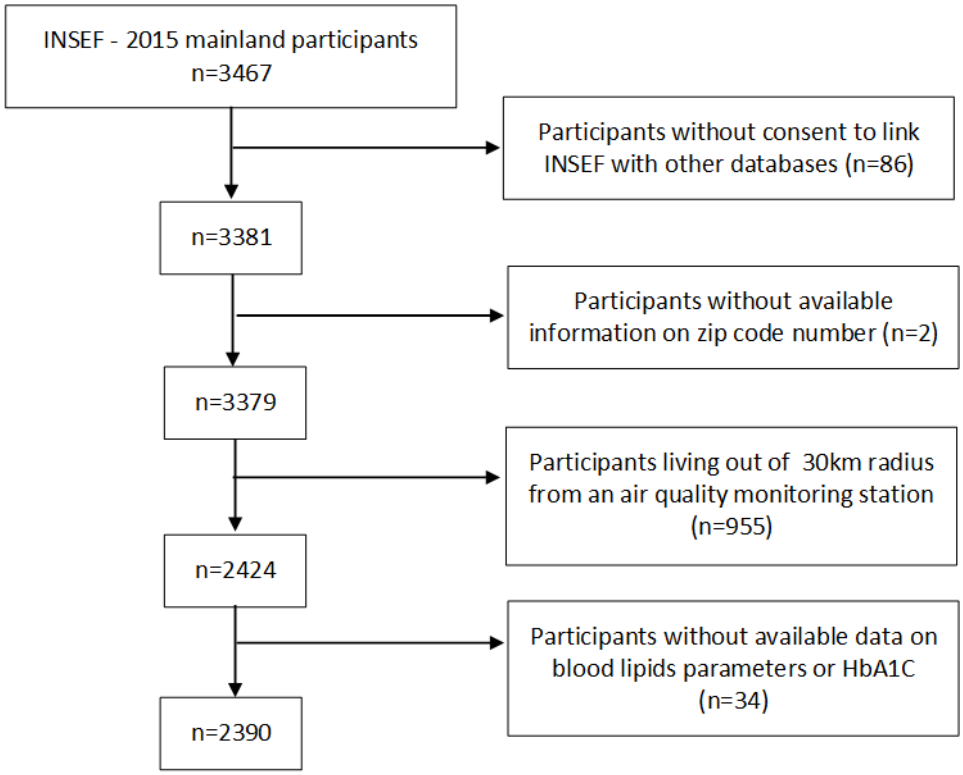

Figure 13 – Participant selection flow diagram.

Health data

Health data collection was performed by trained health professionals, according to the European Health Examination Survey (EHES) procedures (154). HbA1C was measured in fresh non-fasting whole blood samples and blood lipids (TC, HDL, LDL and TG) were measured in fresh non-fasting serum samples, in the 12 regional collaborating laboratories that participated in the National Program for External Quality Assessment (PNAEQ) to assure comparability and reliability of blood tests results.

Waist-to-height ratio (WHtR) was used as proxy of Abdominal Obesity (AO) because. in the absence of more objective measures of central obesity and adiposity, it is the most suitable proxy measure of the VAT quantity (233). WHtR was assessed using waist circumference and height measurements, assuming that participants with a Waist-to-height ratio (WHtR) ≥0.5 had AO.

Sociodemographic (age, sex, educational level and occupation), lifestyle (smoking, excessive alcohol consumption, sedentary and unhealthy diet) and health status variables

(diagnosed-dyslipidaemia, diagnosed diabetes, lipid-lowering medication usage and diabetes medication usage) were obtained by self-report through the interview.

Regarding educational level, we considered the highest level of education completed, grouped into 3 categories, according the 2011 International Standard Classification of Education (ISCED) (155): low education (levels 0-2 of the ISCED 2011), medium education (levels 3-4 of the ISCED 2011) and high education (levels 5-8 of the ISCED 2011). Occupation was grouped according to the International Standard Classification of Occupations (ISCO-08) (156) into 2 categories: white-collar occupation (Managers, Professionals, Technicians & Associate Professional, Clerical Support Workers and Services & Sales Workers) and blue–collar occupation (Skilled Agricultural Workers, Craft & Related trades Workers, Plant & Machine Operators and Elementary occupations).

Regarding the lifestyles related variables, smokers include current daily and occasional smokers, and excessive alcohol consumption was defined as reporting 3 or more days/week of consumption of at least one of the following alcoholic beverages: wine, beer, brandy/bagasse, Port wine/Martini/liqueur, Whisky/Gin/Vodka. Unhealthy diet was defined as no consumption of fruit and vegetables at least once a day and sedentary was defined as reporting reading, watching television or other sedentary activities as the best description of leisure time activities during the last 12 months.

Environmental exposure assessment

We obtained PM_{10} values from QualAr database, available online at the Portuguese Environment Agency (APA) website (https://qualar.apambiente.pt/). We assumed the period of 1-year PM_{10} exposure as being representative of participants' long-term PM_{10} exposure as they reported to live in the same place at least one year before the INSEF examination day. Only background stations with data collection efficiency of at least 75% were considered. The geographic distribution of the participants (zip code number) and the 24-background air quality monitoring stations are shown in **Figure 14.**

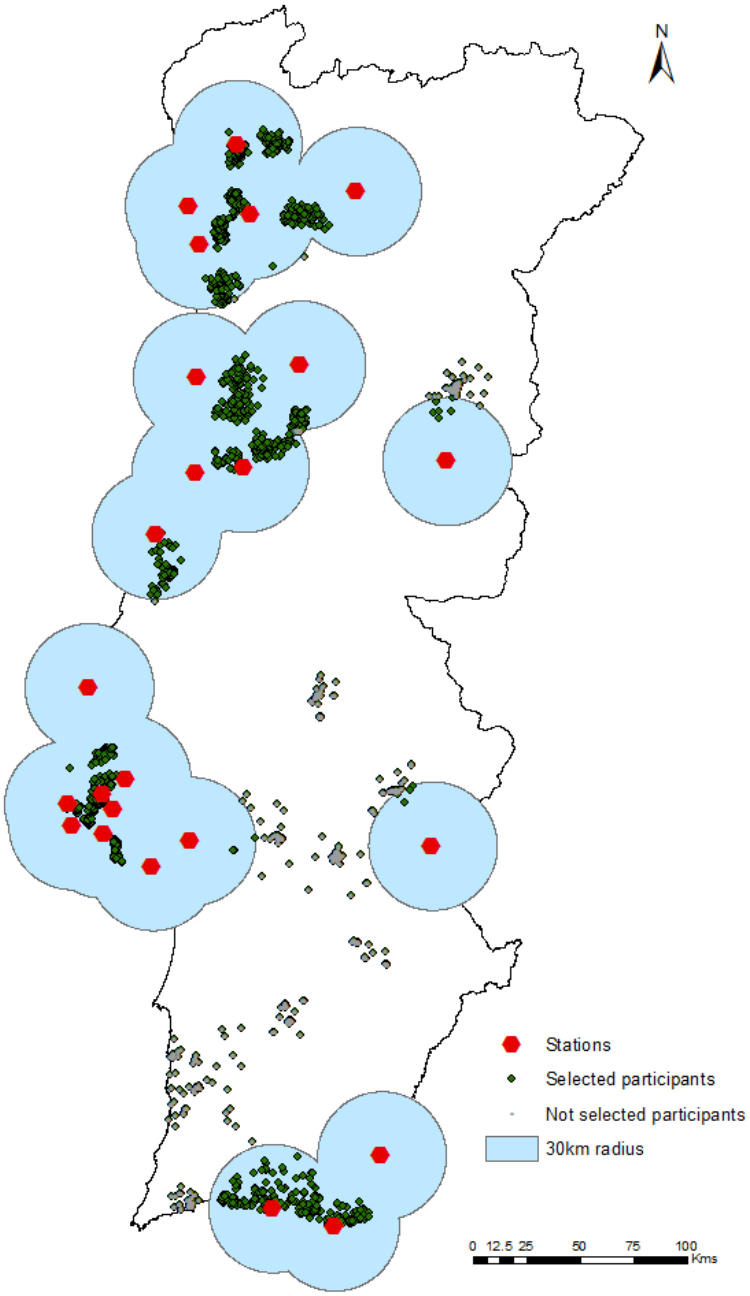

Figure 14 – Geographic distribution of the participants and the 24 background air quality monitoring stations with available PM_{10} data during the study period. Red points represent the air quality monitoring stations, green points represent the INSEF Portuguese mainland participants and blue circles represents the 30 km radius from each station. The grey points not covered by the blue circles are the excluded participants. (names of the air quality monitoring stations: Alverca, Arcos, Cerro, Douro Norte, Ervedeira, Fernando Pó, Fornelo do Monte, Frossos-Braga, Fundão, Ílhavo, Instituto Geofísico de Coimbra, Joaquim Magalhães, Laranjeiro, Loures-Centro, Lourinhã, Malpique, Mem Martins, Mindelo-Vila do Conde, Montemor-o-Velho, Olivais, Paços de Ferreira, Quinta do Marquês, Sobreiras-Lordelo do Ouro and Terena).

Daily average PM_{10} concentrations were calculated, in all INSEF fieldwork days, using the 24-hour observations values from each station. One-year average PM_{10} concentrations were estimated using the preceding 365-daily average PM_{10} concentrations values. For each individual the allocated 1-year average PM_{10} concentration was the weighted average of 1-year averages PM_{10} concentrations of all stations within 30 km from that participant's. This average was weighted by the inverse of the squared distance between the residence and the air quality monitoring stations.

For each individual, the allocated 1-year average temperatures were obtained using data from the National Oceanic and Atmospheric Administration database (www.ncdc.noaa.gov) and we assumed the 1-year average value of the closest temperature monitoring station as being representative of the individual exposure.

Statistical analysis

The statistical analysis was performed using the R program (version 3.6.3) (234). The significance level for all analysis was set at 5%. Sampling weights were used in data analysis. All estimates were weighted to account for different selection probabilities resulting from complex sample design and to match the population distribution in terms of geographic region, age group and sex, in 2015. T-test and the Wilcoxon test were used to access differences of quantitative variables according to their adherence to the normal distribution or not. Proportions were compared using Pearson's chi-squared test.

Conceptual Model

We constructed a directed acyclic graph (DAG) shown in Figure S1 based on literature review to select the minimal sufficient adjustment set of variables needed to account for confounding of the exposure-outcome relationship. This analysis was performed using the "DAGitty" R package (235).

Primary analysis

Regression coefficients of effect (β) of PM_{10} on TC, TG, LDL-C, HDL-C and HbA1C with the corresponding 95% confidence intervals (CI) were obtained by generalized linear regression models analyses for each 1-$\mu g/m^3$ increment of PM_{10}. Then, percent change with

corresponding 95% CIs were calculated by using the formula 100 × [exp (β)−1]. We used the svyglm function from the "survey" R package to run each Gaussian family model with a link function log (family= gaussian(link = "log")).

First, an unadjusted exposure-outcome model was fitted for each outcome. Then, a second model confounder-adjusted for sex (Male/Female), age group (50 years; ≥50 years), educational level (Low education/Medium education/High education), occupation (White-collar occupation/Blue-collar occupation), smoking (smoker/no smoker), excessive alcohol consumption (Yes/No), sedentary (Yes/No), unhealthy diet (Yes/No) and Individual allocated 1-year average temperature (continuous) was performed for each outcome.

To determine whether abdominal obesity is a potential mediator between PM_{10} exposure and parameters levels, we performed a mediation analysis according to Johnson and his colleagues (75). To determine if abdominal obesity interact with PM_{10} levels, an interaction term (PM_{10}*AO) was introduced in the final model of each outcome (177). If the *p-value* were statistically significant ($p<0.05$), an AO-stratified analysis was performed.

Sensitivity analysis

To assess the sensitivity of our analysis to the 30 km radius criteria, we also fit the models for each outcome considering only participants living within a 20 km radius of an air quality monitoring station with available PM_{10} measurements.

Additionally, to evaluate the sensitivity of our analysis regarding the exposure assessment method, we also fit the models considering the modelled PM_{10} concentrations obtained by the application of an air quality modelling system composed by the Weather Research & Forecasting (WRF, version 3.7.1) (165) and Comprehensive Air Quality Model with Extensions (CAMx, version 6.40) (166).The WRF-CAMx system has been extensively applied for Portugal and worldwide and it is described in more detail elsewhere (167–169). It returns surface hourly average concentrations of simulated species by grid cell (5×5 km^2) that were used to compute PM_{10} daily averages in 2014 and 2015. Participants living within a 30-km radius of at least one air quality monitoring station were linked to the correspondent grid cell and grid cell's PM_{10} daily averages were considered as being representative of the individual exposure. The preceding 365-daily average PM_{10} concentrations at the INSEF examination day were considered to obtain the individual allocated 1-year average PM_{10} concentrations.

To assess the sensitivity of our analysis to the choice of abdominal obesity assessed by the Waist-to-height ratio (WHtr) as the VAT proxy we also use Waist-to-hip ratio as a proxy of VAT and repeat the stratified analysis.

Finally, we also repeat the primary analysis after excluding the participants with diagnosed dyslipidaemia or taking lipid-lowering medication (in the TG, CT, HDL-C and LDL-C models) and diabetic participants or taking medication for diabetes treatment (in the HbA1C model).

Results

General characteristics of participants

Included and excluded participants were similar regarding the majority of the analysed characteristics. Differences between the two groups were only found regarding the percentage of smokers, prevalence of diagnosed diabetes and medicated diabetic participant's percentage (Table S5).

Among the 2390 participants in our study, 52.59% were females, 52.76% aged between 25 and 49 years old, 58.44% had low education level and 62.61% had a white-collar occupation. Most participants reported to be non-smokers (79.05%), non-excessive alcohol consumers (63.93%), to have a healthy diet (63.90%) and to be not sedentary (57.47%). The prevalence of diagnosed-dyslipidaemia and diagnosed-diabetes was 24.95% and 7.78% respectively, and 19.34% of the participants reported to take lipid-lowering medication and 7.09% reported to take diabetes medication. Individual allocated 1-year average temperature was 15.7ºC and individual allocated 1-year average PM_{10} concentration was 17.6µg/m^3 (**Table 16**).

The Individual allocated 1-year average PM_{10} concentration values ranged between 10.45µg/m^3 and 26.16µg/m^3 (median = 18.51µg/m^3, IQR=15.27-19.28µg/m^3). The mean concentrations of TC, TG, HDL-C, LDL-C were 193.65mg/dL, 147.35 mg/dL, 54.03 mg/dL and 128.18mg/dL, respectively. The mean percentage of HbA1C was 5.45%. When comparing participants with and without abdominal obesity, differences were found regarding age, level of education, occupation, lifestyles related variables, diagnosed dyslipidaemia and diabetes, medicated participants and outcome variables (**Table 16**).

Primary analysis

There was an association between PM_{10} and blood TG levels after adjustment for age, sex, educational level, occupation, variables, lifestyles and annual mean temperatures. Per each 1 µg/m^3 PM_{10} increment there was a 1.70% (95% CI: 0.11-3.32) increase in the TG values of the participants. No associations were found for the remaining blood lipid parameters and HbA1C (**Table 17**).

Table 16 – General characteristics of the study participants, according to their abdominal obesity condition.

Characteristics	Participants with AO (n=1831)	Participants without AO (n=536)	Total participants (n=2390)
Sex (n=2390) - %			
Males	48.80	42.84	47.41
Females	51.20	57.15	52.59
Age (n=2390) - %			
25-49 years old	**42.66**	**82.96**	**52.76**
50-74 years old	**57.34**	**17.04**	**47.24**
[a] Level of Education (n=2389) - %			
Low education	**66.37**	**35.07**	**58.44**
Medium education	**19.00**	**29.38**	**21.86**
High education	**14.63**	**35.55**	**19.70**
[b] Occupation (n=2203) - %			
White-collar occupation	**57.98**	**76.18**	**62.61**
Blue-collar occupation	**42.02**	**23.82**	**37.39**
Lifestyles variables - %			
[c] Smokers (n=2390)	**17.44**	**32.22**	**20.95**
[d] Excessive alcohol consumers (n=2388)	**40.21**	**24.49**	**36.07**
[e] Unhealthy Diet (n=2388)	**33.69**	**43.36**	**36.10**
[f] Sedentary (n=2375)	**46.01**	**39.15**	**44.53**
Diagnosed Dyslipidemia (n=2373) - %	**30.64**	**8.26**	**24.95**
Dyslipidemia medication (n=2390) - %	**24.33**	**4.45**	**19.33**
Diagnosed Diabetes (n=2384) - %	**9.99**	**0.10**	**7.78**
Diabetes medication (n=2390) - %	**9.12**	**0.10**	**7.09**
Individual allocated 1-year average temperature (n=2390) - ºC (mean±sd)	15.58±1.42	15.90±1.44	15.66±1.43
Individual allocated 1-year average PM_{10} (n=2390) - µg/m^3(mean±sd)	17.54±3.01	17.87±2.74	17.63±2.95
Outcome variables			
TC (n=2390) - mg/dL (mean±sd)	**196.45±37.50**	**184.25±34.98**	**193.65±37.74**
HDL-C (n=2390) - mg/dL (mean±sd)	**52.04±13.16**	**59.82±14.54**	**54.03±14.02**
LDL-C (n=2390) - mg/dL (mean±sd)	**131.73±34.14**	**116.94±31.70**	**128.18±34.39**
TG (n=2390) - mg/dL (mean±sd)	**162.63±102.61**	**99.87±56.25**	**147.35±97.36**
HbA1C (n=2357) - % (mean±sd)	**5.55±0.76**	**5.17±0.45**	**5.45±0.72**

[a] Low education: levels 0-2 of the ISCED 2011 (155); Medium education: levels 3-4 of the ISCED 2011 (155), High education: levels 5-8 of the ISCED 2011 (155). [b] White-collar occupation: Managers, Professionals, Technicians & Associate Professional, Clerical Support Workers and Services & Sales Workers (156); Blue–collar occupation: Skilled Agricultural Workers, Craft & Related trades Workers, Plant & Machine Operators and Elementary occupations (156). [c] Smokers include current daily and occasional smokers.[d]3 or more days/week of consumption of at least one of the following alcoholic beverages (wine, beer, brandy/bagasse, Port wine/Martini/liqueur, Whisky/Gin/Vodka). [e] No consumption of fruit and vegetables at least once a day. [f] Reading, watching TV or other sedentary activities declared as the best description of the leisure time activities during the last 12 months.

Results in bold are those with statistically significant difference between participants with versus without AO, according to the Pearson's chi-squared test. (*p*<0.05). Abbreviations: AO- Abdominal Obesity; TC - Total Cholesterol; HDL-C – High density Lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; TG – Triglycerides.; PM- Particulate matter; sd – standard deviation

We detected an interaction between PM_{10} and Abdominal Obesity in the TG analyses (Interaction term: 1.024, 95%CI:1.002-1.046, *p-value*: 0.034), and, consequently, we present a stratified analysis in **Table 17**. As we can see, the association between PM_{10} and TG levels were only found in participants with abdominal obesity. Per each 1 $\mu g/m^3$ PM_{10} increment, there was a 1.84% (95%CI: 0.02-3.69) increase in the TG values of the participants with abdominal obesity (**Table 17**).

We found that there was no association between the exposure (PM_{10}) and the mediator (Abdominal Obesity), a condition required to perform the mediation analysis. Consequently, the mediation analysis could not be done and we assumed that, based on our results, there was no evidence to suggest that abdominal obesity mediate the association between PM_{10} and blood lipid or glucose levels.

Sensitivity analysis

When we restricted our sample to the participants living within a 20 km radius of an air quality monitoring station with available PM_{10} values, similar results to those from the primary analysis were found (Table S6). Additionally, we found an association between PM_{10} and blood CT levels after adjustment for confounding in the all participant's analysis and also in the participants with abdominal obesity. Per each 1 $\mu g/m^3$ PM_{10} increment there was a 0.53% (95%CI:0.11-0.94) increase in the TG values of the participants with abdominal obesity (Table S7).

When we considered the individual allocated 1-year average PM_{10} concentrations obtained by the air quality modelling system (WRF-CAMx), no associations were found (Table S3). We obtained similar results to those from the primary analysis when we excluded participants with diagnosed dyslipidaemia or taking lipid-lowering medication (in the TG, CT, HDL-C and LDL-C modelling) and diabetic participants or taking medication for diabetes treatment (in the HbA1C modelling) (Table S8). The changing of the abdominal obesity measure from waist-to-height ratio to waist-to-hip ratio also did not modify the obtained results (Table S9).

Table 17 – Percent changes in TG, TC, HDL-C, LDL-C and HbA1C per 1 µg/m^3 increment of PM_{10} among all participants, participants with AO and participants without AO.

	% Change per 1 µg/m^3 of PM_{10} increment				
	TG	TC	HDL-C	LDL-C	HbA1C
All included participants (n=2390)					
Not adjusted model	0.19 (-1.08; 1.48)	0.09 (-0.52; 0.70)	0.12 (-0.43; 0.68)	-0.89 (-1.87; 0.11)	-0.09 (-0.37; 0.19)
***Adjusted model**	**1.70** **(0.11; 3.32)**	0.59 (-0.07; 1.24)	-0.20 (-0.74; 0.33)	0.47 (-0.20; 1.15)	-0.01 (-0.48; 0.47)
Participants with AO (n=1831)[a]					
Not adjusted model	0.92 (-0.60; 2.46)	1.75 (-0.45; 0.80)	-0.21 (-0.69; 0.27)	-0.64 (-1.66; 0.39)	-0.09 (-0.45; 0.28)
***Adjusted model**	**1.84** **(0.02; 3.69)**	0.62 (-0.02; 1.27)	-0.38 (-0.97; 0.21)	0.56 (-0.22; 1.35)	-0.03 (-0.62; 0.57)
Participants without AO (n=536)					
Not adjusted model	-1.76 (-3.60; 0.12)	0.03 (-0.71; 0.78)	0.64 (-0.37; 1.66)	**-1.32** **(-2.39; -0.24)**	0.20 (-0.19; 0.60)
***Adjusted model**	0.75 (-1.90; 3.47)	0.62 (-0.38; 1.63)	0.46 (-0.52; 1.45)	0.23 (-0.66; 1.12)	0.07 (-0.45; 0.60)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 1-year average temperature. [a] Participants without available data on waist or height measurements (n=23) and consequently without AO data were excluded from the stratified analysis. Abbreviations: AO- Abdominal Obesity; TC - Total Cholesterol; HDL-C – High density Lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; TG – Triglycerides.; PM- Particulate matter.

Discussion

Key findings

Our results showed that at least 1-year PM_{10} exposure interacts with abdominal obesity to increase non-fasting blood TG levels by about 2% per each 1 $\mu g/m^3$ PM_{10} increase, in individuals with abdominal obesity. For the remaining blood lipid and glucose parameters, no associations were found.

We were able to detect the effect modification of abdominal obesity (as a proxy of VAT) and, as hypothesized, the biological mechanism explaining our results could be the interaction between the pre-existing quantity of VAT with PM_{10} that will induce adipokines secretion and subsequent raised blood TG levels (232). On the other hand, we also hypothesized the potential mediation action of abdominal obesity but it was not supported by our results.

All sensitivity analysis strengthen our results except when considering modelled PM_{10} concentrations obtained by an air quality modelling system (WRF-CAMx).

Comparison with other published studies and interpretations

Our results are in concordance with a recent published meta-analysis that reported some epidemiologic evidence supporting the association between PM_{10} and increased blood TG levels (230). However, we detected this association only in participants with abdominal obesity, contrary to the previously reported studies (95,97,98). Moreover, our estimate was much higher but less precise than the one reported by Cai and his colleagues (98) that studied two large European cohorts exposed to a similar levels of PM_{10} concentrations (1.70% blood TG increase per 1 $\mu g/m^3$ PM_{10} increment (95%CI: 0.11-3.32) versus 1.9% blood TG increase per 2 $\mu g/m^3$ PM_{10} increment (95%CI: 1.5-2.4)). Precision differences are probably due to the huge sample size differences (n=2390 versus n=111547) and the estimate magnitude difference can be explained by the different set of adjustment variables.

Taking into account the biological mechanism hypothesized, it makes sense that TG levels are the most sensitive blood lipid parameters because additional adipokine secretion, namely TNF-alpha, will induce uncontrolled fatty acid lipolysis from VAT, leading to inflated fatty acids delivery to the liver, which will act as subtract to promote

mainly TG synthesis (232). Moreover the loss of insulin sensitivity within adipose tissue induced by additional adipokine secretion could be not directly reflected on the blood HbA1C levels, explaining why we did not found an effect of PM_{10} on this blood parameter (232).

Despite our study being the first to report the modification effect of the abdominal obesity regarding the PM_{10} effect on blood lipid levels, it had been recently reported regarding the air pollution effect on other health outcomes, namely blood pressure (236), kidney function (237) and lung function (238). Moreover, previous studies performed in China and United States of America, already reported stronger associations between long-term PM exposure and blood lipids levels in overweight or obese participants (95,239).

Recent evidence suggests a causal link between long-term particulate matter exposure and obesity condition (240,241). We hypothesized a mediation action of abdominal obesity that we were unable to confirm. One possible explanation for this result could be the short period of exposure considered that is probably insufficient to represent the long-term PM_{10} exposure able to contribute to the obesity condition.

Regarding the sensitivity analysis, it strengthen our results, suggesting that estimates from the primary analysis could be underestimated due to the exposure misclassification and outcome misclassification associated to the inclusion of participants with dyslipidaemia or taking lipid lowering medication. When considering modelled PM_{10} concentrations obtained by an air quality modelling system (WRF-CAMx) no statistically significant results were found. These modelled data were obtained using a numerical air quality modelling system (WRF-CAMx) with good performance already reported for Portugal domain applications (166,168). Nevertheless, modelled PM_{10} concentrations, when compared with measured PM_{10} concentrations, could be less representative of the real participants PM_{10} exposure because they are a mathematical representation of the reality with a certain degree of uncertainty of the input data, namely in the atmospheric emissions data.

Strengths and limitations

This is the first Portuguese study that links health data from a National Health Examination Survey and air quality monitoring data. We have done an extensive

bibliographic review and construct a conceptual model previous to the statistical analysis, in order to guarantee that the main confounding variables on the relationship between PM_{10} and blood lipid and glucose values were considered. Moreover, we consider multiple methodologies to obtain PM_{10} concentrations, by both measurements and numerical modelling (WRF-CAMx) approaches, as presented in the sensitivity analysis.

One of the limitations of our study is related to the exposure assessment method, namely the criteria used to select the participants taking into account the distance between their residence and the air quality monitoring stations. In fact, as the sensitivity analyses indicates, we may not be detecting PM_{10} effect on other parameters than TG due to an exposure misclassification. However, the number of air quality monitoring stations in the Portuguese mainland and their spatial distribution does not allow us to apply a more restrictive. Moreover, both measured and modelled PM_{10} concentrations are still problematic regarding their capacity to assess the real individual exposure that have unique activity-patterns and only with the use of new technology, with Global Position Systems (GPS) and mobile devices with low-cost air pollution sensors, we could better assess real individualized exposures and reduce exposure misclassification (56).

Another limitation of our study is related to the size of the particulate matter analysed. It is known that the smaller particles are those penetrating in deep into the lungs having the ability to be translocated into the bloodstream (6), potentially being the major contributors to the endocrine disruption at the adipose tissue level. However, we analysed only PM_{10} levels, the ones available to be analysed in our period of study, and we assumed that they are correlated with smaller particles concentrations, as previously reported (242).

Finally, despite we performed the adjustment for several potential confounders, there is still a possibility of residual confounding. Moreover, effect estimates were based on a single-pollutant model and interactions with gaseous pollutants were not evaluated. It is known that there are important interactions between the atmospheric pollutants, namely the potential additive effects of multiple pollutants and they should be considered in future studies (221,222). In the future, it would also be important to test the hypothetical mechanism in this study, in vitro or even in vivo assays, in order to verify whether the hypothesized biological mechanism could explain the PM effect on blood lipid and glucose levels.

Conclusions

To the best of our knowledge, this is the first study showing the modification action of abdominal obesity regarding the PM_{10} effect on a blood lipid parameter (TG). In comparison with other countries in the world, Portugal presents, in the time period under analysis (2014-2015), relatively low values of PM_{10} (range: 10.45-26.16 $\mu g/m^3$) not exceeding the annual limit value imposed by the European Air Quality Standards (annual mean: 40 $\mu g/m^3$). Even so, it was possible to detect the effect of exposure to PM_{10} on the values of one of the lipid parameters. This supports the statement that, as already some authors argue (243), there is no safe level of air pollutants, with effects on human health occurring even when air pollutants levels meeting the standards. On the other hand, our study also strongly suggests that the presence of comorbidities such as abdominal obesity, which affects the vast majority of the population, not only Portuguese but also worldwide, leaves the population more vulnerable to the effect of air pollutants.

Finally, our study strongly suggests that even at low levels of exposure, PM_{10} interacts with abdominal obesity to increase blood TG levels and our findings suggest that reducing both abdominal obesity prevalence and ambient air pollution below current standards would result in additional health benefits for the Portuguese population.

Funding

INSEF was developed as part of the Pre-defined project financed under the Public Health Initiatives Program. ‘‘Improvement of epidemiological health information to support public health decision and management in Portugal. Towards reduced inequalities, improved health and bilateral cooperation’’ with a 1.500.000€ Grant from Iceland, Liechtenstein and Norway from EEA Grants and the Portuguese Government. The present study was also funded by the Portuguese Foundation for Science and Technology (FCT) (PhD Scholarship Reference: SFRH/BD/129426/2017). J. Ferreira was funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia , I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. CESAM received financial support by FCT/MCTES (UIDP/50017/2020+UIDB/50017/2020), through national funds.

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest or competing interests.

Acknowledgments

The authors are grateful to all the professionals and participants involved in the INSEF. The authors are also grateful to the Portuguese Environmental Agency (APA) for making the air quality data available.

5.2. Association between Ambient particulate matter and blood count parameters

5.2.1. Article 3: Exposure to ambient particulate matter increases blood count parameters with potential to mediate a cardiovascular event: results from a population-based study in Portugal

Vânia Gaio [a,b], Rita Roquette [a,c], Alexandra Monteiro [d], Joana Ferreira [d], Sandra Rafael[d], Carlos Matias Dias [a,b], Baltazar Nunes [a,b]

[a] Department of Epidemiology, Instituto Nacional de Saúde Doutor Ricardo Jorge IP (INSA, IP), Lisboa, Portugal;

[b] NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisboa, Portugal;

[c] Nova IMS Information Management School, Universidade NOVA de Lisboa, Lisboa, Portugal.

[d] CESAM & Department of Environment and Planning, Universidade de Aveiro, Aveiro, Portugal

Abstract

Variations in blood count parameters are potential mechanisms involved in the occurrence of cardiovascular events caused by particulate matter (PM) exposure. This study aims to estimate the effect of PM_{10} exposure on blood count parameters with potential to mediate a cardiovascular event.

We used data from 2211 participants of the 1st Portuguese Health Examination Survey (INSEF, 2015) with available information on blood count parameters and living within a 30 km radius of at least one air quality monitoring station with available PM_{10} measurements. Generalized linear models were used to assess both short (3-days) and long-term effects (1-year) of PM_{10} exposure on blood count parameters.

Both short and long-term PM_{10} effects on blood count parameters were found, with males and females affected in a different way. In the short-term scenario, we found a 2.76%

(CI95%: 0.65-4.87) increase in white blood cells among females per each 10µg/m^3 PM_{10} increment. Additionally, there was a 2.96% (CI95%: 0.80-5.12) increase in red cell distribution width (RDW), per each 10µg/m^3 PM_{10} increment, among males, when considering the long-term scenario.

In conclusion, we detected some sex-differential associations regarding the short and long-term effect of PM_{10} exposure on blood count parameters with potential to mediate a cardiovascular event, namely on the RDW parameter, that were never been described. It is uncertain whether changes in blood count parameters due to PM_{10} exposure constitute an adverse health outcome or it reflects only a normal immunity response. However, due to its potential to trigger cardiovascular events, it is essential to reduce PM_{10} levels exposure to protect the population's cardiovascular health.

Keywords: Particulate matter, INSEF 2015, Blood counts, Leucocytes, Platelets, RDW

Introduction

Ambient particulate matter (PM) is now a well-established risk factor to develop cardiovascular diseases, with more than two thirds of the mortality attributed to PM arising from cardiovascular causes, namely cerebrovascular and ischaemic heart diseases (6,59). Multiple studies have linked PM exposure to both fatal and non-fatal cardiovascular events, but the pathophysiologic mechanisms linking the occurrence of these events with PM exposure are still an area of intensive research and scientific debate (81–83).

Blood count parameters, namely white blood cells (WBC), red blood cells (RBC) and platelets (PLAT) have a crucial role in atherosclerosis, one of the underlying conditions to trigger a cardiovascular event (140). Consequently, it is plausible to hypothesize that changes in blood count parameters caused by PM exposure are potential mechanisms mediating the effects of PM on cardiovascular health. In fact, evidence from animal studies shows that acute exposure to ambient PM seems to produce cytokines in the lung that will stimulate the bone-marrow to release leucocytes and platelets contributing, in conjunction with another proinflammatory and prothrombotic markers, to destabilization of atherosclerotic plaques, making them more vulnerable to rupture and thrombosis (86,150,151). Additionally, the cytokines produced in the lung due to the deposition of PM can potentially interact with erythropoietin in the

bone marrow, lowering the RBC cells production and suppress RBC maturation. This will lead to an immature RBC increase and, thus higher red cell distribution width (RDW) values (86,140), originating an anaemic condition with increased cardiovascular risk in proatherosclerotic situations (152).

Some epidemiologic studies already found associations between both short and long-term PM exposure and blood count parameters, namely WBC (143,144), RBC (121,145) or platelets count (146,147) but results remain controversial (148,149) and studies on this topic are still scarce. Consequently, the present study aims to estimate the short and long-term effects of PM_{10} exposure (PM_{10}: particles with an aerodynamic equivalent diameter ≤10μm) on blood count parameters potentially related to cardiovascular risk (WBC, White blood cells count; PLAT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; and RDW, red cell distribution width) in the adult Portuguese mainland population.

Material and Methods

Study population

This study was conducted using data from the 1st Portuguese National Health Examination Survey (INSEF), collected between February and December 2015 (153). INSEF was a cross-sectional population-based survey led by the National Health Institute Doutor Ricardo Jorge (INSA) in partnership with the five Regional Health Administrations from mainland Portugal, the two Regional Health Secretariats from the Autonomous Regions of Azores and Madeira and the Norwegian Institute of Public Health. This survey included a physical examination, a blood collection and an interview using a structured health questionnaire. The INSEF target population was non-institutionalized individuals aged between 25 and 74 years old, living in Portugal for more than 12 months and who were able to follow an interview in Portuguese. The INSEF sample (n=4911) was selected through complex multistage probabilistic design to be representative of the Portuguese population at national and regional levels (153). For this study, the analysis was restricted to the subsample of INSEF participants from mainland Portugal with informed consent to link data, available data on zip code address number, living within a 30-km radius of an air quality monitoring station with available PM_{10} concentration values and available data on blood count parameters (WBC, PLAT, RBC, HEMOG and RDW) (n=2211). The flow diagram of the participants' selection can be found in the Figure S1 (available in the Supplementary Material section).

The INSEF survey received approval from the Ethics Committee of the Portuguese National Health Institute Doutor Ricardo Jorge, the National Data Protection Authority (Authorization nº 9348/2010) and from the regional Ethics Committees. Moreover, Ethics Committee of the Portuguese National Health Institute Doutor Ricardo Jorge approved the study protocol of this particular research. All participants provided informed consent before data collection.

Health and sociodemographic data

Health data collection occurred at primary care level venues and was performed by trained health professionals. It included core physical measurements (blood pressure, height, weight and waist and hip circumference), a blood collection to perform blood tests, including blood count parameters (WBC, units/µL; PLAT, units/µL; RBC, units/µL; HEMOG, g/dL and RDW, %) and a computer assisted personal interview (CAPI), according to the European Health Examination Survey (EHES) procedures (154).

Blood count was performed in fresh non-fasting whole blood samples in the 12 regional collaborating laboratories that participated in the National Program for External Quality Assessment (PNAEQ) to assure comparability and reliability of blood tests results.Using the WHO criteria, anaemia was defined as the haemoglobin concentrations below 13 g/dL in men and below 12 g/dL in women (244) (WHO 2011)

Sociodemographic (age, sex, educational level and occupation) and lifestyles variables (smoking, excessive alcohol consumption, sedentary and unhealthy diet) were obtained by self-report through the interview.

Regarding educational level, we considered the highest level of education completed, grouped into 3 categories, according to the aggregate levels of education presented in International Labour Organization (ILOSTAT): low education (levels 0-2 of the ISCED 2011), medium education (levels 3-4 of the ISCED 2011) and high education (levels 5-8 of the ISCED 2011). (155,245). Occupation was grouped according to the International Standard Classification of Occupations (ISCO-08) (156) into 2 categories: white-collar occupation (Managers, Professionals, Technicians & Associate Professional, Clerical Support Workers and Services & Sales Workers) and blue–collar occupation (Skilled Agricultural Workers, Craft & Related trades Workers, Plant & Machine Operators and Elementary occupations).

Regarding the lifestyles related variables, smokers include current daily and occasional smokers, and excessive alcohol consumption was defined as reporting 3 or more days/week of consumption of at least one of the following alcoholic beverages: wine, beer, brandy/bagasse, Port wine/Martini/liqueur, Whisky/Gin/Vodka. Self-reported unhealthy diet was based on the question: ‘‘How often do you eat fruit/vegetables (excluding juice and potatoes)?’’ Individuals who reported eating fruit and vegetables less than once a day were considered to have an unhealthy diet. Self-reported sedentary was based on the question: ‘‘Which of these situations best describes your leisure time activities during the last 12 months?’’ Individuals who answered ‘‘Reading, watching TV and other sedentary activities’’ were considered to be sedentary.

Environmental exposure assessment

PM_{10} values were obtained from the QualAr database, available online at the Portuguese Environment Agency (APA) website (https://qualar.apambiente.pt/). APA provides hourly observation data for different atmospheric pollutants, measured by 68 air quality monitoring stations that are classified according to the type of environment (demographic area) they represent (rural, urban and suburban) and the influence in terms of dominant atmospheric emission sources (background, traffic and industrial). The PM_{10} hourly data collected from the air quality monitoring stations reported to the period between February 2014 and December 2015, to cover the previous year of exposure before the INSEF examination day to all participants (INSEF fieldwork occurred between February and December 2015).

We assumed the exposure window period of 1-year as being representative of the long-term PM_{10} exposure for all study participants as they reported to live in the same place (same zip code number) at least one year before the INSEF examination day. Moreover, taking into account the published scientific evidence suggesting that acute particulate matter effects on the occurrence of cardiovascular events are generally larger at very shorter periods of time (few days) (158), we also considered the exposure window period of the 3 previous days before the examination date in order to assess the short-term PM_{10} exposure effects.

To reduce the exposure misclassification, only participants living within a 30 km radius of at least one air quality monitoring station with available PM_{10} values collected during the study exposure window periods (1-year average and 24-hours average in the previous 3 days) were included. Moreover, only background stations with data collection

efficiency of at least 75% (with at least 75% of the hourly values regarding the 24-hours averages and at least 75% of the daily values regarding the 1-year average) were considered. The geographic distribution of the participants (zip code address number) and the 33 background air quality monitoring stations used to assess the individual allocated PM_{10} concentrations are shown in

Figure *15*.

Daily (24-hours) average PM_{10} concentrations were calculated, in all INSEF fieldwork days, using the hourly observation values from each station. One-year average PM_{10} concentrations were estimated for each station, in all INSEF fieldwork days, using the preceding 365-daily average PM_{10} concentrations values.

For each individual the allocated average PM_{10} concentrations (3-day average, 1-year average) was the weighted average of PM_{10} concentrations from all stations within 30 km from that participant's residence. This average was weighted by the inverse of the squared distance between the residence and the air quality monitoring stations. The location of the measurement stations and participant's residences (zip code number) were identified and managed by geographic information system (ArcGIS version 10.4) (246).

For each individual, the allocated ambient temperatures (3-day average, 1-year average) were obtained using data from the National Oceanic and Atmospheric Administration (NOAA) database (www.ncdc.noaa.gov). We assumed the value of the closest temperature monitoring station as being representative of the Individual allocated temperature exposure.

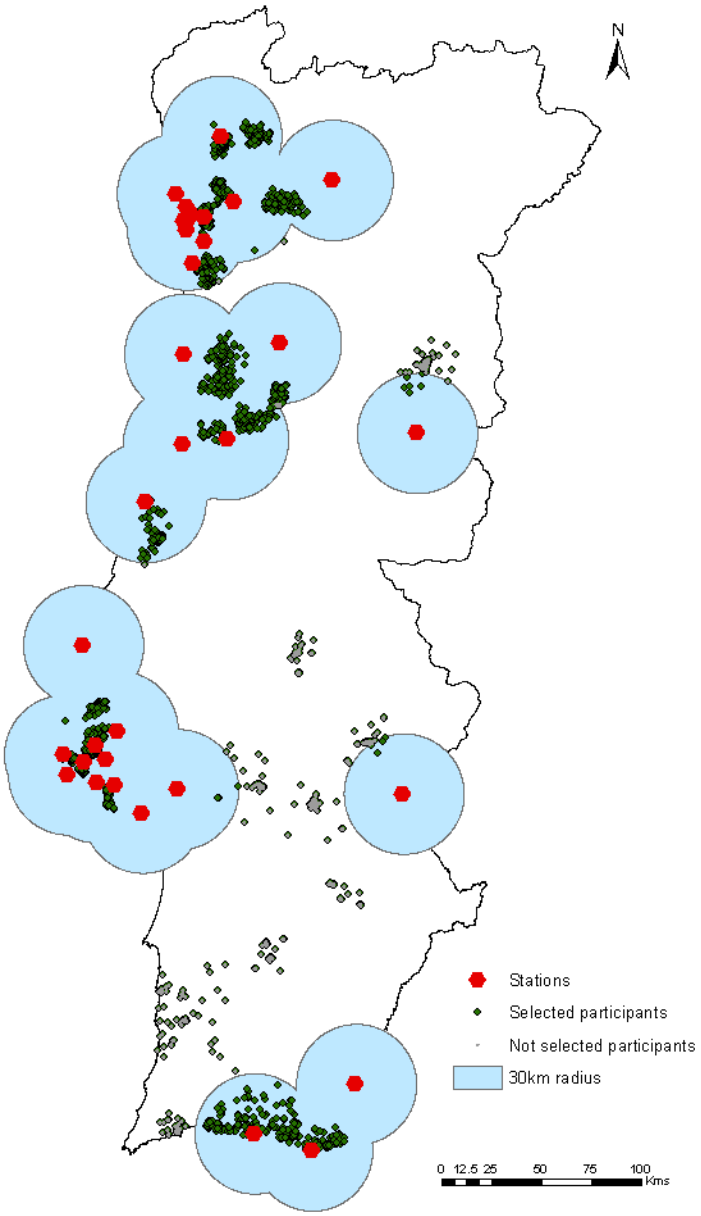

Figure 15 – Geographic distribution of the participants and the 33 background air quality monitoring stations with available PM_{10} data during the study period. Red points represent the air quality monitoring stations, green points represent the INSEF Portuguese mainland participants and blue circles represent the 30 km radius from each station. The grey points not covered by the blue circles are the excluded participants. (names of the air quality monitoring stations: Alverca, Anta-Espinho, Arcos, Avintes, Burgães-Santo Tirso, Cerro, Custóias-Matosinhos, Douro Norte, Ermesinde-Valongo, Ervedeira, Fernando Pó, Fidalguinhos, Fornelo do Monte, Frossos-Braga, Fundão, Ílhavo, Instituto Geofísico de Coimbra, Joaquim Magalhães, Laranjeiro, Leça do Balio-Matosinhos, Loures-Centro, Lourinhã, Malpique, Mem Martins, Mindelo-Vila do Conde,

Montemor-o-Velho, Olivais, Paços de Ferreira, Quinta do Marquês, Reboleira, Sobreiras-Lordelo do Ouro, Terena and VNTelha-Maia).

Statistical analysis

The statistical analysis was performed using the R program (version 3.6.3) (234). The significance level for all analysis was set at 5%. Sampling weights were used in data analysis. All estimates were weighted to account for different selection probabilities resulting from complex sample design and to match the population distribution in terms of geographic region, age group and sex, in 2015.

Regarding the general characteristic's description, the t-test and the Wilcoxon test were used to access differences of quantitative variables according to their adherence to the normal distribution or not. Proportions were compared using Pearson's chi-squared test.

We constructed a directed acyclic graph (DAG) shown in **Figure 16** based on literature review to select a minimal sufficient adjustment set of variables needed to account for confounding of the exposure-outcome relationship. This analysis was performed using the "DAGitty" R package (235). Accordingly, the minimal sufficient adjustment set of variables were age, sex, socioeconomic status (educational level and occupation), lifestyles (smoking, excessive alcohol consumption, unhealthy diet and sedentary) and ambient air temperature.

Primary analysis

Regression coefficients (β) regarding the effect of PM_{10} on WBC, PLAT, RBC, HEMOG and RDW with the corresponding 95% confidence intervals (CI) were obtained by generalized linear regression models analyses for each 10-$\mu g/m^3$ increment of PM_{10}. Then, percent change with corresponding 95% CIs were calculated by using the formula $100 \times [\exp(\beta*10)-1]$. We used the svyglm function from the "survey" R package to run each Gaussian family model with a link function log (family= gaussian(link = "log")).

First, an unadjusted exposure-outcome model was fitted for each outcome (WBC, PLAT, RBC, HEMOG and RDW) in both short and long-term exposure periods, considering respectively the 3-previous days average of PM_{10} concentrations and 1-previous year average of PM_{10} concentrations as exposure variables.

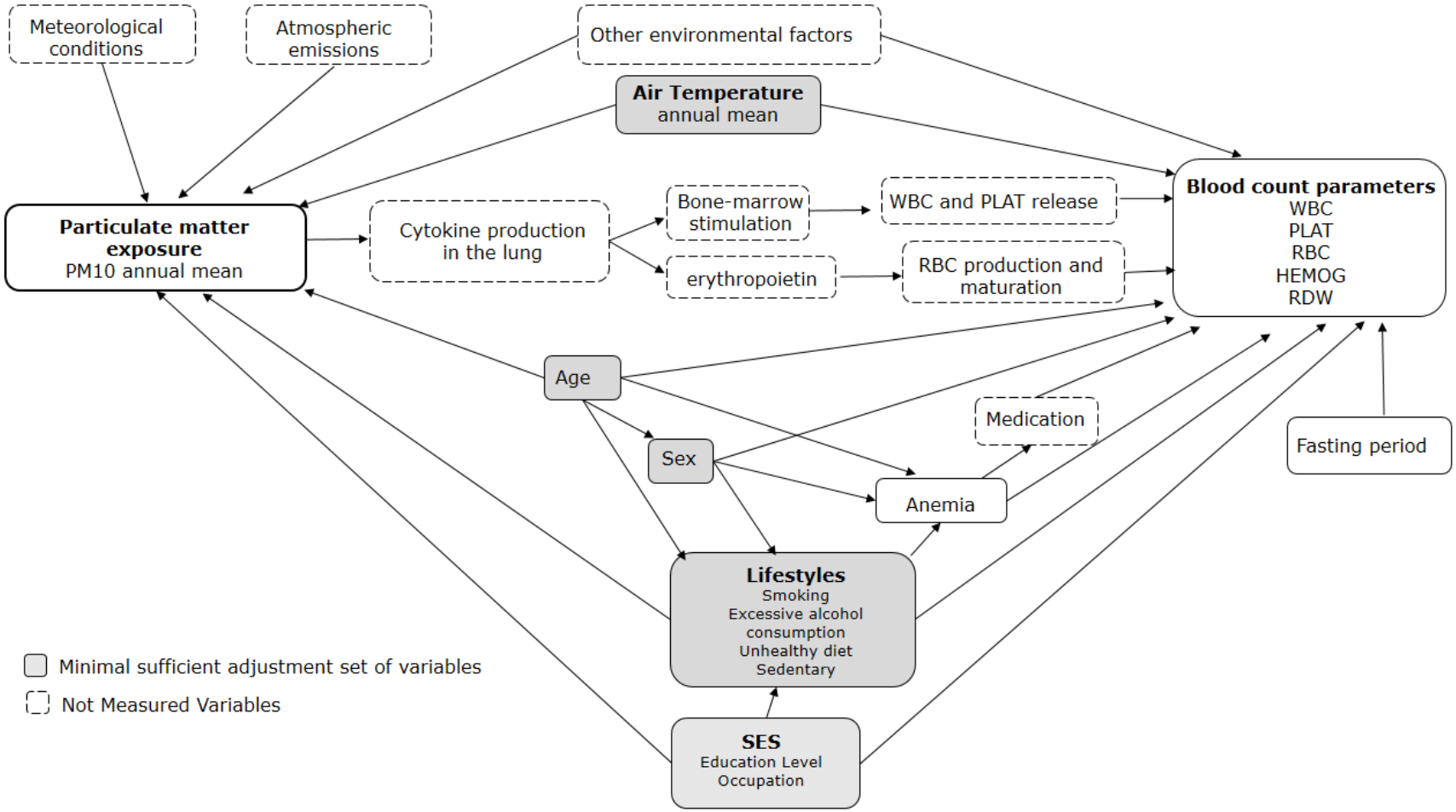

Figure 16 – A Directed Acyclic Graph (DAG) for the association between particulate matter exposure and blood count parameters. Abbreviations: WBC, White blood cells count; PLAT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; and RDW, red cell distribution width; PM, Particulate matter.

Then, a second model confounder-adjusted for sex (2 categories: Male/Female), age group (2 categories:< 50 years old; ≥50 years old), educational level (3 categories: Low education/Medium education/High education), occupation (2 categories: White-collar occupation/Blue –collar occupation), smoking (2 categories: smoker/no smoker), excessive alcohol consumption (2 categories: Yes/No), sedentary (2 categories: Yes/No), unhealthy diet (2 categories: Yes/No) and individual allocated temperature (continuous) was performed for each outcome in both short and long-term exposure periods. Additionally, in the models assessing the short-term effects of PM_{10} exposure, an adjustment for the long-term effect of PM_{10} was also performed by adding the variable 1-previous year average of PM_{10} concentrations.

Moreover, taking into account the published scientific evidence suggesting sex-related differences regarding the PM endocrine-disrupting effects on blood elements, due probably to different hormones levels such as estrogens involved in blood cells formation at bone-marrow level (147,247,248), we also fitted the same models in a sex-stratified analysis (males/females).

Sensitivity analysis

We assess the sensitivity of our analysis to the assumption that PM_{10} concentrations obtained from the air quality monitoring stations within a 30 km from the participant's residence were representative of their exposure. For that, we fit the models for each outcome and for each exposure window period considering only participants living within a 20 km radius of an air quality monitoring station with available PM_{10} measurements.

Additionally, to evaluate the sensitivity of our analysis regarding the exposure assessment method, we also fit the models considering the modelled PM_{10} concentrations obtained by the application of an air quality modelling system composed by the Weather Research & Forecasting (WRF, version 3.7.1) (165) and Comprehensive Air Quality Model with Extensions (CAMx, version 6.40) (166). The WRF-CAMx system has been extensively applied for Portugal and worldwide and it is described in more detail elsewhere (167–169). Briefly, in our study, CAMx was applied to the whole years of 2014 and 2015 following a downscaling approach, using two nested domains, the first domain covering the European region with 25×25 km^2 grid spacing and the second domain over Portugal with a dimension of 475 km by 625 km, gridded as 95 by 125 cells of 5×5 km^2 horizontal spatial resolution. To initialize and drive the WRF meteorological simulation

(165,166). ERA Interim reanalysis data was used, including three dimensional (3D) fields of wind, temperature, and relative humidity and 2D field of surface pressure, among others, from ECMWF (European Centre for Medium Range Weather Forecast) at 6 hours and 0.75 degrees temporal and spatial resolution, respectively. Other inputs comprise anthropogenic emissions of atmospheric pollutants and initial and boundary conditions for the CAMx European domain taken from the outputs (3D concentration fields of gaseous and particulate species) of the global chemical model MOZART (170) at every 6 hours. CAMx returns surface hourly average concentrations of simulated species by grid cell that were used to compute PM_{10} daily averages in 2014 and 2015. Participants living within a 30-km radius of at least one air quality monitoring station (zip code address number) were linked to the correspondent grid cell and PM_{10} daily averages of each grid cell were considered as being representative of the individual exposure. The preceding 365-daily average PM_{10} concentrations at the INSEF examination day were considered to obtain the individual allocated 1-year average PM_{10} concentrations. The preceding 24 hours average on the 3 days before the INSEF examination day were considered to obtain the individual allocated 3-days average PM_{10} concentrations.

Finally, we also repeat the primary analysis after excluding the participants with anaemia to confirm our results in the participants with normal haemoglobin levels.

Additionally, in the models assessing the short-term effects of PM_{10} exposure we also repeat the analysis considering the 2 and 5 previous days average PM_{10} concentrations instead of the 3 previous day´s average PM_{10} concentrations considered in the primary analysis.

Results

General characteristics of Participants

A comparison of the INSEF mainland participants included and excluded from our study is presented in Table S10 of the supplementary material. Included and excluded participants were similar regarding all of the analysed characteristics (Table S10 of the Supplementary Material).

General characteristics of the included participants, in both males and females, are presented in **Table 18**. Among the 2211 participants in our study, 53.4% aged between 25 and 49 years old, 58.2% had low education level and 63.0% had a white-collar occupation. Regarding the lifestyles variables, 21.1% of the participants were smokers,

36.1% were excessive alcohol consumers, 36.1% reported to had an unhealthy diet and 45.1% reported to have a sedentary lifestyle. The prevalence of anaemia was 5.2%, being higher in females (7.5%) when compared to males (2.7%). Sex differences were also found regarding level of education, occupation, percentage of smokers, excessive alcohol consumers and of those reporting to have an unhealthy diet (**Table 18**). Individual allocated 1-year average temperature was 15.9ºC and individual allocated 3-days average was 16.0 ºC.

The distribution of the individual allocated PM_{10} concentrations in the different analysed exposure windows is described in **Table 19** . The mean value of the individual allocated 1-year average PM_{10} concentration was 17.5 µg/m^3 (median = 18.4 µg/m^3, IQR=15.2-19.2 µg/m^3). The mean value of the individual allocated 3-days average PM_{10} concentration was 18.6 µg/m3 (median = 17.2 µg/m^3, IQR=12.0-24.3 µg/m^3) and there were no differences between females and males (Table 2). The mean values of RBC, HEMOG, RDW, WBC and PLAT were 4.7x106units/µL, 14.1 g/dL, 13.2%, 7.3 x 103 units/µL and 233 x 103 units/µL, respectively. Sex differences were found regarding RBC, HEMOG, RDW and PLAT values (**Table 19**).

Table 18 – General characteristics of the study participants, according to sex.

Characteristics	**Females (n=1195)**	**Males (n=1016)**	**Total participants (n=2211)**
Age (n=2211) - %			
25-49 years old	52.8	54.0	53.4
50-74 years old	47.2	46.0	46.6
[a] Level of Education (n=2210) - %			
Low education	**54.6**	**62.2**	**58.2**
Medium education	**21.7**	**21.5**	**21.6**
High education	**23.7**	**16.3**	**20.2**
[b] Occupation (n=2047) - %			
White-collar occupation	**69.6**	**56.1**	**63.0**
Blue-collar occupation	**30.4**	**43.9**	**37.0**
Lifestyles variables - %			
[c] Smokers (n=2211)	**16.2**	**26.5**	**21.1**
[d] Excessive alcohol consumers (n=2210)	**20.3**	**53.4**	**36.1**
[e] Unhealthy Diet (n=2210)	**27.0**	**46.1**	**36.1**
[f] Sedentary (n=2198)	41.7	48.1	45.1
Anaemia (n=2191)- %	**7.5**	**2.7**	**5.2**
Individual allocated Temperature (n=2211) - ºC (mean±sd)			
1-year average	15.9±4.1	16.0±4.0	15.9±4.0
3-days average	16.1±4.0	16.1±4.0	16.0±4.0

[a] Low education: levels 0-2 of the ISCED 2011; Medium education: levels 3-4 of the ISCED 2011, High education: levels 5-8 of the ISCED 2011. [b] White-collar occupation: Managers, Professionals, Technicians & Associate Professional, Clerical Support Workers and Services & Sales Workers; Blue–collar occupation: Skilled Agricultural Workers, Craft & Related trades Workers, Plant & Machine Operators and Elementary occupations. [c] Smokers include current daily and occasional smokers. [d] 3 or more days/week of consumption of at least one of the following alcoholic beverages (wine, beer, brandy/bagasse, Port wine/Martini/liqueur, Whisky/Gin/Vodka). [e] No consumption of fruit and vegetables at least once a day. [f] Reading, watching TV or other sedentary activities declared as the best description of the leisure time activities during the last 12 months. Results in bold are those with statistically significant difference between Females versus Males, according to the Pearson's chi-squared test. (p<0.05).

Table 19 – Distribution of the individual allocated PM_{10} values and blood count parameters, according to sex.

	Total		**Females**		**Males**	
	Mean±sd	Median(Q1-Q3)	Mean±sd	Median(Q1-Q3)	Mean±sd	Median(Q1-Q3)
Individual allocated PM_{10}						
1-year average	17.5±3.0	18.4 (15.2-19.2)	17.5±3.0	18.4 (15.2-19.2)	17.5±3.0	18.4 (15.2-19.2)
3-days average	18.6±8.3	17.2 (12.0-24.3)	18.6±8.4	17.2 (12.0-24.4)	18.6±8.3	17.2 (12.0-24.1)
Blood count parameters						
RBC (10^6units/µL)	**4.7±0.4**	**4.7 (4.4-5.0)**	**4.5±0.3**	**4.5 (4.2-4.7)**	**4.9±0.4**	**5.0 (4.7-5.2)**
HEMOG (g/dL)	**14.1±1.3**	**14.1 (13.2-15.1)**	**13.3±1.0**	**13.3 (12.7-13.9)**	**15.1±1.0**	**15.1 (14.4-15.7)**
RDW (%)	**13.2±0.9**	**13.1 (12.7-13.6)**	**13.3±1.1**	**13.1 (12.7-13.7)**	**13.2±0.8**	**13.1 (12.7-13.5)**
WBC (10^3 units/µL)	7.3±2.0	7.0 (5.9-8.4)	7.3±2.0	7.0 (5.9-8.5)	7.3±1.9	7.0 (6.0-8.3)
PLAT(10^3 units/µL)	**233±56**	**227 (195-266)**	**246±57**	**240 (207-281)**	**219±52**	**215 (184-249)**

Results in bold are those with statistically significant difference between Females versus Males, according to the Wilcoxon test (*p*<0.05). Abbreviations: PM, Particulate matter; sd, standard deviation; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width; WBC, White blood cells count; PLT, platelets count.

Short-term effects of PM on blood count parameters

Results concerning the association of short-term exposure to PM_{10} with blood count parameters are shown in **Figure 17**. There was an association between PM_{10} and WBC values (2.08% WBC increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.42%; 3.73%) after adjustment for age, sex, educational level, occupation, lifestyle variables and individual allocated 3-day average mean temperature, respectively. In the sex-stratified analysis, this association maintains only among females (2.76% WBC increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.65%; 4.87). The estimates and respective 95% confidence intervals presented in **Figure 17** are available in Table S11 of the supplementary material.

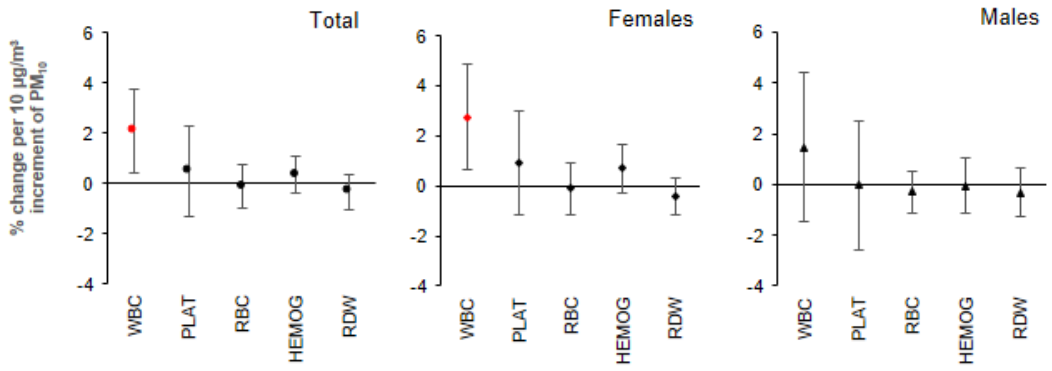

Figure 17 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the short-term exposure scenario (3 previous day - average PM_{10} concentrations). Estimates in red are those statistically significant ($p<0.05$). Abbreviations: PM, Particulate matter; WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width.

Regarding the sensitivity analysis, when we restricted our analysis to participants without anaemia (**Figure 18**), similar results were found. Per each 10 µg/m^3 PM_{10} increment, there was a 2.34% (95% CI: 0.69; 3.99) increase in the WBC values after confounding adjustment, when considering the total sample, and a 3.38% (95% CI: 1.15; 5.63) increase in the WBC values, when considering only females.

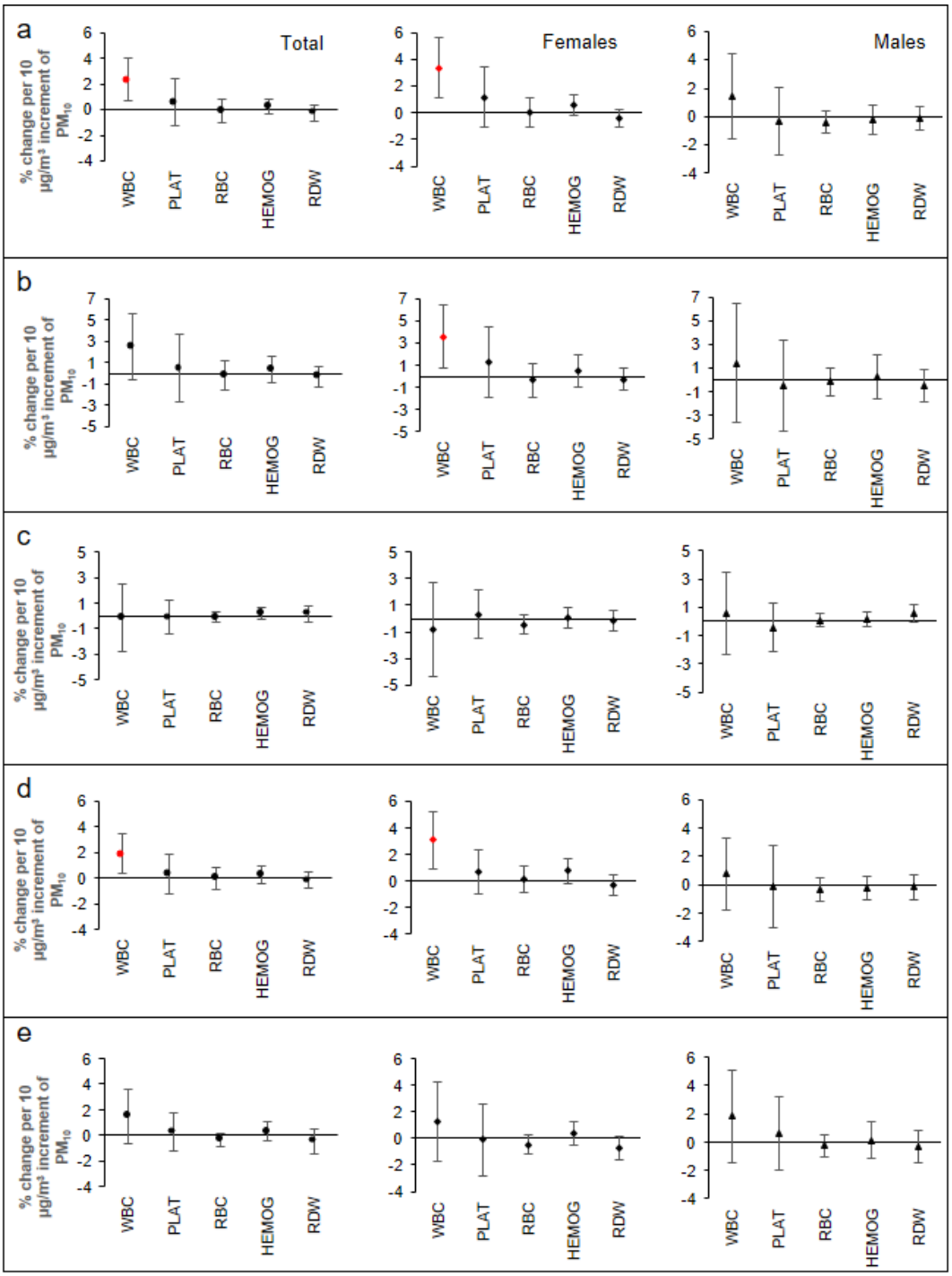

Figure 18 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the short-term exposure scenario (3-previous days - average PM_{10} concentrations) (a) after exclusion of participants with anaemia, (b) after restricting participants to those living within a 20km radius of at least one air quality monitoring station with available PM_{10} measurements, (c) considering the PM_{10} obtained by the air quality modelling system (WRF-CAMx), (d) considering the 2 previous days - average PM_{10} concentrations (e) considering the 5 previous days - average PM_{10} concentrations. Estimates in red are those statistically significant ($p<0.05$). Abbreviations: PM, Particulate matter; WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width.

When we restricted our sample to the participants living within a 20 km radius of an air quality monitoring station with available PM_{10} values, similar results were found when considering the total sample and also when considering only females. Per each 10 µg/m^3 PM_{10} increment, there was a 3.56% (95% CI: 0.72-6.48) increase in the WBC values.

When we considered a different exposure period of time (2 previous days instead of 3 previous days) similar results were found but only among females. Per each 10 µg/m^3 PM_{10} increment, there was a 3.07% (95% CI: 0.96-5.18) increase in the WBC values. On the other hand, when we considered the 5 previous days as the exposure period of time, no associations were found. Additionally, when we considered the individual allocated PM_{10} concentrations obtained by the air quality modelling system (WRF-CAMx), no associations were also found. The estimates and respective 95% confidence intervals presented in **Figure 18** are available in Tables S12 to S16 of the supplementary material.

Long-term effects of PM on blood count parameters

Results concerning the association of long-term exposure to PM_{10} with blood count parameters are shown in **Figure 19**. We found an association between individual allocated 1-year average PM_{10} concentrations and RDW (2.82% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.62%; 5.02%) and PLAT (3.31% PLAT increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.61%; 6.01%) after adjustment for confounding, when considering all participants.

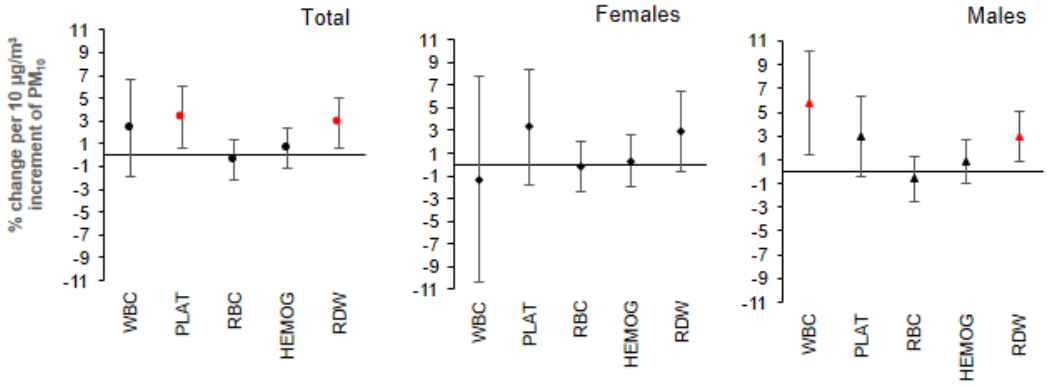

Figure 19 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the long-term exposure scenario (1-year average PM_{10} concentrations). Estimates in red are those statistically significant ($p<0.05$). Abbreviations: PM, Particulate matter; WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width

In the sex-stratified analysis, the association between PM_{10} and RDW was only present among males (2.96% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.80%; 5.12%). In this sex category, we additionally found an association between PM_{10} and WBC (5.78% WBC increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 1.46%; 10.13%). The estimates and respective 95% confidence intervals presented in **Figure 19** are available in Table S17 of the supplementary material.

Regarding the sensitivity analysis, when we restricted our analysis to participants without anaemia (**Figure 20**), similar results were found. There is an association between individual allocated 1-year average PM_{10} concentrations and RDW (2.99% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.81%; 5.18%) and PLAT (4.01% PLAT increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 1.26%; 6.76%) after adjustment for confounding, when considering all participants. In the sex-stratified results, both associations remains among males (2.82% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.69%; 4.97%; and 3.63% PLAT increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.44%; 6.82%) but not among females. Additionally, among males, there is an association between PM_{10} and WBC (5.67% WBC increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.87%; 10.49%).

When we restricted our sample to the participants living within a 20 km radius of an air quality monitoring station with available PM_{10} values, we only found an association between PM_{10} and RDW (3.08% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 1.63%; 4.53%). Through sex-stratified analysis, this association remains among both females (3.09% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.18%; 6.02%) and males (3.38% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 1.02%; 5.76%).

Similarly, when we considered the individual allocated PM_{10} concentrations obtained by the air quality modelling system (WRF-CAMx), we only found an association between PM_{10} and RDW (1.59% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.17%; 3.01%) that only persists among males in the stratified analysis (1.58% RDW increase per each 10 µg/m^3 PM_{10} increment, 95%CI: 0.10%; 3.06%). The estimates and respective 95% confidence intervals presented in **Figure 20** are available in Tables S18 to S20 of the supplementary material.

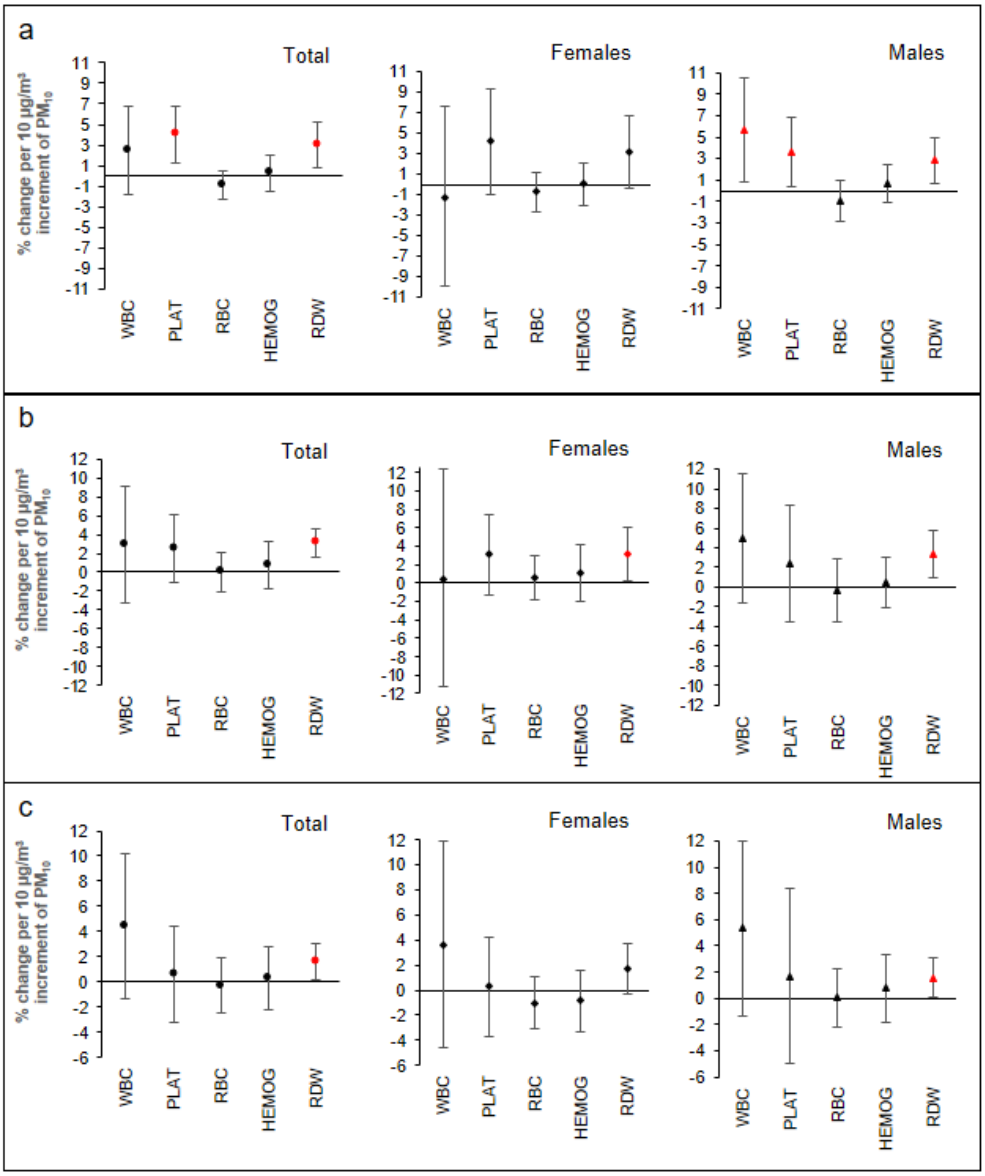

Figure 20 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the long-term exposure scenario (1-year average PM_{10} concentrations) (a) after exclusion of participants with anaemia, (b) after restricting participants to those living within a 20km radius of at least one air quality monitoring station with available PM_{10} measurements, (c) considering the PM_{10} obtained by the air quality modelling system (WRF-CAMx). Estimates in red are those statistically significant ($p<0.05$). Abbreviations: PM, Particulate matter; WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width.

Discussion

Key findings

Concerning the short-term effect of PM_{10} exposure on blood count parameters with potential to mediate a cardiovascular event, our study showed an association between PM_{10} concentrations and WBC, mainly among females. This result was supported by the sensitivity analysis when considering only participants without anaemia, when considering a more restrictive criteria to the exposure assessment (participants living within a 20 km radius of an air quality monitoring station with available PM_{10} values) and when considering the 2-previous days instead of 3-previous days as the exposure period of time.

Regarding the long-term effect of PM_{10} exposure on blood count parameters, we found an association between PM_{10} concentrations and RDW values, but mainly among males, and this result was supported to all the performed sensitivity analysis. The associations between PM_{10} and WBC and PLAT, in the long-term scenario, were found only among males and they disappear on the sensitivity approach, when considering a more restrictive criteria to the exposure assessment (participants living within a 20 km radius of an air quality monitoring station with available PM_{10} values) and when considering the PM_{10} obtained by the air quality modelling system (WRF-CAMx).

Comparison with other published studies and interpretations

Despite the few number of epidemiologic studies reporting associations between both short and long-term PM_{10} exposure and blood count parameters, our results are in agreement with some of them. Lee and her colleagues (144), in 2018, found that increased WBC counts were associated with the long-term (1-year average) PM_{10} exposure (0.76% WBC increase per each 4.4 $\mu g/m^3$ PM_{10} increment, 95%CI: 0.42%; 1.10%) but not with the short-term (3-day average) PM_{10} exposure. In our study, we also found an association between the long-term (1-year average) PM_{10} exposure and WBC values, but only in males (5.78% WBC increase per each 10 $\mu g/m^3$ PM_{10} increment (95%CI: 1.46%; 10.13%). Additionally, we also found that increased WBC counts were significantly associated with the short-term exposure (3-day average) to PM_{10} but only among females (2.76% WBC increase per each 10 $\mu g/m^3$ PM_{10} increment, 95%CI: 0.65%; 4.87%). However, some methodological differences between the two studies

could also explain the different obtained results (A hospital-based cohort study in South Korea versus a Health Examination Survey-based study in Portugal).

Taking into account the biological mechanism hypothesized to be the most contributor to the PM_{10} effect on WBC, it makes sense that the PM_{10} effects occurs soon in a short-term exposure period. Due to the PM_{10} deposition on lung, cytokines will be produced and will stimulate the bone marrow to produce and release WBC that have a crucial role on the inflammatory process (86). In fact, experimental studies shows that after an acute exposure to PM_{10} levels, the WBC elevation is a rapid mechanism, being detectable 24-hours after exposure (249,250). However, this process will depend on the dose to which individuals are exposed and, in the Portuguese context, it is expected that the most frequent exposure will be less acute and possibly more prolonged over time (242). Consequently, the PM_{10} exposure effect on the real context of the population will be different and comparison with experimental studies should be done with caution. Additionally, there are already some studies that support the importance of the long-term exposure effect of PM_{10} in blood parameters (146,249,250) and other biological mechanisms must be considered in addition to the one described in our study.

Regarding our findings on the RDW parameter, we found an association between long-term exposure to PM_{10} and this blood parameter, mainly among males, which is well supported by the sensitivity analysis. To the best of our knowledge, this is the first study describing this association, although being biologically expected because cytokines produced in the lung due to the deposition of PM can potentially interact with erythropoietin in the bone marrow, suppressing RBC maturation and, thus, increase immature RBC which is reflected in the RDW parameter values (86,140). RDW has been identified as an independent prognostic biomarker of multiple cardiovascular diseases (141,142,251), therefore we consider this result to be of special relevance in particular to explain the effect of PM_{10} in triggering cardiovascular events.

Another very interesting result of our study was the sex-differences regarding not only to the type of blood element affected by the PM_{10} exposure but also regarding the temporal pattern of the effects. Actually, our results suggest that females are the most affected in the short-term exposure scenario, being WBC the most affected blood parameters. On the other hand, in the long-term scenario, males seem to be the most affected, mainly at the RDW parameter. A recent study (252) reported that PM_{10} have a significant effect on human eosinophils, a WBC subtype, for both women and men, but with different temporal patterns, with women showing a lag of 0–5 days and men showing a lag of 20–28 days.

Consequently, we must consider the hypothesis that, among males, the PM_{10} short-term effect on WBC, that we were not able to detect, occurs in a later lag day, which was not the target of our study. These results suggest a sex-differential response to PM_{10} exposure whose underlying biological mechanism, to the best of our knowledge, is unknown. However, previous studies already detected some of these sex-related differences and authors argue that it was possibly due to the effects of different hormones levels, such as estrogens (147,247,253). Moreover, the endocrine-disrupting potential of the PM might also interfere with hormone signalling with expected different effect on males and females. (147,248).

Concerning our results on sensitivity analysis, when we restricted our analysis to participants without anaemia, results were confirmed and, in some cases, estimates were more robust after this restriction. When considering a more restrictive criteria to the exposure assessment (participants living within a 20 km radius of an air quality monitoring station with available PM_{10}values), results on the association between short-term PM_{10} exposure and WBC was reinforced but only among females. It also supports the results on the association between long-term PM_{10} exposure and RDW in both sexes. This result, suggest us that exposure misclassification could be present and could be the reason for not detecting associations between PM_{10} and the RBC or even HEMOG, in our principal analysis, when considering all participants living within a 30 km radius of an air quality monitoring station. Consequently, we think that if we restricted more this distance criteria, assessing the PM_{10} exposure in a more personalized way, which was not possible due to the huge sample reduction, associations will be probably found regarding the remaining blood count parameters.

When considering the modelled PM_{10} concentrations obtained by the air quality modelling system (WRF-CAMx), in the sensitivity analysis, only results on the association between long-term PM_{10} exposure and RDW, among males, were supported. One possible explanation for this result is that modelled PM_{10} concentrations, when compared with measured PM_{10} concentrations, could be less representative of the real participants PM_{10} exposure because they are a mathematical representation of the reality with a certain degree of uncertainty of the input data, namely in the atmospheric emissions data. Consequently, it is expected that modelled PM_{10} concentrations are associated with a higher degree of individual exposure misclassification (and not representative of high spatial detail) and only the strong association with the RDW was detected when considering this PM_{10} exposure assessment methodology.

Strengths and limitations

In the present study we detect multiple associations regarding the short and long-term effect of PM_{10} exposure on blood count parameters with potential to mediate a cardiovascular event and also sex-differences were reported regarding these associations, which have not been described/identified before, to the best of our knowledge. We were careful to perform an extensive bibliographic review and construct a conceptual model before the statistical analysis, in order to guarantee that the main confounding variables on the relationship between PM_{10} and blood count parameters were considered. Moreover, due to the diversity observed regarding the air pollution exposure assessment methodology (230), we tried to consider multiple methodologies to obtain PM_{10} concentrations, by using both measurements and numerical modelling (WRF-CAMx) approaches, as presented in the sensitivity analysis.

One of the main limitations of our study is related to the exposure assessment method, due the high distance considered between participant's residence and the air quality monitoring stations (30 km) in the inclusion criteria. In fact, as the sensitivity analysis indicates, exposure misclassification could be present in our study. However, the number and the spatial distribution of air quality monitoring stations in the Portuguese mainland does not allow us to apply a more restrictive distance due to a substantial sample reduction that could compromise the power of the estimates. Even with a more restrictive distance, the air quality data available (both monitoring and modelled-based PM_{10} concentrations) are limited to assess the real individual exposure. A better assessment of this exposure could be possible through the use of new technology, with Global Position Systems (GPS) and mobile devices with low-cost air pollution sensors (56) that would capture the unique activity-patterns of the real individual exposure.

Finally, even with the adjustment for several potential confounders, there is still a possibility of the observed estimates to have been affected by residual confounding due to other unmeasured confounders. Moreover, effect estimates presented in this study were based on a single-pollutant model, due the scarce available air quality monitoring data for the remaining air pollutants. However, it is known that there are important interactions between the atmospheric pollutants, namely the potential additive effects of multiple pollutants and they should be considered in future studies (221,222).

Conclusions

In the present study we detected some associations regarding the short and long-term effect of PM_{10} exposure on blood count parameters with potential to mediate a cardiovascular event and also we report sex-differences regarding these associations that, to the best of our knowledge, were never been described. It is uncertain whether changes in blood count parameters due to PM_{10} exposure constitute an adverse health outcome or it reflect only a normal immunity response in healthy individuals. However, due to its potential to trigger cardiovascular events, more studies are essential to better understand the effects of PM_{10} on these parameters.

Finally, even at relatively low-levels of PM_{10} concentrations, in Portugal, it was possible to detect the PM_{10} exposure effect on blood count parameters with potential to mediate a cardiovascular event, suggesting that there is no safe level of air pollutants. Our findings suggest that reducing PM_{10} levels would result in additional benefits concerning the cardiovascular health of the population.

Acknowledgments

The authors are grateful to all the professionals and participants involved in the INSEF. The authors are also grateful to the Portuguese Environmental Agency (APA) for making the air quality data available.

Funding

INSEF was developed as part of the Pre-defined project financed under the Public Health Initiatives Program. ‘‘Improvement of epidemiological health information to support public health decision and management in Portugal. Towards reduced inequalities, improved health and bilateral cooperation’’ with a 1.500.000€ Grant from Iceland, Liechtenstein and Norway from EEA Grants and the Portuguese Government. The present study was also funded by the Portuguese Foundation for Science and Technology (FCT) (PhD Scholarship Reference: SFRH/BD/129426/2017). J. Ferreira is funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia , I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. Thanks are also due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020), through national funds.

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest or competing interests.

Acknowledgments

The authors are grateful to all the professionals and participants involved in the INSEF. The authors are also grateful to the Portuguese Environmental Agency (APA) for making the air quality data available.

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest or competing interests.

5.3. Association between Ambient particulate matter and blood pressure

5.3.1. Article 4: Investigating the association between ambient particulate matter (PM_{10}) exposure and blood pressure values: results from the link between a Health Examination Survey and air quality data

Vânia Gaio [*a,b], Rita Roquette [a,c], Alexandra Monteiro [d], Joana Ferreira [d], Carlos Matias Dias [a,b], Baltazar Nunes [a,b]

[a] Department of Epidemiology, Instituto Nacional de Saúde Doutor Ricardo Jorge IP (INSA, IP), Lisboa, Portugal;

[b] NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisboa, Portugal;

[c] Nova IMS Information Management School, Universidade NOVA de Lisboa, Lisboa, Portugal.

[d] CESAM & Department of Environment and Planning, Universidade de Aveiro, Aveiro, Portugal

Abstract

Introduction and objectives: High blood pressure (BP) remains a major modifiable cardiovascular (CV) risk factor. Several epidemiologic studies have been performed to assess the association between air pollution exposure and this CV risk factor but results remain inconsistent. This study aims to estimate the effect of short-term PM_{10} exposure (3-day average concentration) on diastolic (DBP) and systolic (SBP) blood pressure values of the resident mainland Portuguese population.

Methods: Our study was based on available DBP and SBP data from 2272 participants of the first Portuguese Health Examination Survey (INSEF, 2015) living within a 30km radius of at least one air quality monitoring station with available PM_{10} measurements. We used data from the air quality-monitoring network of the Portuguese Environment

Agency to obtain the individual allocated PM_{10} concentrations. Generalized linear models were used to assess the effect of PM_{10} exposure on DBP and SBP values.

Results: No statistically significant association was found between PM_{10} exposure and both DBP and SBP values (0.42% DBP change per 10 µg/m^3 of PM_{10} increment (95%CI:-0.85; 1.70) and 0.47% SBP change per 10 µg/m^3 of PM_{10} increment (95%CI:-0.86; 1.79)). Results remain unchanged after restricting the analysis to hypertensive or obese participants or changing the PM_{10} assessment methodology.

Conclusions: Taking into account the PM_{10} levels observed in 2015, our results suggests that exposure to PM_{10} concentrations have a small or no effect on the blood pressure values. Other air pollutants and mixtures of pollutants that were not included in our study should considered in future studies.

Key-words: Particulate matter; blood pressure; INSEF 2015; Air quality data

Introduction

High blood pressure (BP) or hypertension remains a major modifiable cardiovascular (CV) risk factor, being responsible for at least 45% of all deaths due to heart disease and 51% of deaths due to stroke (126,127). Worldwide, it is estimated that around 31% of adults aged 25 years and over had hypertension, defined as systolic BP (SBP) ≥140 mmHg and/or diastolic BP (DBP) ≥90 mmHg (128). In Portugal, according to the Portuguese Health Examination Survey (INSEF), the overall prevalence of hypertension, in the population aged 25 to 74 years of age, was 36.0%, in 2015, defined as having a SBP ≥140 mmHg and, or, a DBP ≥90 mmHg and, or, taking antihypertensive medication (129).

The causes of high blood pressure are complex and are related to genetic, behavioural and environmental factors, including air pollution exposure (254). Over the last decades, several epidemiological studies assessed the association between air pollution exposure, including particulate matter (PM), and BP values. Evidence from a recent meta-analysis which pooled studies published before May 2017, supports the association between PM_{10} and a DBP elevation. The authors of this meta-analysis found a DBP elevation of 0.86 mmHg, 95%CI (0.37; 1.35) and 0.15 mmHg, 95%CI (0.01-0.29)

per each 10 µg/m^3 increase of PM_{10} in long (≥30 days) and short-term (<30 days) periods of exposure, respectively (17). The compiled evidence in this systematic review failed to support the association between PM_{10} and SBP values.

Direct PM translocation into de blood, oxidative stress, inflammatory mediator's production and neuroendocrine activation are the potential biological mechanisms responsible for the pathophysiological mechanisms through which PM exposure can raise blood pressure, including vascular dysfunction and aortic stiffness (58), sodium retention at the kidney level (135) and chronic kidney disease (136). According to Newman and his colleagues, the first three pathophysiological responses associated with BP elevation will occur in a few hour to days after exposure and are reversible whereas the last physiological response will occur mainly in a long-term exposure context and will be irreversible (137).

To the best of our knowledge, in Portugal, the association between PM exposure and blood pressure values has never been published before. Consequently, our study aims to assess/investigate the association between individually allocated environmental PM_{10} concentrations and individual level systolic (SBP) and (DBP) diastolic blood pressure, considering a short period of time (previous 3 days before blood pressure measurements) as the window of exposure, in the mainland resident Portuguese population aged between 25 and 74 years old, in 2015.

Methods

Study population

This study used data from the first Portuguese National Health Examination Survey (INSEF), collected between February and December 2015. This survey was described in more detail by Nunes and his colleagues (153). The present analysis was restricted to the subsample of INSEF participants from mainland Portugal (n=3467) who consented to have their data linked, provided a residence zip code number, were living within a 30-km radius of an air quality monitoring station with available PM_{10} concentration values and had blood pressure values measured in INSEF (n=2272). The INSEF survey received approval from the Ethics Committee of the Portuguese National Health Institute Doutor Ricardo Jorge, the National Data Protection Authority (Authorization nº 9348/2010) and from the regional Ethics Committees.

Health related data

Health data collection in INSEF was performed by trained health professionals, according to the European Health Examination Survey (EHES) procedures (154). Blood pressure values were measured according to the previous published study of Rodrigues and colleagues (129).

Sociodemographic (age, sex, educational level and occupation), lifestyle (smoking, excessive alcohol consumption, sedentary and unhealthy diet) and health status variables (diagnosed-dyslipidaemia, diagnosed diabetes, lipid-lowering medication usage and diabetes medication usage) were obtained by self-report through the interview. Smoker's definition includes current daily and occasional smokers and excessive alcohol consumption was defined as reporting three or more days/week of consumption of at least one of the following alcoholic beverages: wine, beer, brandy/spirits, Port wine/Martini/liqueur, Whisky/Gin/Vodka. Unhealthy diet was defined as no consumption of fruit and vegetables at least once a day and sedentary was defined as reporting reading, watching television or other sedentary activities as the best description of leisure time activities during the last 12 months.

Environmental exposure assessment

Hourly validated PM_{10} values were obtained from the QualAr database, available online at the Portuguese Environment Agency (APA) website (https://qualar.apambiente.pt/) and the individual allocated PM_{10} concentrations were obtained as previously reported by Gaio and colleagues (255). Briefly, for each individual the allocated 3-day average PM_{10} concentration was the weighted average of 3-day averaged PM_{10} concentrations of all stations within a 30 km radius from each participant's residence. This average was weighted by the inverse of the squared distance between the residence and the air quality monitoring stations.

The individually-allocated 3-day average temperatures were also obtained using data from the National Oceanic and Atmospheric Administration database (www.ncdc.noaa.gov) and we assumed the 3-day average value of the closest temperature monitoring station as being representative of the individual exposure.

Statistical analysis

The statistical analysis was performed using the R statistical package version 3.6.3 (234). All estimates were weighted to account for different selection probabilities resulting from the complex sample design of INSEF 2015 and to match the geographic region, age group and sex population distribution of 2015. T-test and the Wilcoxon test were used to access differences of quantitative variables according to their adherence to the normal distribution or not. Proportions were compared using Pearson's chi-squared test. The significance level for all analysis was set at 5%.

Regression coefficients of effect (β) of PM_{10} on DBP and SBP with the corresponding 95% confidence intervals (CI) were obtained by generalized linear regression models analyses for each 1 $\mu g/m^3$ increment of PM_{10}. We used the svyglm function from the "survey" R package to run each Gaussian family model with a link log function (family= gaussian (link = "log")).Then, percent change per each 10 $\mu g/m^3$ increment of PM_{10} with corresponding 95% CIs were calculated by using the formula $100 \times [\exp(\beta) - 1]*10$. Additionally, to obtain estimates comparable with previous studies, we also run the models using the (family= gaussian (link = "identity")) to obtain results in mmHg increase per each 10 $\mu g/m^3$ increment of PM_{10} and these results are presented as supplementary data.

First, an unadjusted exposure-outcome model was fitted for each outcome. Then, a second model confounder-adjusted for sex (Male/Female), age group (25-49 years; 50-74 years), educational level (Low education/Medium education/High education), occupation (White-collar occupation/Blue–collar occupation), smoking (smoker/no smoker), excessive alcohol consumption (Yes/No), sedentary (Yes/No), unhealthy diet (Yes/No) and individual allocated 3-day average temperature (continuous) was performed for each outcome. Additionally, as the objective was to assess only the short-term effects of PM_{10} exposure, an adjustment for the long-term effect of PM_{10} was also included by adding the variable 1-previous year average of PM_{10} concentrations.

To assess the sensitivity of our analysis to the 30 km radius criteria, we also fit the models considering only participants living within a 20 km radius of an air quality monitoring station with available PM_{10} measurements. To evaluate the sensitivity of our analysis regarding the exposure assessment method, we also fit the models considering

the modelled PM_{10} concentrations obtained by the application of a numerical air quality model - the WRF-CAMx modelling system, that has been extensively applied over Portugal region with satisfactory skills, as previously described (255).

Finally, we also repeat the analysis after excluding the participants taking anti-hypertensive medication and also when considering only more vulnerable groups, namely restricting the analysis to the hypertensive participants (defined as measured SBP ≥140 mmHg or measured DBP ≥90 mmHg or self-report of taking antihypertensive medication) or participants with abdominal obesity (assessed by using the formula: waist to height ratio >0.5).

Results

Participants 'characteristics and PM_{10} values description

Included and excluded participants were similar regarding all the analysed general characteristics (Table S21). Among the 2272 participants in our study, 52.8% were females, 53.0% aged between 25 and 49 years old, 57.8% had low education level and 63.6% had a white-collar occupation. Most participants reported to be non-smokers (78.5%), non-excessive alcohol consumers (64.2%), to have a healthy diet (73.6%) and to be not sedentary (55.0%). The prevalence of hypertension was 36.4% and 25.1% of the participants reported to take antihypertensive medication. The mean values of DBP and SBP were 74.4 mmHg and 125.1 mmHg, respectively and the Individual allocated 3-day average temperature was 16.1 ºC. Differences between males and females were found regarding level of education, occupation, smoking, alcohol consumption, unhealthy diet, hypertension prevalence and blood pressure values (**Table 20**).

The individual allocated 3-day average PM_{10} concentration values ranged between 3.31µg/m^3 and 64.35µg/m^3 (median = 17.19µg/m^3, IQR=12.40-24.27µg/m^3) and differences between males and females were not found (**Figure 21**). The distribution of the 3-day average PM_{10} concentration values are above the daily limit value given by the EU Ambient Air Quality Directives (not to be exceed on more than 35/year) and also by the World Health Organization air quality guidelines (not to be exceed more than 3 days/year).

Association between PM_{10} and DBP and SBP values

There was no association between individual allocated 3-day average PM_{10} concentration and DBP or SBP values (0.42% DBP increase per each 10 µg/m^3 PM_{10} increment, 95%CI: -0.85%; 1.70% and 0.47% SBP increase per each 10 µg/m^3 PM_{10} increment, 95%CI: -0.86%; 1.79%). Correspondent estimates in mmHg increase per each 10 µg/m^3 increment of PM_{10} are presented as supplementary data, in Table S29 (0.33 mmHg DBP increase per each 10 µg/m^3 PM_{10} increment, 95%CI: -0.62%; 1.27% and 0.57 mmHg SBP increase per each 10 µg/m^3 PM_{10} increment, 95%CI: -1.10%; 2.54%). Similar results were obtained in the stratified analysis, when considering only males or females (**Table 21**).

Table 20 – Description of the general characteristics of the study participants.

Characteristics	Total (n=2272)	Males (n=1037)	Females (n=1235)
Age (n=2272) - %			
25-49 years old	53.0	53.6	52.4
50-74 years old	47.0	46.4	47.6
[a] Level of Education (n=2271) - %			
Low education	**57.8**	**62.1**	**54.0**
Medium education	**21.9**	**21.9**	**21.9**
High education	**20.3**	**16.0**	**24.1**
[b] Occupation (n=2102) - %			
White-collar occupation	**63.6**	**56.1**	**70.7**
Blue-collar occupation	**36.4**	**43.9**	**29.3**
Lifestyles variables - %			
[c] Smokers (n=2272)	**21.5**	**27.0**	**16.6**
[d] Excessive alcohol consumers (n=2271)	**35.8**	**53.5**	**20.0**
[e] Unhealthy Diet (n=2271)	**36.4**	**46.0**	**27.8**
[f] Sedentary (n=2258)	45.0	42.0	48.0
[g] Hypertension (n=2272) - %	**36.4**	**40.8**	**32.4**
[h] Hypertensive medication (n=2272)	25.1	24.4	25.7
Diastolic Blood Pressure (n=2272) – mmHg (mean, sd)	**74.0 (10.2)**	**76.1 (9.9)**	**72.0 (9.9)**
Systolic Blood Pressure(n=2272) mmHg (mean, sd)	**125.1 (16.0)**	**130.0 (14.3)**	**120.8 (16.3)**
Individual allocated			
3-day average Temperature (n=1887) - ºC (mean, sd)	16.1 (4.0)	16.1 (4.0)	16.1 (4.0)
3-day average PM_{10} (n=2272) - ºC (mean, sd)	18.7 (8.3)	18.6 (8.2)	18.7 (8.4)

[a] Low education: levels 0–2 of the ISCED 2011; medium education: levels 3–4 of the ISCED 2011; high education: levels 5–8 of the ISCED 2011; [b] White-collar occupation: Managers, Professionals, Technicians and Associate Professional, Clerical Support Workers and Services and Sales Workers; Blue-collar occupation: Skilled Agricultural Workers, Craft and Related trades Workers, Plant and Machine Operators and Elementary occupations; [c] Smokers include current daily and occasional smokers; [d] 3 or more days/week of consumption of at least one of the following alcoholic beverages (wine, beer, brandy/spirits, port wine/martini/liqueur, whisky/gin/vodka); [e] No consumption of fruit and vegetables at least once a day; [f] Reading, watching TV or other sedentary activities declared as the best description of the leisure time activities during the last 12 months; [g] defined as defined as measured SBP ≥140 mmHg or measured DBP ≥90 mmHg or self-report of taking antihypertensive medication; [h] self-report of taking antihypertensive medication.Results in bold are those with statistically significant difference between females versus males, according to the Pearson's chi-square test ($p<0.05$)

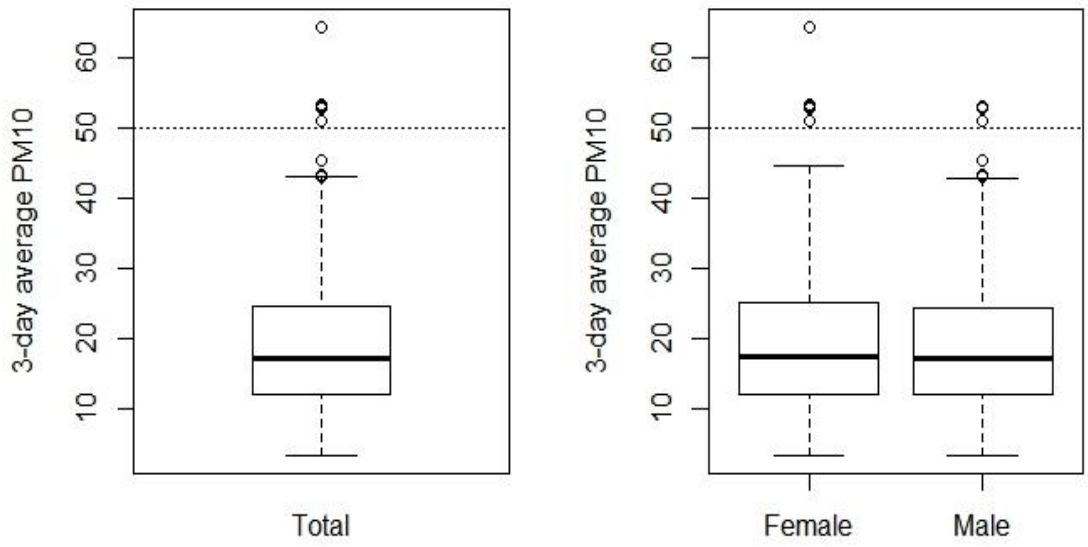

Figure 21 - Boxplot of the PM_{10} distribution according to sex. The dashed lines represent the daily limit value given by the EU Ambient Air Quality Directives (not to be exceed on more than 35 days/year) and also by the World Health Organization air quality guidelines (not exceeded more than 3 days/year).

Table 21 – Percent changes in DBP and SBP per 10 µg/m^3 increment of PM_{10} according to sex.

	% Change per 10 µg/m^3 of PM_{10} increment	
	DBP	SBP
Total (n=2272)		
Unadjusted model	-0.73 (-1.92; 0.47)	-0.60 (-1.88; 0.69)
*Adjusted model	0.42 (-0.85; 1.70)	0.47 (-0.86; 1.79)
Males (n=1037)		
Unadjusted model	-0.61(-1.95; 0.73)	-0.50 (-1.87; 0.88)
*Adjusted model	-0.04(-1.09; 1.02)	0.68 (-0.81; 2.18)
Females (n=1235)		
Unadjusted model	-0.80 (-2.84; 1.25)	-0.64 (-2.75; 1.46)
*Adjusted model	0.76 (-1.42; 2.94)	0.16 -1.98; 2.31)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average of PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

Sensitivity analysis

Regarding the sensitivity analysis, when we restricted our sample to the participants living within a 20 km radius of an air quality monitoring station with available PM_{10} values, similar results were found when considering the total sample and also when considering both males or females. Per each 10 $\mu g/m^3$ PM_{10} increment, there was a 0.51% (95% CI: -1.35; 2.37) increase in the DBP values and a 0.75% (95% CI: -1.02; 2.53) increase in the SBP values, being these results not statistically significant (Table S22). When we considered different exposure periods of time (2 previous days or 5 previous days instead of 3 previous days) similar results were found (Table S23 and S24). Additionally, when we considered the individual allocated PM_{10} concentrations obtained by the air quality modelling system (WRF-CAMx), no associations were neither found (Table S25). Finally, when we restricted our analysis to participants not taking hypertensive medication, or when we considered only more susceptible individuals like those obese or diagnosed with hypertension, results remain similar (Tables S26, S27 and S28).

Discussion

Main finding of this study

In this study, no statistically significant association was found between PM_{10} exposure and both DBP and SBP values. However, the point estimates obtained were similar or even higher than those from the last published meta-analysis about the global association between ambient air pollution and blood pressure (17) (DBP: 0.33 *versus* 0.15 mmHg increase per each 10 µg/m^3 PM_{10} increment; SBP: 0.57 *versus* 0.21 mmHg increase per each 10 µg/m^3 PM_{10} increment). These results were consistently maintained by the sensitivity analysis performed, namely when considering a more restrictive criteria to the exposure assessment (participants living within a 20 km radius of an air quality monitoring station with available PM_{10} values), when considering the 2 or 5-previous days instead of 3-previous days as the exposure period of time, when considering an individual allocated PM_{10} concentrations obtained from an air quality model instead of the direct measurements from the air monitoring stations or when considering only hypertensive, participants with abdominal obesity or participants not taking anti-hypertensive medication.

What is already known on this topic

According to a recent systematic review and meta-analysis performed on the association between ambient air pollution and blood pressure, that pools studies published before May 2017 (17), evidence already supported the association between short-term PM_{10} exposure and DBP values (0.15 mmHg, 95%CI: 0.01;0.29, per each 10 µg/m^3 increase of PM_{10}), which our study was unable to confirm. However, the point estimate obtained in our study was higher than the one reported by the cited meta-analysis (0.33 *versus* 0.15 mmHg increase per each 10 µg/m^3 PM_{10} increment). Additionally, the same meta-analysis did not support the association between PM_{10} exposure and SBP values (0.21 mmHg, 95%CI: -0.01; 0.43, per each 10 µg/m^3 increase of PM_{10}), which is in concordance to our results.

It is important to mention that the polled estimates of the meta-analysis previously mentioned (17) were obtained using data from studies mostly from America and Asia regions where the levels of PM_{10} are higher in comparison to the majority of the European countries. When we consider only the pooling of the estimates from studies performed

in European countries (256–259), we found a -0.01 mmHg DBP decrease (95%CI: -0.14; 0.13) per each 10 μg/m^3 increase of PM_{10}. Accordingly, when considering only European countries, the published meta-analysis also did not support the association between short-term exposure to PM_{10} and diastolic (DBP) or systolic (SBP) blood pressure values, which is corroborated by our study.

After the publication of the meta-analysis previously cited (17), additional studies have been published on this issue but the association between short-term PM_{10} exposure and blood pressure values remains controversial (260,261). The majority of the studies, mostly performed in China, provide evidence of a positive association between PM_{10} and blood pressure values (260,262) but there are some other studies that did not support this association (261). For an instance, in a panel study performed in Italy and Sweden, with a range of PM_{10} values comparable to those from Portugal, no associations were found between PM_{10} levels and either systolic (SBP) or diastolic (DBP) blood pressure but short-term exposure to PM_{10} results in reductions in carotid elasticity among elderly population which means that PM exposure could be associated with other important CV outcomes (261).

What this study adds

This study suggests us that exposure to PM_{10} concentrations in the levels observed in Portugal, similar to other European countries, have a small or no effect on the blood pressure values, however it could have impact in other important cardiovascular outcomes. In fact, in a recent published study performed in the Portuguese population (255), a significant association between short-term PM_{10} exposure (3-day average PM_{10} concentration) and white blood cells (WBC) among females was found (a 2.76% WBC increase, 95% CI: 0.65–4.87, per each 10μg/m3 PM_{10} increment) suggesting that PM_{10} exposure could impact on other outcomes with potential to mediate a cardiovascular event. Additionally, it is also important to mention that studies reporting significant results are more likely to be published and the possibility of a publication bias on this issue cannot be rejected (263,264).

The point estimates obtained in this study were not statistically significant, which will be probably due to the lack of statistical power to estimate effects of this magnitude with a sample size of just over two thousand participants. However, even if the point

estimates had statistical significance, we considered that obtained estimates would represent a small effect of the exposure to PM_{10} concentrations on blood pressure, taking into account the daily mean values of PM_{10} concentrations observed in the Portuguese context, in 2015 (3-day average PM_{10}: 18.7 $\mu g/m^3$).

Limitations of this study

Our study have some limitations. A first one is related to the exposure assessment method, due to the high distance considered between participants' residence and the air quality monitoring stations (30 km) in the inclusion criteria. The number and the spatial distribution of air quality monitoring stations in the Portuguese mainland does not allow us to apply a more restrictive distance due to a substantial sample reduction that could compromise the power of the estimates and result in selection bias. However, our results remain similar when we apply more restrictive criteria (including only participants living within a 20 km radius of an air quality monitoring station with available PM_{10} measurements) or when we used data from a numerical model.

A second limitation of our study is related to the possibility of an erroneous assumption on the temporal relationship between exposure to PM_{10} and the BP changes. In fact, the considered time window of exposure, that was the 3 previous days in our study, varied a lot in the previously published studies, ranging from days to months and even years. Despite the time window of PM exposure that is causally-linked to BP levels is not well established, according to the perspective of Newman and his collaborators (137), it will be on the order of a few days given the rapid occurrence of the physiological responses whereby PM influences on BP. Consequently, we assumed that the 3 previous days' average PM_{10} concentrations were adequate to represent the individual exposure of each participant. Additionally, we also tested the associations when considering 2 and 5 previous days instead of the 3 previous days and results remain the same.

Finally, even considering the adjustment for several potential confounders, there is still a possibility that the observed estimates have been affected by residual confounding due to other unmeasured confounders. In addition, effect estimates presented in this study were based on a single-pollutant model and considering only PM_{10}, due the scarce available air quality monitoring data for the remaining air pollutants,

but there are important interactions between the atmospheric pollutants, namely the potential additive effects of multiple pollutants, that should be considered in future studies (221,222).

Conclusions

To the best of our knowledge, this is the first study assessing the association between an air pollutant exposure and the blood pressure values in Portugal. Our results suggest that exposure to the PM_{10} levels observed in 2015, have a small or no effect on the blood pressure values. However, we only analysed one of a large amount of air pollutants, and future studies should investigate whether the exposure to other air pollutants, including particulate matter of a smaller size, can explain some of the causal link between short-term exposure to ambient air pollution and diastolic or systolic blood pressure values.

Funding

INSEF was developed as part of the Pre-defined project financed under the Public Health Initiatives Program. ‘‘Improvement of epidemiological health information to support public health decision and management in Portugal. Towards reduced inequalities, improved health and bilateral cooperation’’ with a 1.500.000€ Grant from Iceland, Liechtenstein and Norway from EEA Grants and the Portuguese Government. The present study was also funded by the Portuguese Foundation for Science and Technology (FCT) (PhD Scholarship Reference: SFRH/BD/129426/2017). J. Ferreira was funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia , I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. CESAM received financial support by FCT/MCTES (UIDP/50017/2020+UIDB/50017/2020), through national funds.

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest or competing interests.

Acknowledgments

The authors are grateful to all the professionals and participants involved in the INSEF. The authors are also grateful to the Portuguese Environmental Agency (APA) for making the air quality data available.

6. Discussion

The overall purpose of developing this thesis was to improve scientific knowledge on the relationship between exposure to environmental air particulate matter and cardiovascular related biomarkers. The hypothesis of this research was that exposure to ambient particulate matter (PM_{10}) is associated with changes in the values of some cardiovascular risk biomarkers in the Portuguese resident population, in 2015. This section includes the overall discussion of the key findings, implications to public health as well as strengths and limitations of all the developed research that is included in the four articles presented in the previous section.

6.1. Key findings

Concerning objective 1 of this thesis, that aimed to systematically review and meta-analyse studies on the association between AAP and biomarkers of dyslipidaemia, I consider that it was successfully achieved. In addition to its usefulness in summarising the state of knowledge prior to design the analysis strategy in the **Article 2** of this thesis, it has also been useful to other researchers all over the world, having so far more than 25 citations. At this time, given that some additional studies have already been carried out after the meta-analysis presented in **Article 1**, including the one described in **Article 2** of this thesis, it would be pertinent to update the analysis in order to ascertain whether the latest studies corroborate the finding previous described.

Still regarding the systematic review, that also included other air pollutants beyond PM_{10}, the recommendation presented in **Article 1** to further investigate exposure to gaseous pollutants that are less frequently included in studies of the association between AAP exposure and dyslipidaemia biomarkers. However, in the studies developed in this thesis, only PM_{10} were considered because it was the pollutant that, in the studied period (2014-2015) had the greatest coverage of measurements from the Portuguese air quality stations. For the remaining pollutants, there was a higher percentage of missing values in the time series of the hourly measured values and a small percentage of participants was covered. However, taking into account that the systematic review also found evidence of an association between NO_2 and TG values, data on NO_2 should be explored more deeply in the future to try to study the combined effect of PM_{10} and NO_2 on this outcome.

Table 22 summarize the key findings of this thesis, answering to the delineated objectives 2 to 5, that aimed to assess the association between PM_{10} exposure and each studied biomarker group. The described key findings support the case for a positive association between PM_{10} exposure and some biomarkers of cardiovascular risk, namely those related to dyslipidaemia (TG in people with abdominal obesity) and inflammatory response (WBC in females and RDW in males). Consequently, it suggests that reduction of PM_{10} below current air quality standards would result in additional cardiovascular health benefits for the population.

Table 22 – Summary of the Key findings of this thesis.

Biomarker Group	**Parameter**	**Long-term Exposure**	**Short-term Exposure**
		1-year average PM_{10}	3-day average PM_{10}
Dyslipidaemia (Article 2)	TC	×	
	HDL-C	×	
	LDL-C	×	
	TG	✓*	
Diabetes(Article 2)	HbA1c	×	
Inflammatory response (Article 3)	**WBC**	×	✓**
	PLAT	×	×
	RBC	×	×
	RDW	✓***	×
	HEMOG	×	×
Hypertension (Article 4)	DBP		×
	SBP		×

✓Represents the significant associations found in this thesis. × represent the no statistically significant associations found in this thesis. Grey zones represents associations that were not assessed in this thesis. *Only in participants with abdominal Obesity; **only in females; ***only in males. Abbreviations: TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TG, Triglycerides; HbA1c, Glycated haemoglobin; DBP, Diastolic blood pressure; SBP, systolic blood pressure; WBC, White blood cells; PLAT, Platelets; RBC, Red blood cells; RDW, Red cell distribution width; HEMOG, Haemoglobin; DBP, Diastolic blood pressure; SBP, systolic blood pressure.

It is important to mention that **Article 2** describes, to the best of my knowledge, the first study demonstrating that, even at relatively low levels of exposure (below the EU limits), long-term exposure to PM_{10} interacts with abdominal obesity (as a proxy of Visceral Adipose Tissue) to increase non-fasting blood TG levels by about 2% per each 1 $\mu g/m^3$ PM_{10} increment. It supports the hypothesis that PM could act as an

environmental endocrine disruptor through adipokines dysregulation at the adipose tissue level and this biological mechanism should be considered to be explored deeply in future in vitro and in vivo animal experiments.

Additionally, **Article 3** reports some sex-differential associations regarding the short and long-term effect of PM_{10} exposure on blood count parameters with potential to mediate a cardiovascular event, namely on the RDW parameter, that to the best of my knowledge were never been described. It is uncertain whether changes in blood count parameters due to PM_{10} exposure constitute an adverse health outcome or it reflects only a normal immunity response. Nevertheless, due to its potential to trigger cardiovascular events, exploring PM deleterious effects on immunity system must also be considered.

As presented in **Table 22**, associations between PM_{10} exposure and the remaining studied biomarkers were not supported by the studies developed in this thesis. A possible explanation to not identify these associations in the Portuguese context, some of them already well recognised in other parts of the globe, could be the high PM diversity not only in terms of concentration but also in terms of constituents. In fact, according to the last report of the Portuguese Environment Agency (APA) (42), the levels of pollutants measured in Portugal for the time period under analysis (2014-2015) are largely lower (annual mean values above 20 $\mu g/m^3$ for the years 2014- 2015) when compared to levels reported for other parts of the world, namely Asian or American countries (annual mean values around 80 $\mu g/m^3$ and 55 $\mu g/m^3$ for the years 2014-2015 in China and USA, respectively) (265,266). At the European level, PM_{10} values have been decreasing over the years (267) and therefore, epidemiological studies carried out in European Countries that may have identified an association between PM_{10} exposure and certain outcomes could no longer be comparable to the current context of exposure used in this thesis. Additionally, PM are a heterogeneous and complex mixture of solid and liquid particles suspended in the atmosphere, which can in turn transport a wide variety of compounds harmful to human health, varying in particle size and chemical composition on a range of temporal and spatial scales (28). Consequently, it is plausible to expect that PM exposure could affect different health outcomes in different parts of the globe as its constituents will diverge and possibly will have different health effects.

Concerning objective 6, that aimed to assess the potential mediation/modifier effect of abdominal obesity on the association between exposure to PM_{10} and biomarkers

of dyslipidaemia, diabetes and blood pressure (**Articles 2** and **4**) it was only possible to identify the modification action of abdominal obesity regarding the association between PM_{10} and biomarkers of dyslipidaemia. I consider that it was also an important result obtained through this thesis as obesity is a particular important cardiovascular risk factor, highly prevalent in the Portuguese population (268), meaning that a high percentage of the population could be at risk regarding the potential PM effect on blood lipid levels.

Finally, concerning objective 7 that aimed to compare the usage of two different indirect methods to assign the individual-level exposure (PM_{10} values obtained by direct measurements from the air quality monitoring stations *versus* PM_{10} values obtained by air quality modelling system) in assessing associations between PM_{10} and biomarkers of cardiovascular risk, only the positive association between PM_{10} and RDW was detected using both indirect methods. For the remaining described associations, they are not found significant when using PM_{10} values obtained by air quality modelling system. Although this could be due to measurement errors associated with the usage of the direct measurements from the air quality monitoring stations, our results suggest that using modelled PM_{10} values could be a less sensitive method to assess individual exposure and only the most robust association was identified. Additional discussion on the exposure assessment methodology is presented in the strengths and limitations subsection of this thesis.

6.2. Public Health implications

Exposure to AAP is largely beyond the control of individuals and requires action by public authorities at the national and international levels (43). Results obtained within this thesis provide scientific arguments for taking actions to improve air quality, even when the standard air quality limits are target. The results show that cardiovascular related effects can occur at air pollution concentrations lower than established guidelines and, as previous reported by World Health Organization, there is no evidence of a safe level of PM exposure or a threshold below which no adverse health effects occur (43). Furthermore, the positive associations found in this research reinforce the requirement for the reduction of pollution levels which must gain an additional strength to contribute to the reduction of the cardiovascular diseases' burden (6).

In Portugal, the few epidemiologic studies that have been published on the association between air pollution exposure and cardiovascular outcomes were focused on risk of mortality or hospital admissions due to cardiorespiratory diseases (11-14). This was probably due to data availability, but the result was that subclinical and clinical cardiovascular risk conditions are less well understood, requiring more research, since the percentage of the Portuguese population affected by these less severe health effects is much larger that the percentage of the population affected by the more severe health effects. Consequently, it is important to analyse the air pollution effects on these less severe effects as done in the studies performed in this thesis, given their prevention potential. Additionally, results of this thesis contribute to increase the knowledge base of the biologic plausibility explaining the results of previous epidemiologic studies that reported an association between air pollution and increased cardiovascular mortality and hospital admissions due to cardiovascular diseases in the adult Portuguese population (11-14). In future experimental studies, including in animal models and *in vitro*, it would be important to explore deeply the hypothetical biological mechanisms presented in this thesis to explain the identified associations.

To my knowledge, studies presented in this thesis that linked air quality data collected through air quality monitoring network of the Environmental Portuguese Agency and individual data from a Health Examination Survey have never been performed previously in the Portuguese population. Based on an adequate study design and a wide span of measured variables, the associations between PM_{10} and biomarkers of cardiovascular risk were assessed with reduced confounding bias. Methodology

approach presented in this thesis can be used to study the association between other air pollutants and other health outcomes in a perspective to improve the use of environmental and health data already collected and available to all the scientific community instead of collecting new data.

6.3. Strengths and limitations

Specific studies limitations and strengths were previously discussed in each of the articles' discussion sections. This section is dedicated to the overall thesis limitations and strengths. One limitation of the studies developed in thesis is related to the study design. Longitudinal studies investigating changes in biomarkers of cardiovascular risk in relation to PM exposure are required to confirm the findings as studies described in **Articles 2**, **3** and **4** are cross-sectional studies. Additionally, all the studies developed in this thesis are related to the covered population. It only included adults from the general population and not children and elderly that could be more vulnerable population groups. In fact, PM exposure is nearly ubiquitous, affecting everyone, but those at greatest risk are children, people aged 65 and over, people with pre-existing comorbidities namely CV and respiratory diseases, people who work outdoors and people of lower socio-economic status because they are more likely to live in areas with worst air quality (72). Specific studies in these sub population groups must be considered in the future.

Another possible limitation is related to the exposure assessment methodology. When using data from air quality stations, I make the assumption that measurements at a single site are representative of air quality over a larger area. Data from air quality models was also considered to test if results persist as it is expected that increased resolution associated with the air quality modelling (5 km instead of 30 km) would result in reduced measurement error of the exposure and more robust associations with cardiovascular outcomes (54). In this way, we were only able to find the association between PM_{10} values and RDW, when using PM_{10} modelled values, and the remaining associations were not obtained when using this method. Consequently, we could not discharge the possibility of a misclassification of exposure error associated with the use of data from air quality stations. However, more advanced modelling techniques may also bring additional uncertainty into the resulting estimates and interpretation of results (269). In this particular case, the modelled PM_{10} concentrations that were also analysed in this thesis, when compared with the measured PM_{10} concentrations, could be less representative of the real participants PM_{10} exposure because they are a mathematical representation of the reality with a certain degree of uncertainty of the input data, namely in the atmospheric emissions data, being potentially associated with a higher degree of individual exposure misclassification. As exposure assessment methods vary by study, making the results from the associations between PM and cardiovascular related

outcomes difficult to compare, it will be very important, in future studies, to assess which method is more appropriate to use in this kind of study.

In this thesis, only PM_{10} concentrations were considered as exposure, and these particles do not penetrate very deep into the respiratory system. However, the biological mechanisms that were assumed to potentially explain the associations described in this thesis only make sense based on the assumption that particles penetrate deep into the respiratory system. Consequently, to study $PM_{2.5}$ instead of PM_{10} would be more appropriate taking into account the objectives of this thesis, however there is no data available with sufficient coverage of the Portuguese territory to allow its study using the INSEF data. Even so, PM_{10} levels were analysed, the ones available to be analysed in our period of study, and it was assumed that they are correlated with smaller particles concentrations, as previously reported (242).

7. Conclusions

Despite the downward trend of PM concentrations in most recent years not only in Portugal but also in other European Countries and in other parts of the world (265,267) air pollution continues to be a key global public health issue to solve and it is largely beyond the control of individuals, requiring action by public authorities at the national and international levels (43). PM is being considered an important risk factor for cardiovascular diseases, which continue to be the leading cause of death in many countries including in Portugal. However, while scientific evidence supporting the association between PM and CV mortality and morbidity is well-established at both international and national level, the pathophysiologic mechanisms linking PM and CVD are not entirely known, being a currently research area with a lot of scientific debate. In this sense, epidemiological studies on the association between PM and biomarkers of subclinical and clinical CV risk conditions are essential to establish the biologic plausibility of the association between PM and CV mortality and morbidity.

In **Article 1**, that approaches the systematic review and meta-analysis of epidemiologic evidence on the association between ambient air pollution exposure and lipid profile parameters, despite the few studies included in the meta-analysis, it suggests some epidemiologic evidence supporting the association between PM_{10} (and also NO_2) exposures and increased TG levels. Due to the very low level of evidence, more studies are essential to support the association and it was recommended further investigation of the gaseous pollutants exposure, that were less frequently included, to explore the potential additive effects of multiple pollutants, as well as developing more robust spatiotemporal models to assess air pollution exposure more precisely.

Article 2 suggested that even at low levels of exposure, long-term PM_{10} exposure interacts with abdominal obesity to increase blood triglycerides. It supports the hypothesis that PM could act as an environmental endocrine disruptor through adipokines dysregulation at the adipose tissue level and this biological mechanism should be considered to be explored deeply in future in vitro and in vivo animal experiments.

In **Article 3**, a long-term exposure to PM_{10} was associated with higher RDW values among males and a short-term exposure to PM_{10} as associated with higher WBC values among females. It is still uncertain whether changes in blood count parameters due to

PM_{10} exposure constitute an adverse health outcome or if it reflects only a normal immune response but due to its potential to trigger cardiovascular events, exploring PM deleterious effects on immunity system must also be considered in experimental studies using animal models or in vitro studies.

Finally, **Article 4** suggests that exposure to PM_{10} concentrations in the levels observed in Portugal, similar to other European countries, have a small or no effect on the blood pressure values.

Overall, results obtained within this thesis provide more scientific arguments for taking actions to improve air quality, even when the standard air quality limits are targeted. It indicates that cardiovascular related effects can occur at air pollution concentrations lower that those considered in establishing guidelines. Finally, to my knowledge, this thesis describes a methodology never performed in Portugal to link air quality data and health data that could be used to study other air pollutants and other health outcomes. Consequently, studies developed in this thesis are just the first of many linkage studies that can be done between data obtained through national health surveys and environmental data that could answer to important research questions with public health relevance.

8. References

1. World Health Organization [Internet]. Ambient air pollution. [cited 2019 May 21]. Available from: https://www.who.int/health-topics/air-pollution#tab=tab_2.
2. Vallero DA. Chapter 18 - Air Pollution: Atmospheric Wastes. In: Letcher TM, Vallero DA, editors. Waste (Second Edition). Academic Press; 2019.pp.453–475.
3. European Environment Agency. Air quality in Europe - 2019 report. No 10/2019. Luxembourg: Publications Office of the European Union; 2019.
4. Karagulian F, Belis CA, Dora CFC, Prüss-Ustün AM, Bonjour S, Adair-Rohani H, et al. Contributions to cities' ambient particulate matter (PM): A systematic review of local source contributions at global level. Atmospheric Environment. 2015;120:475–83.
5. Lelieveld J, Pozzer A, Pöschl U, Fnais M, Haines A, Münzel T. Loss of life expectancy from air pollution compared to other risk factors: a worldwide perspective. Cardiovascular research. 2020;116(11):1910–7.
6. Miller MRM, Newby DED. Air pollution and cardiovascular disease: Car sick. Cardiovascular Research. 2020;116(2):279–94.
7. Yang Y, Ruan Z, Wang X, Yang Y, Mason TG, Lin H, et al. Short-term and long-term exposures to fine particulate matter constituents and health: A systematic review and meta-analysis. Environmental Pollution. 2019;247:874–82.
8. Lee KK, Shah SA, McAllister DA, Hunter A, Nair H, Whiteley W, et al. Air pollution and stroke: A systematic review and meta-analysis. Circulation. 2014;130.suppl_2: A9900.
9. Zhao L, Liang HR, Chen FY, Chen Z, Guan WJ, Li JH. Association between air pollution and cardiovascular mortality in China: A systematic review and meta-analysis. Oncotarget. 2017;8(39):66438–48.
10. Shah ASV, Langrish JP, Nair H, McAllister DA, Hunter AL, Donaldson K, et al. Global association of air pollution and heart failure: A systematic review and meta-analysis. The Lancet. 2013;382(9897):1039–48.
11. Cruz AMJ, Sarmento S, Almeida SM, Silva A V., Alves C, Freitas MC, et al. Association between atmospheric pollutants and hospital admissions in Lisbon. Environmental Science and Pollution Research. 2015;22(7):5500–10.
12. Almeida SM, Silva A V., Sarmento S. Effects of exposure to particles and ozone on hospital admissions for cardiorespiratory diseases in Setúbal, Portugal. Journal

of Toxicology and Environmental Health - Part A: Current Issues. 2014;77(14–16):837–48.

13. Almeida SP de, Casimiro E, Calheiros J. Short-term association between exposure to ozone and mortality in Oporto, Portugal. Environmental Research. 2011;111(3):406–10.

14. Camacho I, Camacho J, Camacho R, Góis A, Nóbrega V. Influence of Outdoor Air Pollution on Cardiovascular Diseases in Madeira (Portugal). Water, Air, and Soil Pollution. 2020;231(3):1–15.

15. Liu C, Chen R, Sera F, Vicedo-Cabrera AM, Guo Y, Tong S, et al. Ambient particulate air pollution and daily mortality in 652 cities. New England Journal of Medicine. 2019;381(8):705–15.

16. Yang BY, Fan S, Thiering E, Seissler J, Nowak D, Dong GH, et al. Ambient air pollution and diabetes: A systematic review and meta-analysis. Environmental Research. 2020;180:108817–27.

17. Yang BY, Qian Z, Howard SW, Vaughn MG, Fan SJ, Liu KK, et al. Global association between ambient air pollution and blood pressure: A systematic review and meta-analysis. Environmental Pollution. 2018; 235:576–588.

18. Almetwally AA, Bin-Jumah M, Allam AA. Ambient air pollution and its influence on human health and welfare: an overview. Environmental Science and Pollution Research. 2020;27(20):24815–30.

19. Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E. Environmental and Health Impacts of Air Pollution: A Review. Frontiers in Public Health. 2020;8:14–27.

20. European Environment Agency [Internet]. Infographic: Many factors contribute to air pollution concentration levels. [updated 2020 Nov 23; cited 2021 May 23]. Available from: https://www.eea.europa.eu/media/infographics/many-factors-contribute-to-air-1/view.

21. Kelly FJ, Fussell JC. Air pollution and public health: emerging hazards and improved understanding of risk. Environmental Geochemistry and Health. 2015;37(4):631–649.

22. Fowler D, Brimblecombe P, Burrows J, Heal MR, Grennfelt P, Stevenson DS, et al. A chronology of global air quality. Philosophical transactions Series A, Mathematical, physical, and engineering sciences. 2020;378(2183):20190314.

23. World Health Organization. Air Quality Guidelines. Global update 2005. Copenhagen: Regional Office for Europe; 2006.

24. Health Effects Institute. State of Global Air 2017: A Special Report on Global Exposure to Air Pollution and Its Disease Burden Boston, MA:Health Effects Institute; 2017.

25. Pinault LL, Weichenthal S, Crouse DL, Brauer M, Erickson A, Donkelaar A van, et al. Associations between fine particulate matter and mortality in the 2001 Canadian Census Health and Environment Cohort. Environmental Research. 2017;159:406–15.

26. Di Q, Wang Y, Zanobetti A, Wang Y, Koutrakis P, Choirat C, et al. Air pollution and mortality in the medicare population. New England Journal of Medicine. 2017;376(26):2513–22.

27. Intergovernmental Panel on Climate Change. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report; 2014.

28. Alemayehu YA, Asfaw SL, Terfie TA. Exposure to urban particulate matter and its association with human health risks. Environmental Science and Pollution Research. 2020;27(22):27491–506.

29. Kampa M, Castanas E. Human health effects of air pollution. Environmental Pollution. 2008;151(2):362–7.

30. Falcon-Rodriguez CI, Osornio-Vargas AR, Sada-Ovalle I, Segura-Medina P. Aeroparticles, composition, and lung diseases. Frontiers in Immunology. 2016;7:3.

31. Bell ML. Assessment of the health impacts of particulate matter characteristics. Research report (Health Effects Institute). 2012;(161):5–38.

32. Kastury F, Smith E, Juhasz AL. A critical review of approaches and limitations of inhalation bioavailability and bioaccessibility of metal(loid)s from ambient particulate matter or dust. Science of the Total Environment. 2017;574:1054–74.

33. Chen RH, Wang BQ, Wang ZB, Yao S. The pollution character analysis and risk assessment for metals in dust and PM10 around road from China. Biomedical and Environmental Sciences. 2015;28(1):44–56.

34. Valavanidis A, Fiotakis K, Vlachogianni T. Airborne particulate matter and human health: Toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. Journal of Environmental Science and Health - Part C Environmental Carcinogenesis and Ecotoxicology Reviews. 2008;26(4):339–62.

35. Terzano C, Di Stefano F, Conti V, Graziani E, Petroianni A. Air pollution ultrafine

particles: Toxicity beyond the lung. European Review for Medical and Pharmacological Sciences. 2010;14(10):809–21.

36. Lippmann M, Chen LC. Health effects of concentrated ambient air particulate matter (CAPs) and its components Health effects of ambient air CAPs M. Lippmann and L.-C. Chen. Critical Reviews in Toxicology. 2009;39(10):865–913.

37. Miller M, Shaw C, Langrish J. From particles to patients: Oxidative stress and the cardiovascular effects of air pollution. Future Cardiology. 2012;8(4):577–602.

38. Harrison R. Airborne particulate matter. Philosophical transactions of the Royal Society. 2020;378:20190319.

39. European Environment Agency [Internet]. Portugal - Air pollution country fact sheet. [updated 2020 Nov 23; cited 2021 May 21]. Available from: https://www.eea.europa.eu/themes/air/country-fact-sheets/2020-country-fact-sheets/portugal.

40. Harrison RM, Beddows DC. Efficacy of Recent Emissions Controls on Road Vehicles in Europe and Implications for Public Health. Scientific Reports. 2017;7(1):1–5.

41. EU. Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe. 2008.

42. Guerra MMD, Ribeiro R, Rodrigues S. Relatório do Estado do Ambiente 2019. Lisboa: Agência Portuguesa do Ambiente; 2019.

43. World Health Organization. Review of evidence on health aspects of air pollution - REVIHAAP Project. Copenhagen: WHO Regional Office for Europe; 2013.

44. Hertel O, De Leeuw FAAM, Raaschou-Nielsen O, Jensen SS, Gee D, Herbarth O, et al. Human exposure to outdoor air pollution (IUPAC Technical Report). Pure and Applied Chemistry. 2001;73(6):933–58.

45. Khafaie MA, Ojha A, Salvi SS, Yajnik CS. Methodological Approach In Air Pollution Health Effects Studies. Journal of Air Pollution and Health. 2016;1(13):219–26.

46. Zhang JJ, Lioy PJ. Human exposure assessment in air pollution systems. The Scientific World Journal. 2002;2:497–513.

47. Zou B, Wilson JG, Zhan FB, Zeng Y. Air pollution exposure assessment methods utilized in epidemiological studies. Journal of Environmental Monitoring. 2009;11(3):475–90.

48. Yang L, Hou XY, Wei Y, Thai P, Chai F. Biomarkers of the health outcomes

associated with ambient particulate matter exposure. Science of the Total Environment. 2017;579:1446–59.

49. Park YM. Assessing personal exposure to traffic-related air pollution using individual travel-activity diary data and an on-road source air dispersion model. Health and Place. 2020;63: 102351.

50. Steinle S, Reis S, Sabel CE. Quantifying human exposure to air pollution-Moving from static monitoring to spatio-temporally resolved personal exposure assessment. Science of the Total Environment. 2013;443:184–93.

51. Yarza S, Hassan L, Shtein A, Lesser D, Novack L, Katra I, et al. Novel approaches to air pollution exposure and clinical outcomes assessment in environmental health studies. Atmosphere. 2020;11(2):122–39.

52. Evangelopoulos D, Katsouyanni K, Keogh RH, Samoli E, Schwartz J, Barratt B, et al. PM2.5 and NO2 exposure errors using proxy measures, including derived personal exposure from outdoor sources: A systematic review and meta-analysis. Environment International. 2020;137:105500–14.

53. Bo M, Salizzoni P, Clerico M, Buccolieri R. Assessment of indoor-outdoor particulate matter air pollution: A review. Atmosphere. 2017;8(8):136–53.

54. McGuinn LA, Ward-Caviness C, Neas LM, Schneider A, Di Q, Chudnovsky A, et al. Fine particulate matter and cardiovascular disease: Comparison of assessment methods for long-term exposure. Environmental Research. 2017;159:16–23.

55. Hoek G, Beelen R, de Hoogh K, Vienneau D, Gulliver J, Fischer P, et al. A review of land-use regression models to assess spatial variation of outdoor air pollution. Atmospheric Environment. 2008;42(33):7561–78.

56. Hoek G. Methods for Assessing Long-Term Exposures to Outdoor Air Pollutants. Current environmental health reports. 2017;4:450–62.

57. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux A V., et al. Particulate Matter Air Pollution and Cardiovascular Disease. Circulation. 2010;121(21):2331–78.

58. Rajagopalan S, Al-Kindi SG, Brook RD. Air Pollution and Cardiovascular Disease: JACC State-of-the-Art Review. Journal of the American College of Cardiology. 2018;72(17):2054–70.

59. Cohen AJ, Brauer M, Burnett R, Anderson HR, Frostad J, Estep K, et al. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. The Lancet. 2017;389(10082):1907–18.

60. World Health Organization. Quantification of health effects of exposure to air pollution : report on a WHO working group, Bilthoven, Netherlands 20-22 November 2000. Copenhagen : WHO Regional Office for Europe; 2001.

61. Samet J, Buist S, Bascom R, Garcia J, Lipsett M, Mauderly J, et al. What constitutes an adverse health effect of air pollution? American Journal of Respiratory and Critical Care Medicine. 2000;161(2I):665–73.

62. Schraufnagel DE, Balmes JR, Cowl CT, De Matteis S, Jung SH, Mortimer K, et al. Air Pollution and Noncommunicable Diseases: A Review by the Forum of International Respiratory Societies' Environmental Committee, Part 2: Air Pollution and Organ Systems. Chest. 2019;155(2):417–26.

63. Borchers Arriagada N, Horsley JA, Palmer AJ, Morgan GG, Tham R, Johnston FH. Association between fire smoke fine particulate matter and asthma-related outcomes: Systematic review and meta-analysis. Environmental Research. 2019;179: 108777.

64. Ling SH, van Eeden SF. Particulate matter air pollution exposure: role in the development and exacerbation of chronic obstructive pulmonary disease. International journal of chronic obstructive pulmonary disease. 2009;4:233–43.

65. Turner MC, Andersen ZJ, Baccarelli A, Diver WR, Gapstur SM, Pope CA, et al. Outdoor air pollution and cancer: An overview of the current evidence and public health recommendations. CA: A Cancer Journal for Clinicians. 2020;70(6):460–479.

66. Loomis D, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, et al. IARC evaluation of the carcinogenicity of outdoor air pollution. Environnement, Risques et Sante. 2014;13(4):347–52.

67. Ngoc LTN, Park D, Lee Y, Lee YC. Systematic review and meta-analysis of human skin diseases due to particulate matter. International Journal of Environmental Research and Public Health. 2017;14(12):1458–68.

68. Braithwaite I, Zhang S, Kirkbride JB, Osborn DPJ, Hayes JF. Air pollution (Particulate matter) exposure and associations with depression, anxiety, bipolar, psychosis and suicide risk: A systematic review and meta-analysis. Environmental Health Perspectives. 2019;127(12):1260021–43.

69. Tsai TL, Lin YT, Hwang BF, Nakayama SF, Tsai CH, Sun XL, et al. Fine particulate matter is a potential determinant of Alzheimer's disease: A systemic review and meta-analysis. Environmental Research. 2019;177: 108638.

70. Carré J, Gatimel N, Moreau J, Parinaud J, Léandri R. Does air pollution play a role

in infertility?: A systematic review. Environmental Health: A Global Access Science Source. 2017;16(1):1–16.

71. Sapkota A, Chelikowsky AP, Nachman KE, Cohen AJ, Ritz B. Exposure to particulate matter and adverse birth outcomes: A comprehensive review and meta-analysis. Air Quality, Atmosphere and Health. 2012;5(4):369–81.

72. Holgate ST. Every breath we take: The lifelong impact of air pollution' - A call for action. Clinical Medicine, Journal of the Royal College of Physicians of London. 2017;17(1):8–12.

73. Instituto Nacional de Estatística. Causas de Morte 2017. Lisboa: Instituo Nacional de Estatística; 2019.

74. Kyu HH, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2018;392(10159):1859–922.

75. Roth GA, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2018;392(10160):2170.

76. Gaio V, Rodrigues AP, Kislaya I, Barreto M, Namorado S, Dias CM. Estimation of the 10-Year Risk of Fatal Cardiovascular Disease in the Portuguese Population: Results from the First Portuguese Health Examination Survey (INSEF 2015). Acta Médica Portuguesa. 2020;33(13).

77. Shah ASV, Lee KK, McAllister DA, Hunter A, Nair H, Whiteley W, et al. Short term exposure to air pollution and stroke: Systematic review and meta-analysis. BMJ (Online). 2015;350.

78. Luo C, Zhu X, Yao C, Hou L, Zhang J, Cao J, et al. Short-term exposure to particulate air pollution and risk of myocardial infarction: a systematic review and meta-analysis. Environmental Science and Pollution Research. 2015;22(19):14651–62.

79. Song X, Liu Y, Hu Y, Zhao X, Tian J, Ding G, et al. Short-term exposure to air pollution and cardiac arrhythmia: A meta-analysis and systematic review. International Journal of Environmental Research and Public Health. 2016;13(7).

80. Franchini M, Mengoli C, Cruciani M, Bonfanti C, Mannucci PM. Association between particulate air pollution and venous thromboembolism: A systematic

literature review. European Journal of Internal Medicine. 2016;27:10–13.

81. Hamanaka RB, Mutlu GM. Particulate Matter Air Pollution: Effects on the Cardiovascular System. Frontiers in Endocrinology. 2018;9.

82. Pranata R, Vania R, Tondas AE, Setianto B, Santoso A. A time-to-event analysis on air pollutants with the risk of cardiovascular disease and mortality: A systematic review and meta-analysis of 84 cohort studies. Journal of Evidence-Based Medicine. 2020;13(2):102–15.

83. Scheers H, Jacobs L, Casas L, Nemery B, Nawrot TS. Long-Term Exposure to Particulate Matter Air Pollution Is a Risk Factor for Stroke: Meta-Analytical Evidence. Stroke. 2015;46(11):3058–66.

84. Choi HS, Ashitate Y, Lee JH, Kim SH, Matsui A, Insin N, et al. Rapid translocation of nanoparticles from the lung airspaces to the body. Nature Biotechnology. 2010;28(12):1300–3.

85. Schisler JC, Ronnebaum SM, Madden M, Channell M, Campen M, Willis MS. Endothelial inflammatory transcriptional responses to an altered plasma exposome following inhalation of diesel emissions. Inhalation Toxicology. 2015;27(5):272–80.

86. Van Eeden SF, Hogg JC. Systemic inflammatory response induced by particulate matter air pollution: The importance of bone-marrow stimulation. Journal of Toxicology and Environmental Health - Part A. 2002;65(20):1597–613.

87. Nakane H. Translocation of particles deposited in the respiratory system: A systematic review and statistical analysis. Environmental Health and Preventive Medicine. 2012;17(4):263–74.

88. Block ML, Elder A, Auten RL, Bilbo SD, Chen H, Chen JC, et al. The outdoor air pollution and brain health workshop. NeuroToxicology. 2012;33(5):972–84.

89. Franklin BA, Brook R, Arden Pope C. Air pollution and cardiovascular disease. Current Problems in Cardiology. 2015;40(5):207–38.

90. Atkinson AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, et al. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clinical Pharmacology and Therapeutics. 2001;69(3):89–95.

91. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJL. Selected major risk factors and global and regional burden of disease. Lancet. 2002;360(9343):1347–60.

92. World Health Organization. Global Health Risks: Mortality and burden of disease attributable to selected major risks. Bulletin of the World Health Organization.

2009;87:646–646.

93. World Health Organization. Global status report on noncommunicable diseases. Chapter 1: Burden: mortality, morbidity and risk factors. WHO; 2010.

94. Barreto M, Gaio V, Kislaya I, Antunes L, Silva AC, Vargas P, et al. 1ºInquérito Nacional de Saúde com Exame Físico (INSEF, 2015): Estado de Saúde. Lisboa: Instituto Nacional de Saúde Doutor Ricardo Jorge; 2016.

95. Yang B-Y, Bloom MS, Markevych I, Qian ZM, Vaughn MG, Cummings-Vaughn LA, et al. Exposure to ambient air pollution and blood lipids in adults: The 33 Communities Chinese Health Study. Environment international. 2018;119:485–92.

96. Lee S, Park H, Kim S, Lee EK, Lee J, Hong YS, et al. Fine particulate matter and incidence of metabolic syndrome in non-CVD patients: A nationwide population-based cohort study. International Journal of Hygiene and Environmental Health. 2019;222(3):533–40.

97. Shanley RP, Hayes RB, Cromar KR, Ito K, Gordon T, Ahn J, et al. Particulate Air Pollution and Clinical Cardiovascular Disease Risk Factors. Epidemiology (Cambridge, Mass). 2016 Mar;27(2):291–8.

98. Cai Y, Hansell AL, Blangiardo M, Burton PR, de Hoogh K, Doiron D, et al. Long-term exposure to road traffic noise, ambient air pollution, and cardiovascular risk factors in the HUNT and lifelines cohorts. European heart journal. 2017 Aug;38(29):2290–6.

99. Eze IC, Schaffner E, Foraster M, Imboden M, Von Eckardstein A, Gerbase MW, et al. Long-term exposure to ambient air pollution and metabolic syndrome in adults. PLoS ONE. 2015;10(6).

100. Bell G, Mora S, Greenland P, Tsai M, Gill E, Kaufman JD. Association of Air Pollution Exposures With High-Density Lipoprotein Cholesterol and Particle Number: The Multi-Ethnic Study of Atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2017 May;37(5):976–82.

101. Li J, Zhou C, Xu H, Brook RD, Liu S, Yi T, et al. Ambient Air Pollution Is Associated With HDL (High-Density Lipoprotein) Dysfunction in Healthy Adults. Arteriosclerosis Thrombosis And Vascular Biology. 2019 Mar;39(3):513–22.

102. Yang BY, Guo Y, Markevych I, Qian ZM (min), Bloom MS, Heinrich J, et al. Association of Long-term Exposure to Ambient Air Pollutants with Risk Factors for Cardiovascular Disease in China. JAMA Network Open. 2019;2(3):e190318.

103. Liu C, Ying Z, Harkema J, Sun Q, Rajagopalan S. Epidemiological and

experimental links between air pollution and type 2 diabetes. Toxicologic Pathology. 2013;41(2):361–73.

104. Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB, et al. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation. 2009;119(4):538–46.

105. Reyes-Caballero H, Rao X, Sun Q, Warmoes MO, Penghui L, Sussan TE, et al. Air pollution-derived particulate matter dysregulates hepatic Krebs cycle, glucose and lipid metabolism in mice. Scientific Reports. 2019;9(1):17423.

106. Deng J, Liu S, Zou L, Xu C, Geng B, Xu G. Lipolysis response to endoplasmic reticulum stress in adipose cells. Journal of Biological Chemistry. 2012;287(9):6240–9.

107. Tan H-H, Fiel MI, Sun Q, Guo J, Gordon RE, Chen L-C, et al. Kupffer cell activation by ambient air particulate matter exposure may exacerbate non-alcoholic fatty liver disease. Journal of Immunotoxicology. 2009;6(4):266–75.

108. Zheng Z, Xu X, Zhang X, Wang A, Zhang C, Hüttemann M, et al. Exposure to ambient particulate matter induces a NASH-like phenotype and impairs hepatic glucose metabolism in an animal model. Journal of Hepatology. 2013;58(1):148–54.

109. Rao X, Patel P, Puett R, Rajagopalan S. Air pollution as a risk factor for type 2 diabetes. Toxicological Sciences. 2015;143(2):231–41.

110. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Research and Clinical Practice. 2018;138:271–81.

111. Sarwar N, Gao P, Kondapally Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. The Lancet. 2010;375(9733):2215–22.

112. Barreto M, Kislaya I, Gaio V, Rodrigues APAP, Santos AJAJ, Namorado S, et al. Prevalence, awareness, treatment and control of diabetes in Portugal: Results from the first National Health examination Survey (INSEF 2015). Diabetes Research and Clinical Practice. 2018;140:271–78.

113. Kerner W, Brückel J. Definition, classification and diagnosis of diabetes mellitus. Diabetologie und Stoffwechsel. 2014;9(1):S96–9.

114. Bonora E, Tuomilehto J. The pros and cons of diagnosing diabetes with A1C.

Diabetes Care. 2011;34(SUPPL. 2): S184–S190.

115. Rajagopalan S, Brook RD. Air pollution and type 2 diabetes: Diabetes. 2012;61(12):3037–45.

116. Liu F, Chen G, Huo W, Wang C, Liu S, Li N, et al. Associations between long-term exposure to ambient air pollution and risk of type 2 diabetes mellitus: A systematic review and meta-analysis. Environmental Pollution. 2019;252:1235–45.

117. Eze IC, Hemkens LG, Bucher HC, Hoffmann B, Schindler C, Künzli N, et al. Association between ambient air pollution and diabetes mellitus in Europe and North America: Systematic review and meta-analysis. Environmental Health Perspectives. 2015;123(5):381–9.

118. Janghorbani M, Momeni F, Mansourian M. Systematic review and metaanalysis of air pollution exposure and risk of diabetes. European Journal of Epidemiology. 2014;29(4):231–42.

119. Balti E V., Echouffo-Tcheugui JB, Yako YY, Kengne AP. Air pollution and risk of type 2 diabetes mellitus: A systematic review and meta-analysis. Diabetes Research and Clinical Practice. 2014;106(2):161–72.

120. Ma R, Zhang Y, Sun Z, Xu D, Li T. Effects of ambient particulate matter on fasting blood glucose: A systematic review and meta-analysis. Environmental Pollution. 2020;258:113589.

121. Elbarbary M, Honda T, Morgan G, Kelly P, Guo Y, Negin J. Ambient air pollution exposure association with diabetes prevalence and glycosylated hemoglobin (HbA1c) levels in China. Cross-sectional analysis from the WHO study of AGEing and adult health wave 1. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering. 2020;55(10):1149–62.

122. Honda T, Pun VC, Manjourides J, Suh H. Associations between long-term exposure to air pollution, glycosylated hemoglobin and diabetes. International Journal of Hygiene and Environmental Health. 2017;220(7):1124–32.

123. Sade MY, Kloog I, Liberty IF, Schwartz J, Novack V, Yitshak Sade M, et al. The association between air pollution exposure and glucose and lipids levels. The Journal of clinical endocrinology and metabolism. 2016;101(6):2460–7.

124. Wolf K, Popp A, Schneider A, Breitner S, Hampel R, Rathmann W, et al. Association between long-term exposure to air pollution and biomarkers related to insulin resistance, subclinical inflammation, and adipokines. Diabetes. 2016;65(11):3314–26.

125. Elbarbary M, Honda T, Morgan G, Guo Y, Guo Y, Kowal P, et al. Ambient air pollution exposure association with anaemia prevalence and haemoglobin levels in Chinese older adults. International Journal of Environmental Research and Public Health. 2020;17(9):3209.

126. Lawes CM, Hoorn S Vander, Rodgers A. Global burden of blood-pressure-related disease, 2001. The Lancet. 2008;371(9623):1513–8.

127. Stanaway JD, Afshin A, Gakidou E, Lim SS, Abate D, Abate KH, et al. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Stu. The Lancet. 2018;392(10159):1923–94.

128. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation. 2016;134(6):441–50.

129. Rodrigues AP, Gaio V, Kislaya I, Graff-Iversen S, Cordeiro E, Silva AC, et al. Sociodemographic disparities in hypertension prevalence: Results from the first Portuguese National Health Examination Survey. Revista Portuguesa de Cardiologia. 2019;38(8):547–55.

130. WHO. Hypertension control. Report of a WHO Expert Committee. World Health Organization technical report series; 1996.

131. Carretero OA, Oparil S. Essential hypertension. Part I: Definition and etiology. Circulation. 2000;101(3):329–35.

132. Cai Y, Zhang B, Ke W, Feng B, Lin H, Xiao J, et al. Associations of Short-Term and Long-Term Exposure to Ambient Air Pollutants With Hypertension. Hypertension. 2016;68(1):62–70.

133. Liang R, Zhang B, Zhao X, Ruan Y, Lian H, Fan Z. Effect of exposure to PM2.5 on blood pressure: A systematic review and meta-analysis. Journal of Hypertension. 2014;32(11):2130–41.

134. Zhang H, Qian J, Zhao H, Wang J, Zhu H, Zhou Y, et al. A study of the association between atmospheric particulate matter and blood pressure in the population. Blood Pressure. 2016;25(3):169–76.

135. Tsai DH, Riediker M, Wuerzner G, Maillard M, Marques-Vidal P, Paccaud F, et al. Short-term increase in particulate matter blunts nocturnal blood pressure dipping and daytime urinary sodium excretion. Hypertension. 2012;60(4):1061–9.

136. Wu MY, Lo WC, Chao C Ter, Wu MS, Chiang CK. Association between air

pollutants and development of chronic kidney disease: A systematic review and meta-analysis. Science of the Total Environment. 2020;706:135522.

137. Newman JD, Rajagopalan S, Levy P, Brook RD. Clearing the air to treat hypertension. Journal of Human Hypertension. 2020;*34*(11): 759-763.

138. Madjid M, Fatemi O. Components of the complete blood count as risk predictors for coronary heart disease: In-depth review and update. Texas Heart Institute Journal. 2013;40(1):17–29.

139. Chmielewski PP, Strzelec B. Elevated leukocyte count as a harbinger of systemic inflammation, disease progression, and poor prognosis: A review. Folia Morphologica (Poland). 2018;77(2):171–8.

140. Lassale C, Curtis A, Abete I, Van Der Schouw YT, Verschuren WMM, Lu Y, et al. Elements of the complete blood count associated with cardiovascular disease incidence: Findings from the EPIC-NL cohort study. Scientific Reports. 2018;8(1):1–11.

141. Fava C, Cattazzo F, Hu Z-D, Lippi G, Montagnana M. The role of red blood cell distribution width (RDW) in cardiovascular risk assessment: useful or hype? Annals of Translational Medicine. 2019;7(20):581–581.

142. Parizadeh SM, Jafarzadeh-Esfehani R, Bahreyni A, Ghandehari M, Shafiee M, Rahmani F, et al. The diagnostic and prognostic value of red cell distribution width in cardiovascular disease; current status and prospective. BioFactors. 2019;45(4) 507–516.

143. Gondalia R, Holliday KM, Baldassari A, Justice AE, Stewart JD, Liao D, et al. Leukocyte traits and exposure to ambient particulate matter air pollution in the women's health initiative and atherosclerosis risk in communities study. Environmental Health Perspectives. 2020;128(1):017004.

144. Lee H, Myung W, Jeong BH, Choi H, Jhun BW, Kim H. Short- and long-term exposure to ambient air pollution and circulating biomarkers of inflammation in non-smokers: A hospital-based cohort study in South Korea. Environment International. 2018;119:264–73.

145. Poursafa P, Kelishadi R, Amini A, Amini A, Amin MM, Lahijanzadeh M, et al. Association of air pollution and hematologic parameters in children and adolescents. Jornal de Pediatria. 2011;87(4):350–6.

146. Hou J, Duan Y, Liu X, Li R, Tu R, Pan M, et al. Associations of long-term exposure to air pollutants, physical activity and platelet traits of cardiovascular risk in a rural Chinese population. Science of the Total Environment. 2020;738:140182.

147. Zhang Z, Chan TC, Guo C, Chang L yun, Lin C, Chuang YC, et al. Long-term exposure to ambient particulate matter (PM2.5) is associated with platelet counts in adults. Environmental Pollution. 2018;240:432–9.

148. Dabass A, Talbott EO, Venkat A, Rager J, Marsh GM, Sharma RK, et al. Association of exposure to particulate matter (PM2.5) air pollution and biomarkers of cardiovascular disease risk in adult NHANES participants (2001-2008). International Journal of Hygiene and Environmental Health. 2016;219(3):301–10.

149. Liao D, Heiss G, Chinchilli VM, Duan Y, Folsom AR, Lin HM, et al. Association of criteria pollutants with plasma hemostatic/inflammatory markers: A population-based study. Journal of Exposure Analysis and Environmental Epidemiology. 2005;15(4):319–28.

150. Mukae H, Vincent R, Quinlan K, English D, Hards J, Hogg JC, et al. The effect of repeated exposure to particulate air pollution (PM10) on the bone marrow. American journal of respiratory and critical care medicine. 2001 Jan;163(1):201–9.

151. Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and the triggering of myocardial infarction. Circulation. 2001;103(23):2810–5.

152. Mozos I. Mechanisms linking red blood cell disorders and cardiovascular diseases. Vol. 2015, BioMed Research International. 2015.

153. Nunes B, Barreto M, Gil APAP, Kislaya I, Namorado S, Antunes L, et al. The first Portuguese National Health Examination Survey (2015): design, planning and implementation. Journal of public health (Oxford, England). 2019;41(3):511–7.

154. Tolonen H. EHES manual. Part B. Fieldwork procedures. Helsinki; 2013.

155. UNESCO. The international standard classification of education 2011. Comparative Social Research; 2012.

156. International Labour Office. International Standard Classification of Occupations: ISCO-08. Vol. I, International Standard Classification of Occupations: ISCO-08. 2012.

157. Anderson JO, Thundiyil JG, Stolbach A. Clearing the Air: A Review of the Effects of Particulate Matter Air Pollution on Human Health. Journal of Medical Toxicology. 2012;8:166–75.

158. Kim SY, Peel JL, Hannigan MP, Dutton SJ, Sheppard L, Clark ML, et al. The temporal lag structure of short-term associations of fine particulate matter chemical constituents and cardiovascular and respiratory hospitalizations.

Environmental Health Perspectives. 2012;120(8):1094–9.

159. Kirkley AG, Sargis RM. Environmental endocrine disruption of energy metabolism and cardiovascular risk. Current Diabetes Reports. 2014;14(6):494-511.

160. Hu D, Jia X, Cui L, Liu J, Chen J, Wang Y, et al. Exposure to fine particulate matter promotes platelet activation and thrombosis via obesity-related inflammation. Journal of Hazardous Materials. 2021;413:125341.

161. Cosselman KE, Krishnan RM, Oron AP, Jansen K, Peretz A, Sullivan JH, et al. Blood pressure response to controlled diesel exhaust exposure in human subjects. Hypertension. 2012; 59(5):943–48.

162. Chen Z, Salam MT, Toledo-Corral C, Watanabe RM, Xiang AH, Buchanan TA, et al. Ambient air pollutants have adverse effects on insulin and glucose homeostasis in mexican americans. Diabetes care. 2016;39(4):547–54.

163. Parker JD, Woodruff TJ, Akinbami LJ, Kravets N. Linkage of the US National Health Interview Survey to air monitoring data: An evaluation of different strategies. Environmental Research. 2008; 106(3):384–92.

164. Kravets N, Parker J. Linkage of the Third National Health and Nutrition Examination Survey to air quality data. 2008;2(149):1–16.

165. Skamarock W, Klemp J, Dudhi j. A Description of the Advanced Research WRF Version 3. Colorado: Natl Cent for Atmos Res Boulder; 2008.

166. ENVIRON. User's guide on Comprehensive Air Quality Model with Extensions (CAMx), version 6.50. report. 2018.

167. Ferreira J, Lopes D, Rafael S, Relvas H, Almeida SSM, Miranda AI, et al. Modelling air quality levels of regulated metals: limitations and challenges. Environmental Science and Pollution Research. 2020;27(27):33916–28.

168. Sá E, Martins H, Ferreira J, Marta-Almeida M, Rocha A, Carvalho A, et al. Climate change and pollutant emissions impacts on air quality in 2050 over Portugal. Atmospheric Environment. 2016;131:209–24.

169. Wang X, Wei W, Cheng S, Li J, Zhang H, Lv Z. Characteristics and classification of PM2.5 pollution episodes in Beijing from 2013 to 2015. Science of the Total Environment. 2018;612:170–9.

170. NCAR. Model for Ozone and Related chemical Tracers, version 4 (MOZART-4). National Center of Atmosferic Research; 2010.

171. Galindo N, Varea M, Gil-Moltó J, Yubero E, Nicolás J. The influence of meteorology on particulate matter concentrations at an urban mediterranean

location. Water, Air, and Soil Pollution. 2011;215(1):365–372.

172. Zhou X, Lin H, Zhang S, Ren J, Wang Z, Zhang Y, et al. Effects of climatic factors on plasma lipid levels: A 5-year longitudinal study in a large Chinese population. Journal of Clinical Lipidology. 2016;10(5):1119–1128.

173. Modesti PA, Rapi S, Rogolino A, Tosi B, Galanti G. Seasonal blood pressure variation: Implications for cardiovascular risk stratification review-article. Hypertension Research. 2018;41(7):475–82.

174. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. Journal of Personality and Social Psychology. 1986;51(6):1173–82.

175. VanderWeele TJ. Explanation in causal inference: Developments in mediation and interaction. International Journal of Epidemiology. 2016;45(6):1904-08.

176. Valeri L, VanderWeele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: Theoretical assumptions and implementation with SAS and SPSS macros. Psychological Methods. 2013;18(2):137–150.

177. Van Der Weele TJ, Knol MJ. A tutorial on interaction. Epidemiologic Methods. 2014;3(1):33-72.

178. World Health Organization [Internet]. Ambient (outdoor) air quality and health. [updated 2018 May 2; cited 2019 May 20]. Available from: https://www.who.int/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health.

179. Brook RD. Cardiovascular effects of air pollution. Clinical Science. 2008;115(5–6):175–87.

180. Simkhovich BZ, Kleinman MT, Kloner RA. Air pollution and cardiovascular injury. Journal of the American College of Cardiology. 2008;52(9):719–26.

181. Meo SA, Suraya F. Effect of environmental air pollution on cardiovascular diseases. European Review for Medical and Pharmacological Sciences. 2015;19(24):4890–7.

182. World Health Organization. Ambient air pollution: a global assessment of exposure and burden of disease.Geneva: World Health Organization; 2016.

183. Araujo JA. Particulate air pollution, systemic oxidative stress, inflammation, and atherosclerosis. Air Quality Atmosphere And Health. 2011;4(1):79–93.

184. Li R, Navab M, Pakbin P, Ning Z, Navab K, Hough G, et al. Ambient ultrafine

particles alter lipid metabolism and HDL anti-oxidant capacity in LDLR-null mice. Journal of Lipid Research. 2013;54(6):1608–15.

185. Yeatts K, Svendsen E, Creason J, Alexis N, Herbst M, Scott J, et al. Coarse particulate matter (PM2.5-10) affects heart rate variability, blood lipids, and circulating eosinophils in adults with asthma. Environmental health perspectives. 2007;115(5):709–14.

186. Chuang K-J, Yan Y-H, Cheng T-J. Effect of Air Pollution on Blood Pressure, Blood Lipids, and Blood Sugar: A Population-Based Approach. Journal Of Occupational And Environmental Medicine. 2010;52(3):258–62.

187. Bind M-A, Peters A, Koutrakis P, Coull B, Vokonas P, Schwartz J. Quantile Regression Analysis of the Distributional Effects of Air Pollution on Blood Pressure, Heart Rate Variability, Blood Lipids, and Biomarkers of Inflammation in Elderly American Men: The Normative Aging Study. Environmental health perspectives. 2016;124(8):1189–98.

188. Fioravanti S, Cesaroni G, Badaloni C, Michelozzi P, Forastiere F, Porta D. Traffic-related air pollution and childhood obesity in an Italian birth cohort. Environmental research. 2018;160:479–86.

189. Moher D, Liberati A, Tetzlaff J, Altman DG, Grp P. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. Plos Medicine. 2009;6(7): e1000097.

190. Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen M-R, Wiklund O, et al. ESC/EAS Guidelines for the management of dyslipidaemias. European Heart Journal. 2011;32(14):1769–818.

191. NCEP-ATP III. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. Circulation. 2002;106(25):3143–421.

192. Joint Committee for Developing Chinese Guidelines. Chinese guidelines on prevention and treatment of dyslipidemia in adults. Chinese Journal of Epidemioogy. 2007;35:390–419.

193. United States Environmental Protection Agency [Internet]. Vocabulary catalog list detaileintegrated risk information system (IRIS) glossary [cited 2019 May 19]. Available from: https://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details%C2%BC&glossaryName%C2%BCIRIS%20Glossary

194. Atkinson RW, Cohen A, Mehta S, Anderson HR. Systematic review and meta-analysis of epidemiological time-series studies on outdoor air pollution and health in Asia. Air Quality Atmosphere and Health. 2012;5(4):383–91.

195. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, et al. Chapter 7: Systematic reviews of etiology and risk . In: Aromataris E, Munn Z, editors. Joanna Briggs Institute; 2017.

196. Balshem H, Helfand M, Schuenemann HJ, Oxman AD, Kunz R, Brozek J, et al. GRADE guidelines: 3. Rating the quality of evidence. Journal of Clinical Epidemiology. 2011;64(4):401–6.

197. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology - A proposal for reporting. Jama-Journal of the American Medical Association. 2000;283(15):2008–12.

198. Harrop O. Air Quality Assessment and Management: A Practical Guide. London: Clay's Library of Health and the Environment; 2002.

199. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated 2011; cited 2018 May 21]. The Cochrane Collaboration. Available from. http://handbook.cochrane.org. 2011.

200. Higgins JPTT, White IR, Anzures-Cabrera J. Meta-analysis of skewed data: Combining results reported on log-transformed or raw scales. Statistics in Medicine. 2008;27(29):6072–92.

201. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. British Medical Journal. 2003;327(7414):557–60.

202. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Bmj-British Medical Journal. 1997;315(7109):629–34.

203. Schwarzer G. meta: an R package for meta-analysis. R. News.2007;7(3):40–45.

204. Gordon M, Lumley T. Forestplot: Advanced Forest Plot Using "grid" Graphics; 2017.

205. Chuang K-J, Yan Y-H, Chiu S-Y, Cheng T-J. Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. Occupational and environmental medicine. 2011;68(1):64–8.

206. Sorensen M, Hjortebjerg D, Eriksen KT, Ketzel M, Tjonneland A, Overvad K, et al. Exposure to long-term air pollution and road traffic noise in relation to cholesterol: A cross-sectional study. Environment international. 2015;85:238–43.

207. Wallwork RS, Colicino E, Zhong J, Kloog I, Coull BA, Vokonas P, et al. Ambient

Fine Particulate Matter, Outdoor Temperature, and Risk of Metabolic Syndrome. American journal of epidemiology. 2017;185(1):30–9.

208. Poursafa P, Amin MM, Mansourian M, Hajizadeh Y, Sadeghian B, Kelishadi R. Association of Exposure to Fine Particulate Matter and Risk Factors of Non-Communicable Diseases in Children and Adolescents. International Journal of Pediatrics. 2017;5(10):5863–70.

209. Tan C, Wang Y, Lin M, Wang Z, He L, Li Z, et al. Long-term high air pollution exposure induced metabolic adaptations in traffic policemen. Environmental Toxicology and pharmacology. 2018;58:156–62.

210. Wang M, Zheng S, Nie Y, Weng J, Cheng N, Hu X, et al. Association between Short-Term Exposure to Air Pollution and Dyslipidemias among Type 2 Diabetic Patients in Northwest China: A Population-Based Study. International Journal of Environmental Research and Public Health. 2018;15(4):631–43.

211. Ghosh R, Gauderman WJ, Minor H, Youn HA, Lurmann F, Cromar KR, et al. Air pollution, weight loss and metabolic benefits of bariatric surgery: a potential model for study of metabolic effects of environmental exposures. Pediatric Obesity. 2018;13(5):312–20.

212. McGuinn LA, Schneider A, McGarrah RW, Ward-Caviness C, Neas LM, Di Q, et al. Association of long-term PM2.5 exposure with traditional and novel lipid measures related to cardiovascular disease risk. Environment International. 2019;122:193–200.

213. Shin J, Choi J, Kim KJ. Association between long-term exposure of ambient air pollutants and cardiometabolic diseases: A 2012 Korean Community Health Survey. Nutrition Metabolism and Cardiovascular Diseases. 2019;29(2):144–51.

214. Wu X, Broadwin R, Basu R, Malig B, Ebisu K, Gold EB, et al. Associations between fine particulate matter and changes in lipids/lipoproteins among midlife women. Science of the Total Environment. 2019;654:1179–86.

215. Li H, Chen R, Cai J, Cui X, Huang N, Kan H. Short-term exposure to fine particulate air pollution and genome-wide DNA methylation: A randomized, double-blind, crossover trial. Environment International. 2018;120:130–6.

216. Miller M, Cannon CP, Murphy SA, Qin J, Ray KK, Braunwald E, et al. Impact of triglyceride levels beyond low-density lipoprotein cholesterol after acute coronary syndrome in the PROVE IT-TIMI 22 trial. Journal of the American College of Cardiology. 2008;51(7):724–30.

217. Watts GF, Karpe F. Triglycerides and atherogenic dyslipidaemia: extending

treatment beyond statins in the high-risk cardiovascular patient. Heart. 2011;97(5):350–6.

218. Ahn CH, Choi SH. New drugs for treating dyslipidemia: Beyond statins. Diabetes and Metabolism Journal. 2015;39(2):87–94.

219. Fiordelisi A, Piscitelli P, Trimarco B, Coscioni E, Iaccarino G, Sorriento D. The mechanisms of air pollution and particulate matter in cardiovascular diseases. Heart Failure Reviews. 2017;22(3):337–47.

220. Wu X, Basu R, Malig B, Broadwin R, Ebisu K, Gold EB, et al. Association between gaseous air pollutants and inflammatory, hemostatic and lipid markers in a cohort of midlife women. Environment International. 2017;107:131–9.

221. Oakes M, Baxter L, Long TC. Evaluating the application of multipollutant exposure metrics in air pollution health studies. Environment International. 2014; 69:90–9.

222. Davalos AD, Luben TJ, Herring AH, Sacks JD. Current approaches used in epidemiologic studies to examine short-term multipollutant air pollution exposures. Annals of Epidemiology. 2017; 27:145–153.

223. Cohen JC, Noakes TD, Benade AJS. Serum Triglyceride Responses To Fatty Meals - Effects Of Meal Fat-Content. American Journal of Clinical Nutrition. 1988;47(5):825–7.

224. Ginsberg HN, Karmally W, Siddiqui M, Holleran S, Tall AR, Rumsey SC, et al. A Dose-response Study of The Effects of Dietary-cholesterol on Fasting and Postprandial Lipid And Lipoprotein Metabolism In Healthy-young Men. Arteriosclerosis and Thrombosis. 1994;14(4):576–86.

225. Farzadfar F, Finucane MM, Danaei G, Pelizzari PM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in serum total cholesterol since 1980: systematic analysis of health examination surveys and epidemiological studies with 321 country-years and 3.0 million participants. Lancet. 2011;377(9765):578–86.

226. An Z, Jin Y, Li J, Li W, Wu W. Impact of Particulate Air Pollution on Cardiovascular Health. Current Allergy and Asthma Reports. 2018;18(3):1–7.

227. Burnett R, Chen H, Szyszkowicz M, Fann N, Hubbell B, Pope CA, et al. Global estimates of mortality associated with longterm exposure to outdoor fine particulate matter. Proceedings of the National Academy of Sciences of the United States of America. 2018;115(38): 9592–97.

228. Shin WY, Kim JH, Lee G, Choi S, Kim SR, Hong YC, et al. Exposure to ambient fine particulate matter is associated with changes in fasting glucose and lipid

profiles: A nationwide cohort study. BMC Public Health. 2020;20(1):1-11.

229. Mao S, Chen G, Liu F, Li N, Wang C, Liu Y, et al. Long-term effects of ambient air pollutants to blood lipids and dyslipidemias in a Chinese rural population. Environmental Pollution. 2020; 256:113403.

230. Gaio V, Roquette R, Dias CMCM, Nunes B. Ambient air pollution and lipid profile: Systematic review and meta-analysis. Environmental Pollution. 2019;254:113036.

231. Hassan L, Pecht T, Goldstein N, Haim Y, Kloog I, Yarza S, et al. The effects of ambient particulate matter on human adipose tissue. Journal of Toxicology and Environmental Health - Part A: Current Issues. 2019;82(9):564-576.

232. Gutierrez DA, Puglisi MJ, Hasty AH. Impact of increased adipose tissue mass on inflammation, insulin resistance, and dyslipidemia. Current Diabetes Reports. 2009;9(1):26-32.

233. Swainson MG, Batterham AM, Tsakirides C, Rutherford ZH, Hind K. Prediction of whole-body fat percentage and visceral adipose tissue mass from five anthropometric variables. PLoS ONE. 2017;12(5): e0177175

234. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020.

235. Textor J, van der Zander B, Gilthorpe MS, Liśkiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: The R package “dagitty.” International Journal of Epidemiology. 2016;45(6):1887–94.

236. Dong GH, Wang J, Zeng XW, Chen L, Qin X Di, Zhou Y, et al. Interactions between Air Pollution and Obesity on Blood Pressure and Hypertension in Chinese Children. Epidemiology. 2015;26(5):740–47.

237. Jeong SM, Park JH, Kim HJ, Kwon H, Hwang SE. Effects of abdominal obesity on the association between air pollution and kidney function. International Journal of Obesity. 2020;44(7):1568–76.

238. Xing X, Hu L, Guo Y, Bloom MS, Li S, Chen G, et al. Interactions between ambient air pollution and obesity on lung function in children: The Seven Northeastern Chinese Cities (SNEC) Study. Science of the Total Environment. 2020;699: 134397.

239. Kim JS, Chen Z, Alderete TL, Toledo-Corral C, Lurmann F, Berhane K, et al. Associations of air pollution, obesity and cardiometabolic health in young adults: The Meta-AIR study. Environment International. 2019;133,105180.

240. Deschenes O, Wang H, Wang S, Zhang P. The effect of air pollution on body weight and obesity: Evidence from China. Journal of Development Economics.

2020;145:102461.

241. Yang Z, Song Q, Li J, Zhang Y. Air pollution as a cause of obesity: Micro-level evidence from Chinese cities. International Journal of Environmental Research and Public Health. 2019;16(21):4296–4311.

242. Gama C, Monteiro A, Pio C, Miranda AI, Baldasano JM, Tchepel O. Temporal patterns and trends of particulate matter over Portugal: a long-term analysis of background concentrations. Air Quality, Atmosphere and Health. 2018;11(4):397–407.

243. Barnett AG. It's safe to say there is no safe level of air pollution. Australian and New Zealand Journal of Public Health. 2014;38(5):407–408.

244. World Health Organization. Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity. WHO; 2011.

245. ILOSTAT [Internet]. International Standard Classification of Education (ISCED): Aggregate levels of education. [cited 2019 May 21]. Available from: https:// ilostat.ilo.org/resources/concepts-and-definitions/classificationeducation/. 2020.

246. ESRI [Internet]. Available from: https://www.esri.com/en-us/arcgis/about-arcgis/overview.

247. Nagata Y, Yoshikawa J, Hashimoto A, Yamamoto M, Payne AH, Todokoro K. Proplatelet formation of megakaryocytes is triggered by autocrine-synthesized estradiol. Genes and Development. 2003;17(23):2864–9.

248. Rudel RA, Perovich LJ. Endocrine disrupting chemicals in indoor and outdoor air. Atmospheric Environment. 2009;43(1):170–81.

249. Cozzi E, Wingard CJ, Cascio WE, Devlin RB, Miles JJ, Bofferding AR, et al. Effect of ambient particulate matter exposure on hemostasis. Translational Research. 2007;149(6):324–32.

250. Emmerechts J, Alfaro-Moreno E, Vanaudenaerde BM, Nemery B, Hoylaerts MF. Short-term exposure to particulate matter induces arterial but not venous thrombosis in healthy mice. Journal of Thrombosis and Haemostasis. 2010;8(12):2651–61.

251. Haybar H, Pezeshki SMS, Saki N. Evaluation of complete blood count parameters in cardiovascular diseases: An early indicator of prognosis? Vol. 110, Experimental and Molecular Pathology. 2019;110:104267.

252. Ding Z, Xie Z, Su Y, Qi J, Cui B. The lagged effect of air pollution on human eosinophils: a distributed lag non-linear model. International journal of occupational medicine and environmental health. 2020;33(3):299–310.

253. Biino G, Santimone I, Minelli C, Sorice R, Frongia B, Traglia M, et al. Age- And Sex-Related Variations in Platelet Count in Italy: A Proposal of Reference Ranges Based on 40987 Subjects' Data. PLoS ONE. 2013;8(1): e54289.

254. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. Nature Reviews Nephrology. 2020;16(4):223–237.

255. Gaio V, Roquette R, Monteiro A, Ferreira J, Rafael S, Dias CM, et al. Exposure to ambient particulate matter increases blood count parameters with potential to mediate a cardiovascular event: results from a population-based study in Portugal. Air Quality, Atmosphere and Health. 2021;1–14.

256. Bilenko N, Van Rossem L, Brunekreef B, Beelen R, Eeftens M, Hoek G, et al. Traffic-related air pollution and noise and children's blood pressure: Results from the PIAMA birth cohort study. European Journal of Preventive Cardiology. 2015; 16(1):4–12.

257. Pieters N, Koppen G, van Poppel M, de Prins S, Cox B, Dons E, et al. Blood pressure and same-day exposure to air pollution at school: Associations with nano-sized to coarse PM in children. Environmental Health Perspectives. 2015; 123(7):737–742.

258. Tsai DH, Guessous I, Riediker M, Paccaud F, Gaspoz JM, Theler JM, et al. Short-term effects of particulate matters on pulse pressure in two general population studies. Journal of Hypertension. 2015;33(6):1144–1152.

259. Harrabi I, Rondeau V, Dartigues JF, Tessier JF, Filleul L. Effects of particulate air pollution on systolic blood pressure: A population-based approach. Environmental Research. 2006; 101(1):89–93.

260. Guo P, He Z, Jalaludin B, Knibbs LD, Leskinen A, Roponen M, et al. Short-Term Effects of Particle Size and Constituents on Blood Pressure in Healthy Young Adults in Guangzhou, China. Journal of the American Heart Association. 2021;10(10):e019063.

261. Scheers H, Nawrot TS, Nemery B, Casas L. Changing places to study short-term effects of air pollution on cardiovascular health: a panel study. Environmental Health. 2018;17(1):1–8.

262. Xu N, Lv X, Yu C, Guo Y, Zhang K, Wang Q. The association between short-term exposure to extremely high level of ambient fine particulate matter and blood pressure: a panel study in Beijing, China. Environmental Science and Pollution Research. 2020;27:28113–28122.

263. Mlinarić A, Horvat M, Smolčić VŠ. Dealing with the positive publication bias: Why

you should really publish your negative results. Biochemia Medica. 2017; 27(3):447–52.

264. Koletsi D, Karagianni A, Pandis N, Makou M, Polychronopoulou A, Eliades T. Are studies reporting significant results more likely to be published? American Journal of Orthodontics and Dentofacial Orthopedics. 2009;136(5):e1632–e1635.

265. Environmental Protection Agency [Internet]. Particulate Matter (PM10) Trends. [cited 2019 May 21]. Available from: https://www.epa.gov/air-trends/particulate-matter-pm10-trends.

266. Wang Y, Li W, Gao W, Liu Z, Tian S, Shen R, et al. Trends in particulate matter and its chemical compositions in China from 2013–2017. Science China Earth Sciences. 2019;62(12):1857–1871.

267. European Environment Agency (EEA). Air quality in Europe - 2020 report. Luxembourg: Publications Office of the European Union; 2020.

268. Gaio V, Antunes L, Namorado S, Barreto M, Gil A, Kyslaya I, et al. Prevalence of overweight and obesity in Portugal: Results from the First Portuguese Health Examination Survey (INSEF 2015). Obesity Research and Clinical Practice. 2018;12(1):40–50.

269. Baxter LK, Dionisio KL, Burke J, Ebelt Sarnat S, Sarnat JA, Hodas N, et al. Exposure prediction approaches used in air pollution epidemiology studies: Key findings and future recommendations. Journal of Exposure Science and Environmental Epidemiology. 2013;23(6):654–659.

9. Supplementary materials

Table S1. Search terminology strategy (**Article 1**).

DataBase	Search terminology	Number of Records
PubMed	(“air pollution”[Title/Abstract] OR “air pollutant*”[Title/Abstract] OR “particulate matter”[Title/Abstract] OR “PM10”[Title/Abstract] OR “PM2.5”[Title/Abstract] OR “ozone”[Title/Abstract] OR “O3”[Title/Abstract] OR “sulphur dioxide”[Title/Abstract] OR “SO2”[Title/Abstract] OR “nitrogen dioxide”[Title/Abstract] OR “NO2”[Title/Abstract] OR “carbon monoxide”[Title/Abstract] OR “black carbon”[Title/Abstract] OR “ultrafine particles”[Title/Abstract] OR “NOx”[Title/Abstract]) AND (“lipid profile”[Title/Abstract] OR “high density lipoprotein cholesterol”[Title/Abstract] OR “HDL”[Title/Abstract] OR “low density lipoprotein cholesterol”[Title/Abstract] OR “LDL”[Title/Abstract] OR “cholesterol”[Title/Abstract] OR “triglycerides”[Title/Abstract] OR “dyslipid*”[Title/Abstract] OR “hypercholesterol*”[Title/Abstract], OR “hypertriglycerid*”[Title/Abstract] OR dyslipidemias [MeSH Terms] OR triglycerides [MeSH Terms] OR cholesterol [MeSH Terms]) Filters: Humans	542
Web of Science	TS=(“air pollution” OR "air pollutant*” OR "particulate matter” OR "PM10” OR "PM2.5” OR "ozone” OR “O3” OR "sulphur dioxide” OR “SO2” OR "nitrogen dioxide” OR “NO2” OR "carbon monoxide” OR "black carbon” OR "ultrafine particles” OR "NOx”) AND TS=(“lipid profile” OR "high_density lipoprotein cholesterol” OR "HDL” OR "low_density lipoprotein cholesterol” OR "LDL” OR "cholesterol” OR "triglycerides” OR “dyslipid*” OR “hypercholesterol*” OR “hypertriglycerid*“) NOT TS=(“mice” OR “rat” OR “rats” OR “cell*” OR “in vitro”)	605
Scopus	TITLE-ABS(“air pollution” OR "air pollutant*” OR "particulate matter” OR "PM10” OR "PM2.5” OR "ozone” OR “O3” OR "sulphur dioxide” OR “SO2” OR "nitrogen dioxide” OR “NO2” OR "carbon monoxide” OR "black carbon” OR "ultrafine particles” OR "NOx”) AND TITLE-ABS(“lipid profile” OR "high_density lipoprotein cholesterol” OR "HDL” OR "low_density lipoprotein cholesterol” OR "LDL” OR "cholesterol” OR "triglycerides” OR “dyslipid*” OR “hypercholesterol*” OR “hypertriglycerid*“) NOT TITLE-ABS (“mice” OR “rat” OR “rats” OR “cell*” OR “in vitro”)	363

Table S2. Classification of the included cross-sectional studies based on items from the JBI Checklist (**Article 1**).

Author, year	Items from the JBI Checklist for Analytical Cross Sectional Studies*								Score classification	Quality
	1	2	3	4	5	6	7	8		
Bell et al. 2017	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	High
Cai et al. 2017	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	7	High
Chen et al. 2016	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	High
Chuang et al. 2010	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	High
Chuang et al. 2011	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	High
Eze et al. 2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	High
Fioravanti et al. 2018	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	7	High
McGuinn et al. 2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	High
Poursafa et al. 2017	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	No	6	Moderate
Shanley et al. 2016	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	7	High
Shin et al. 2019	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	7	High
Sorensen et al. 2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	High
Wang et al. 2018	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	7	High
Yang et al. 2018	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	High

*Item 1: Were the criteria for inclusion in the sample clearly defined? Item 2: Were the study subjects and the setting described in detail? Item 3: Was the exposure measured in a valid and reliable way? Item 4: Were objective, standard criteria used for measurement of the condition? Item 5: Were confounding factors identified? Item 6: Were strategies to deal with confounding factors stated? Item 7: Were the outcomes measured in a valid and reliable way? Item 8: Was appropriate statistical analysis used? (Moola et al. 2017).

Table S3. Classification of the included cohort studies based on items from the JBI Checklist (**Article 1**).

Author, year	Items from the JBI Checklist for Analytical Cohort Studies*											Score classification	Quality
	1	2	3	4	5	6	7	8	9	10	11		
Bind et al.2016	Yes	Yes	No	Yes	Yes	Yes	Unclear	Yes	No	No	Yes	7	Moderate
Ghosh et al.2018	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Not applicable	Yes	10	High
Lee et al. 2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	9	High
Li et al.2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Not applicable	Yes	10	High
Sade et al.2016	Yes	Yes	Yes	Yes	Yes	Not applicable	Yes	Unclear	Unclear	Unclear	Yes	8	Moderate
Wallwork et al.2017	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	9	High
Yeatts et al.2007	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Not applicable	Yes	9	High
Wu et al. 2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	9	High

*Item 1: Were the two groups similar and recruited from the same population? Item 2: Were the exposures measured similarly to assign people to both exposed and unexposed groups? Item 3: Was the exposure measured in a valid and reliable way? Item 4: Were confounding factors identified? Item 5: Were strategies to deal with confounding factors stated? Item 6: Were the participants free of the outcome at the start of the study? Item 7: Were the outcomes measured in a valid and reliable way? Item 8: Was the follow up time reported and sufficient to be long enough for outcomes to occur? Item 9: Was follow up complete, and if not, were the reasons to loss to follow up described and explored? Item 10: Were strategies to address incomplete follow up utilized? Item 11: Was appropriate statistical analysis used? (Moola et al. 2017).

Table S4. GRADE classification of the cumulative evidence from the seven meta-analysis (**Article 1**).

Pollutant/outcome	Global Effect Estimate	Quality assessment*					Quality of evidence
		Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	
PM_{10}/TC	0.23 (−0.98 to 1.45)	No	Very serious	No	Yes	No	Very low
NO_2/TC	0.22 (−0.59 to 1.03)	No	Serious	No	Yes	-	Very low
PM_{10}/HDL	0.10 (−0.36 to 0.56)	No	No	No	Yes	No	Very low
NO_2/HDL	−0.45 (−2.65 to 1.81)	No	Very serious	No	Yes	-	Very low
PM_{10}/LDL	0.12 (−1.78 to 2.06)	No	Very serious	No	Yes	-	Very low
PM_{10}/TG	3.14 (1.36 to 4.95)	No	Serious	No	No	No	Very low
NO_2/TG	4.24 (1.37 to 7.19)	No	Serious	No	No	-	Very low

* Risk of bias based on the study design characteristics of the included studies, namely the appropriate eligibility criteria. Inconsistency based the I^2 statistic: if the value was <50%, we considered that there is not inconsistency; if the value was ≥50% and <80%, we considered that there is a serious risk of inconsistency; and if the value was ≥80%, we considered that there is a very serious risk of inconsistency. Indirectness: we considered no indirectness because evidence comes from research that directly compares the outcomes that we are interested in this meta-analysis; Imprecision based on the examination of the 95% confidence intervals. Publication bias based the Egger's test for asymmetry. In the case of only 2 studies, it was not possible to apply Egger's test and publication bias was not assessed (Balshem et al. 2011).

Table S5. Comparison of the general characteristics of the included *versus* excluded INSEF mainland participants (**Article 2**).

Characteristics	Included participants (n=2390)	Excluded participants (n=1077)	Total mainland INSEF participants (n=3467)
Sex (n=3467) - %			
Males	47.41	47.92	47.50
Females	52.59	52.08	52.50
Age group (n=3467) - %			
25-49 years	52.76	51.47	52.53
50-74 years	47.24	48.53	47.47
[a] Level of Education (n=3464) - %			
Low education	58.44	60.85	58.86
Medium education	21.86	20.20	21.57
High education	19.70	18.95	19.57
[b] Occupation (n=3200) - %			
White-collar occupation	62.61	60.13	62.18
Blue-collar occupation	37.39	39.87	37.82
Lifestyles variables - %			
[c] Smokers (n=3465)	**20.95**	**26.21**	**21.87**
[d] Excessive alcohol consumption (n=3465)	36.07	36.00	36.06
[e] Unhealthy Diet (n=3463)	36.10	36.58	36.19
[f] Sedentary (n=3443)	44.53	45.68	44.73
[g] Abdominal Obesity (n=3435) - %	75.21	78.60	75.81
Diagnosed Dyslipidaemia (n=3433) - %	24.95	26.74	25.26
Lipid-Lowering medication (n=3467) - %	19.34	19.92	19.44
Diagnosed Diabetes (n=3453) - %	**7.78**	**10.75**	**8.31**
Diabetes medication (n=3467) - %	**7.09**	**9.87**	**7.58**
Individual allocated 1-year average temperature (n=3465) - ºC(mean ± standard deviation)	15.70±1.4	15.30±1.4	15.60±1.4

[a] Low education: levels 0-2 of the ISCED 2011; Medium education: levels 3-4 of the ISCED 2011, High education: levels 5-8 of the ISCED 2011; [b] White-collar occupation: Managers, Professionals, Technicians & Associate Professional, Clerical Support Workers and Services & Sales Workers; Blue–collar occupation: Skilled Agricultural Workers, Craft & Related trades Workers, Plant & Machine Operators and Elementary occupations. [c] Smokers include current daily and occasional smokers. [d] 3 or more days/week of consumption of at least one of the following alcoholic beverages (wine, beer, brandy/bagasse, Port wine/Martini/liqueur, Whisky/Gin/Vodka). [e] No consumption of fruit and vegetables at least once a day. [f] Reading, watching TV or other sedentary activities declared as the best description of leisure time activities during the last 12 months. [g] Waist-to-height ratio (WHtR) ≥0.5.

Results in bold are those with statistically significant difference between included versus excluded INSEF mainland participants, according to the Pearson's chi-squared test (p<0.05).

Table S6- Percent changes in TG, TC, HDL-C, LDL-C and HbA1C per 1 µg/m^3 increment of PM_{10} among all participants, participants with AO and participants without AO, assuming the criteria of 20 km instead of 30 km to select the air monitoring stations contributing to Individual allocated 1-year average PM_{10} concentrations (**Article 2**).

	% Change per 1 µg/m^3 of PM_{10} increment				
	TG	TC	HDL-C	LDL-C	HbA1C
All included participants (n=1819)					
Not adjusted model	0.27 (-1.00; 1.56)	0.08 (-0.39; 0.49)	0.09 (-0.51; 0.69)	**-1.42 (-2.47; -0.35)**	0.02 (-0.22; 0.27)
***Adjusted model**	**1.78 (0.31; 3.28)**	**0.50 (0.04; 0.96)**	-0.33 (-1.12; 0.46)	-0.12 (-0.83; 0.60)	0.17 (-0.13; 0.46)
Participants with AO (n=1363)[a]					
Not adjusted model	0.97 (-0.71; 2.69)	0.12 (-0.30; 0.53)	-0.22 (-0.78; 0.35)	**-1.30 (-2.37; -0.21)**	-0.01 (-0.29; 0.28)
***Adjusted model**	**2.00 (0.26; 3.76)**	**0.53 (0.11; 0.94)**	-0.60 (-1.26; 0.07)	-0.06 (-0.82; 0.71)	0.13 (-0.23; 0.50)
Participants without AO (n=439)					
Not adjusted model	-1.59 (-4.18; 1.08)	0.06 (-0.82; 0.95)	0.47 (-0.74; 1.69)	**-1.57 (-3.04; -0.08)**	**0.33 (0.02; 0.65)**
***Adjusted model**	-0.12 (-3.27; 3.13)	0.60 (-0.43; 1.64)	0.40 (-1.14; 1.97)	-0.17 (-1.41; 1.08)	0.30 (-0.04; 0.64)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and Individual allocated estimated 1-year average temperature; [a] Participants without available data on waist or height measurements (n=17) and consequently without AO data were excluded from the stratified analysis. **Abbreviations:** AO- Abdominal Obesity; TC - Total Cholesterol; HDL-C – High density Lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; TG – Triglycerides.; PM- Particulate matter.

Table S7- Percent changes in TG, TC, HDL-C, LDL-C and HbA1C per 1 µg/m^3 increment of PM_{10} among all participants, participants with AO and participants without AO, assuming the PM_{10} obtained by the air quality modelling system (WRF-CAMx) (**Article 2**).

	% Change per 1 µg/m^3 of PM_{10} increment				
	TG	TC	HDL-C	LDL-C	HbA1C
All included participants(n=2390)					
Not adjusted model	-0.60	-0.02	0.06	-0.31	-0.07
	(-1.25; 0.05)	(-0.43; 0.38)	(-0.33; 0.46)	(-1.06; 0.45)	(-0.27; 0.14)
***Adjusted model**	0.14	0.04	0.02	-0.20	-0.03
	(-0.89; 1.19)	(-0.42; 0.50)	(-0.44; 0.47)	(-1.02; 0.62)	(-0.25; 0.20)
Participants with AO (n=1831)[a]					
Not adjusted model	-0.03	0.06	-0.10	-0.14	-0.03
	(-0.77; 0.72)	(-0.37; 0.48)	(-0.55; 0.36)	(-0.91; 0.64)	(-0.30; 0.24)
***Adjusted model**	0.43	0.09	-0.08	-0.09	-0.03
	(-0.67; 1.55)	(-0.37; 0.54)	(-0.66; 0.51)	(-0.90; 0.74)	(-0.33; 0.27)
Participants without AO (n=536)					
Not adjusted model	-1.25	-0.11	-0.10	-0.47	0.17
	(-2.75; 0.28)	(-0.62; 0.40)	(-0.76; 0.57)	(-1.48; 0.55)	(-0.05; 0.38)
***Adjusted model**	-0.63	0.01	-1.36	-0.36	0.14
	(-2.02; 0.78)	(-0.61; 0.63)	(-0.51; 0.51)	(-1.54; 0.83)	(-0.05; 0.33)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary. Individual allocated 1-year average temperature was not included because PM_{10} obtained by air quality modelling already taking into account meteorological variables including temperature. [a] Participants without available data on waist or height measurements (n=17) and consequently without AO data were excluded from the stratified analysis. **Abbreviations:** AO- Abdominal Obesity; TC - Total Cholesterol; HDL-C – High density Lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; TG – Triglycerides.; PM- Particulate matter.

Table S8- Percent changes in TG, TC, HDL-C, LDL-C and HbA1C per 1 µg/m^3 increment of PM_{10} among all participants, participants with AO and participants without AO after exclusion of participants with diagnosed dyslipidaemia or taking lipid-lowering medication (in the TG, CT, HDL-C and LDL-C modelling) and diabetic participants or taking medication for diabetes treatment (in the HbA1C modelling) (**Article 2**).

	% Change per 1 µg/m^3 of PM_{10} increment				
	TG	TC	HDL-C	LDL-C	HbA1C
All included participants (n=1709, n=2165 for HbA1C)					
Not adjusted model	-0.03 (-1.65; 1.629	0.09 (-0.70; 0.89)	0.30 (-0.33; 0.94)	-0.95 (-2.03; 0.15)	-0.05 (-0.28; 0.18)
***Adjusted model**	**2.08 (0.40; 3.80)**	0.63 (-0.22; 1.48)	-0.11 (-0.74; 0.52)	0.50 (-0.29; 1.30)	0.01 (-0.33; 0.35)
Participants with AO [a] (n=1208, n=1625 for HbA1C)					
Not adjusted model	0.93 (-1.02; 2.92)	0.21 (-0.60; 2.92)	0.00 (-0.62; 0.63)	-0.64 (-1.71; 0.43)	-0.07 (-0.38; 0.25)
***Adjusted model**	**2.30 (0.22; 4.43)**	0.66 (-0.18; 1.50)	-0.28 (-1.06; 0.50)	0.56 (-0.25; 1.38)	-0.01 (-0.44; 0.42)
Participants without AO (n=482, n=519 for HbA1C)					
Not adjusted model	-1.73 (-3.63; 0.20)	0.10 (-0.74; 0.94)	0.47 (-0.54; 1.49)	-1.20 (-2.48; 0.09)	0.22 (-0.04; 0.49)
***Adjusted model**	1.08 (-1.41; 3.65)	0.74 (-0.30; 1.79)	0.40 (-0.59; 1.40)	0.38 (-0.69; 1.47)	0.10 (-0.28; 0.48)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 1-year average temperature; [a] Participants without available data on waist or height measurements (n=19, n=21 for the HbA1C) and consequently without AO data were excluded from the stratified analysis. **Abbreviations:** AO- Abdominal Obesity; TC - Total Cholesterol; HDL-C – High density Lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; TG – Triglycerides.; PM- Particulate matter.

Table S9- Percent changes in TG, TC, HDL-C, LDL-C and HbA1C per 1 µg/m^3 increment of PM_{10} among all participants, participants with AO and participants without AO, considering waist-to-hip ratio as the measure of Abdominal Obesity. (**Article 2**).

	% Change per 1 µg/m^3 of PM_{10} increment				
	TRIG	TC	HDL-C	LDL-C	HbA1C
All included participants (n=2390)					
Not adjusted model	0.19 (-1.08; 1.48)	0.09 (-0.52; 0.70)	0.12 (-0.43; 0.68)	-0.89 (-1.87; 0.11)	-0.09 (-0.37; 0.19)
***Adjusted model**	**1.70 (0.11; 3.32)**	0.59 (-0.07; 1.24)	-0.20 (-0.74; 0.33)	0.47 (-0.20; 1.15)	-0.01 (-0.48; 0.47)
Participants with AO (n=1583)[a]					
Not adjusted model	0.84 (-0.40; 2.09)	0.11 (-0.52; 0.74)	-0.28 (-0.77; 0.21)	-0.67 (-1.66; 0.33)	-0.11 (-0.52; 0.30)
***Adjusted model**	**1.68 (0.03; 3.35)**	0.50 (-0.20; 1.21)	-0.46 (-1.16; 0.24)	0.41 (-0.34; 1.16)	-0.07 (-0.75; 0.61)
Participants without AO (n=791)					
Not adjusted model	-1.06 (-3.24; 1.18)	0.15 (-0.29; 0.58)	0.62 (-0.34; 1.5)	**-1.19 (-2.10; -0.27)**	**0.17 (0.03; 0.32)**
***Adjusted model**	1.35 (-0.51; 3.25)	0.78 (-0.07; 1.62)	0.51 (-0.46; 1.35)	0.54 (-0.20; 1.28)	0.11 (-0.12; 0.34)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 1-year average temperature. [a] Participants without available data on waist or hip measurements (n=16) and consequently without AO data were excluded from the stratified analysis. **Abbreviations:** AO- Abdominal Obesity; TC - Total Cholesterol; HDL-C – High density Lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; TG – Triglycerides.; PM- Particulate matter.

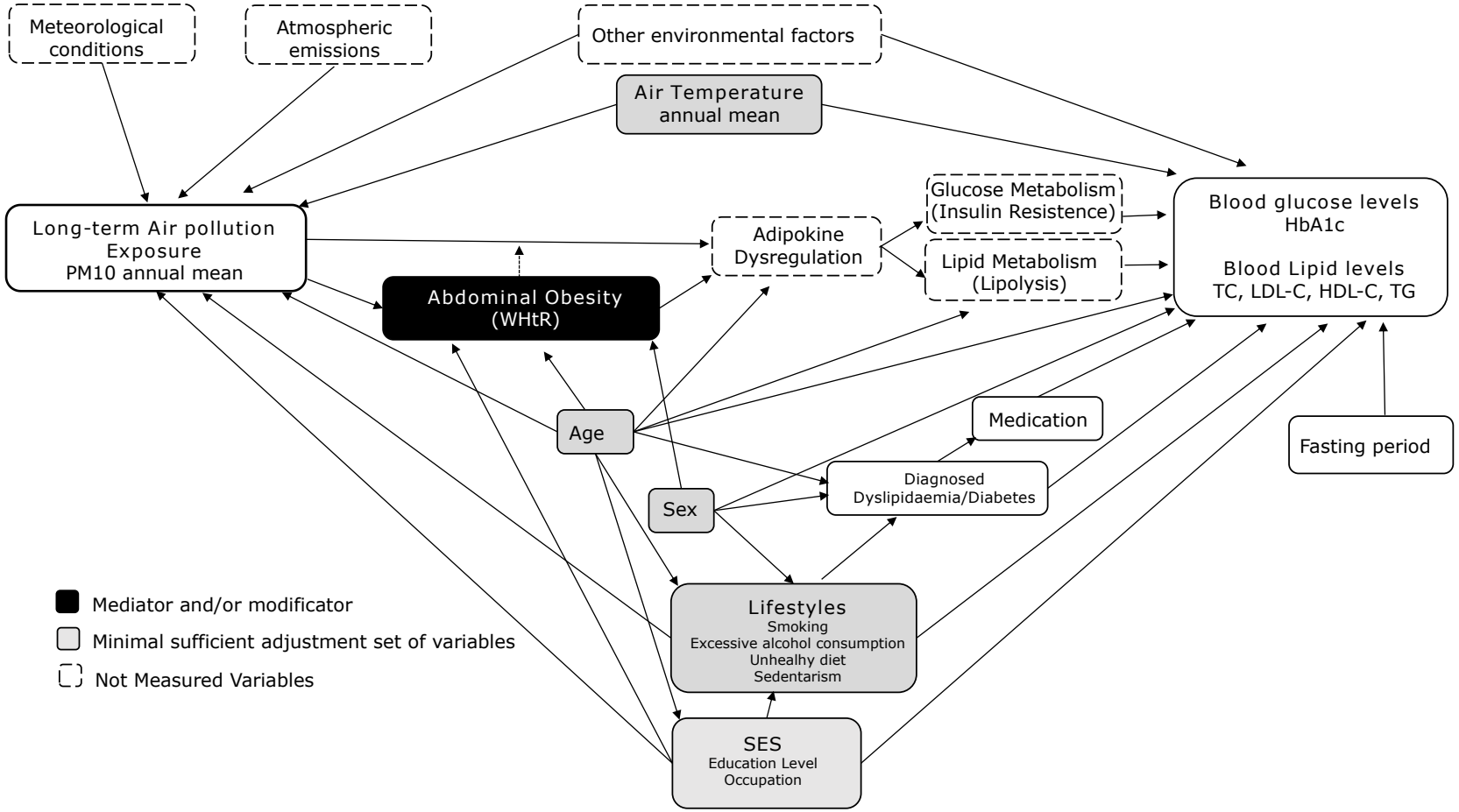

Figure S1- A Directed Acyclic Graph (DAG) for the association between long-term air pollution exposure (PM_{10} annual mean values) and blood lipid and glucose levels. The dashed row representing the modification effect of abdominal obesity was not included in the analysis of the minimal sufficient adjustment set of variables. Abbreviations: HbA1c, Glycated haemoglobin; TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; TG, Triglycerides; PM, Particulate matter; WHtR, Waist-to-Height Ratio (**Article 2**).

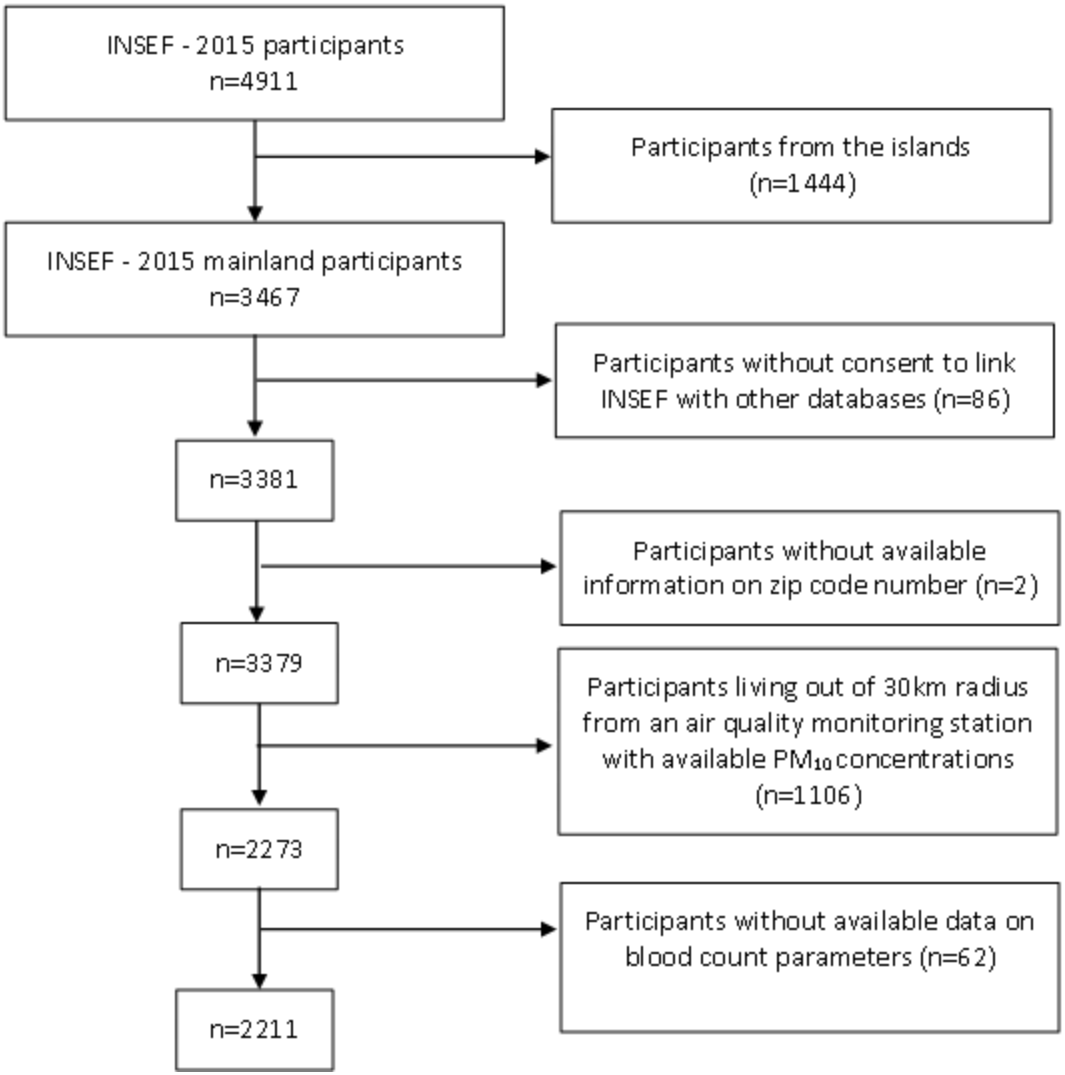

Figure S2. Flow diagram of the Participants' selection (**Article 3**).

Table S10. Comparison of the general characteristics of the included *versus* excluded INSEF mainland participants (**Article 3**).

Characteristics	**Included participants (n=2211)**	**Excluded participants(n=1256)**	**Total mainland INSEF participants (n=3467)**
Sex (n=3467) - %			
Males	47.6	47.1	47.5
Females	52.4	52.9	52.5
Age group (n=3467) - %			
25-49 years	53.4	49.7	52.5
50-74 years	46.6	50.3	47.5
[a] Level of Education (n=3464) - %			
Low education	58.2	61.2	58.9
Medium education	21.6	21.4	21.6
High education	20.2	17.4	19.5
[b] Occupation (n=3200) - %			
White-collar occupation	63.0	59.2	62.2
Blue-collar occupation	37.0	40.8	37.8
Lifestyles variables - %			
[c] Smokers (n=3465)	21.1	24.5	21.9
[d] Excessive alcohol consumption (n=3465)	36.1	36.0	36.1
[e] Unhealthy Diet (n=3463)	36.1	36.4	36.2
[f] Sedentary (n=3443)	45.1	43.5	44.7
[g] Anaemia (n=3389) - %	5.2	5.7	5.4

[a] Low education: levels 0-2 of the ISCED 2011; Medium education: levels 3-4 of the ISCED 2011, High education: levels 5-8 of the ISCED 2011;
[b] White-collar occupation: Managers, Professionals, Technicians & Associate Professional, Clerical Support Workers and Services & Sales Workers; Blue–collar occupation: Skilled Agricultural Workers, Craft & Related trades Workers, Plant & Machine Operators and Elementary occupations.
[c] Smokers include current daily and occasional smokers.
[d] 3 or more days/week of consumption of at least one of the following alcoholic beverages (wine, beer, brandy/bagasse, Port wine/Martini/liqueur, Whisky/Gin/Vodka).
[e] No consumption of fruit and vegetables at least once a day.
[f] Reading, watching TV or other sedentary activities declared as the best description of leisure time activities during the last 12 months.
[g] Hemoglobin concentrations below 13 g/dL in men and below 12 g/dL in women.

Table S11 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the short-term exposure scenario (3 previous days - average PM_{10} concentrations) (**Article 3**).

	Total	**Females**	**Males**
	(n=2211)	**(n=1195)**	**(n=1016)**
RDW			
Unadjusted model	0.20 (-0.47; 0.88)	0.12 (-0.60; 0.84)	0.29 (-0.54; 1.12)
Adjusted model	-0.35 (-1.03; 0.34)	0.00 (-0.00; 0.00)	-0.33 (-1.28; 0.63)
WBC			
Unadjusted model	**1.59 (0.16; 3.01)**	2.03 (-0.36; 4.42)	1.05 (-0.83; 2.93)
Adjusted model	**2.08 (0.42; 3.73)**	**2.76 (0.65; 4.87)**	1.46 (-1.46; 3.73)
PLAT			
Unadjusted model	0.59 (-0.63; 1.82)	0.76 (-1.00; 2.53)	0.28 (-1.90; 2.47)
Adjusted model	0.48 (-1.33; 2.30)	0.91 (-1.16; 2.98)	-0.04 (-2.58; 2.52)
RBC			
Unadjusted model	-0.12 (-0.64; 0.40)	-0.25 (-0.78; 0.28)	-0.09 (-0.82; 1.00)
Adjusted model	-0.12 (-0.99; 0.74)	-0.09 (-1.13; 0.54)	-0.30 (-1.14; 0.95)
HEMOG			
Unadjusted model	0.00 (-0.61; 0.61)	0.24 (-0.38; 0.86)	-0.16 (-1.19; 0.87)
Adjusted model	0.35 (-0.39; 1.09)	0.69 (-0.29; 1.05)	-0.06 (-1.16; 1.67)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 2 previous day-average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S12 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the short-term exposure scenario (3 previous days - average PM_{10} concentrations) after exclusion of participants with anaemia (**Article 3**).

	Total	**Females**	**Males**
	(n=2092)	**(n=1107)**	**(n=985)**
RDW			
Unadjusted model	0.20 (-0.53; 0.94)	0.05 (-0.73; 1.17)	0.38 (-0.41; 1.17)
Adjusted model	-0.26 (-0.90; 0.38)	-0.42 (-1.11; 0.28)	-0.12 (-0.93; 0.69)
WBC			
Unadjusted model	**1.80 (0.33; 3.28)**	**2.57 (0.05; 5.11)**	0.92 (-1.03; 2.86)
Adjusted model	**2.34 (0.69; 3.99)**	**3.38 (1.15; 5.63)**	1.43 (-0.69; 4.41)
PLAT			
Unadjusted model	0.67 (-0.61; 1.95)	0.85 (-1.23; 2.93)	0.43 (-1.58; 2.46)
Adjusted model	0.62 (-1.20; 2.44)	1.19 (-1.07; 3.44)	-0.36 (-2.76; 2.04)
RBC			
Unadjusted model	-0.11 (-0.52; 0.30)	-0.14 (-0.62; 0.34)	-0.07 (-0.78; 0.64)
Adjusted model	-0.11 (-0.98; 0.77)	0.06 (-1.04; 1.17)	-0.40 (-1.16; 0.36)
HEMOG			
Unadjusted model	-0.04 (-0.57; 0.49)	0.25 (-0.24; 0.73)	-0.31 (-1.20; 0.57)
Adjusted model	0.21 (-0.37; 0.78)	0.57 (-0.19; 1.32)	-0.20 (-1.22; 0.82)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 2 previous day-average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S13 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the short-term exposure scenario (3 previous days - average PM_{10} concentrations) after restricting participants to those living within a 20km radius of at least one air quality monitoring station with available PM10 measurements (**Article 3**).

	Total	**Females**	**Males**
	(n=1683)	**(n=905)**	**(n=778)**
RDW			
Unadjusted model	0.18 (-0.87; 1.23)	0.11 (-1.08; 1.31)	0.22 (-0.87; 1.30)
Adjusted model	-0.33 (-1.31; 0.65)	-0.26 (-1.86; 0.76)	-0.46 (-1.28; 0.94)
WBC			
Unadjusted model	1.43 (-0.80; 3.66)	2.24 (-0.85; 5.34)	0.46 (-2.01; 2.94)
Adjusted model	2.52 (-0.59; 5.63)	**3.56 (0.72; 6.48)**	1.45 (-3.56;6.41)
PLAT			
Unadjusted model	0.32 (-1.24; 1.89)	0.70 (-1.22; 2.62)	-0.54 (-3.18; 2.11)
Adjusted model	0.53 (-2.64; 3.72)	1.28 (-1.94; 4.51)	-0.47 (-4.30; 3.37)
RBC			
Unadjusted model	-0.31 (-0.92; 0.29)	-0.33 (-1.02; 1.10)	0.01 (-1.07; 0.37)
Adjusted model	-0.18 (-1.57; 1.22)	-0.35 (-1.90; 1.21)	-0.14 (-1.30; 1.03)
HEMOG			
Unadjusted model	-0.11 (-0.85; 0.62)	0.33 (-1.38; 1.04)	-0.17 (-0.40; 1.05)
Adjusted model	0.42 (-0.82; 1.66)	0.54 (-0.94; 2.01)	0.25 (-1.65; 2.15)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 2 previous day-average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S14 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the short-term exposure scenario (3 previous days - average PM_{10} concentrations) considering the PM10 obtained by the air quality modelling system (WRF-CAMx) (**Article 3**).

	Total	**Females**	**Males**
	(n=2211)	**(n=1195)**	**(n=1016)**
RDW			
Unadjusted model	0.10 (-0.81; 0.61)	-0.28 (-0.95; 0.39)	0.05 (-0.78; 0.89)
Adjusted model	0.17 (-0.46; 0.81)	-0.19 (-0.97; 0.59)	0.58 (0.00; 1.16)
WBC			
Unadjusted model	-0.37 (-2.01; 1.28)	-1.25 (-3.81; 1.31)	0.65 (-0.71; 2.01)
Adjusted model	-0.16 (-2.76; 2.44)	-0.83 (-4.33; 2.69)	0.55 (-2.38; 3.50)
PLAT			
Unadjusted model	**0.70 (0.16; 1.24)**	0.14 (-0.82; 1.10)	0.81 (-0.22; 1.84)
Adjusted model	-0.11 (-1.40; 1.19)	0.32 (-1.52; 2.16)	-0.44 (-2.16; 1.28)
RBC			
Unadjusted model	-0.15 (-0.57; 0.27)	-0.18 (-0.56; 0.19)	0.42 (-0.11; 0.95)
Adjusted model	-0.09 (-0.51; 0.32)	-0.45 (-1.19; 0.30)	0.08 (-0.37; 0.54)
HEMOG			
Unadjusted model	0.00 (-0.42; 0.42)	0.13 (-0.23; 0.49)	0.50 (-0.04; 1.04)
Adjusted model	0.16 (-0.31; 0.64)	0.07 (-0.72; 0.87)	0.13 (-0.35; 0.62)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 2 previous day-average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S15 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the short-term exposure scenario considering a different previous period of time (2 previous days - average PM10 concentrations) (**Article 3**).

	Total	**Females**	**Males**
	(n=2211)	**(n=1195)**	**(n=1016)**
RDW			
Unadjusted model	0.21 (-0.36; 0.79)	0.17 (-0.41; 0.76)	0.25 (-0.58; 1.08)
Adjusted model	-0.21 (-0.84; 0.42)	-0.29 (-1.05; 0.46)	-0.16 (-1.01; 0.69)
WBC			
Unadjusted model	**1.31 (0.05; 2.68)**	1.87 (-0.49; 4.22)	0.65 (-1.16; 2.46)
Adjusted model	**1.90 (0.38; 3.43)**	**3.07 (0.96; 5.18)**	0.78 (-1.76; 3.33)
PLAT			
Unadjusted model	0.47 (-0.50; 1.44)	0.66 (-0.81; 2.13)	0.12 (-2.15; 2.40)
Adjusted model	0.34 (-1.22; 1.90)	0.73 (-0.93; 2.38)	-0.11 (-3.00; 2.80)
RBC			
Unadjusted model	-0.06 (-0.55; 0.43)	-0.08 (-0.64; 0.47)	-0.04 (-0.77; 0.84)
Adjusted model	-0.04 (-0.96; 0.87)	0.11 (-0.91; 1.13)	-0.31 (-1.15; 0.54)
HEMOG			
Unadjusted model	0.08 (-0.68; 0.52)	0.20 (-0.38; 0.79)	-0.26 (-1.20; 0.67)
Adjusted model	0.26 (-0.46; 0.98)	0.74 (-0.18; 1.67)	-0.26 (-1.11; 0.59)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 2 previous day-average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S16 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the short-term exposure scenario considering a different previous period of time (5 previous days - average PM_{10} concentrations) (**Article 3**).

	Total	**Females**	**Males**
	(n=2211)	**(n=1195)**	**(n=1016)**
RDW			
Unadjusted model	0.29 (-0.43; 1.00)	0.07 (-0.78; 0.93)	0.51 (-0.26; 1.28)
Adjusted model	-0.49 (-1.42; 0.44)	-0.72 (-1.62; 0.18)	-0.31 (-1.47; 0.84)
WBC			
Unadjusted model	1.46 (-0.44; 3.37)	1.55 (-1.63; 4.75)	1.35 (-0.70; 3.39)
Adjusted model	1.48 (-0.66; 3.63)	1.27 (-1.70; 4.25)	1.80 (-1.45; 5.06)
PLAT			
Unadjusted model	0.64 (-0.84; 2.12)	0.30 (-2.16; 2.76)	0.89 (-1.46; 3.25)
Adjusted model	0.22 (-1.25; 1.69)	-0.12 (-2.80; 2.56)	0.61 (-1.94; 3.17)
RBC			
Unadjusted model	-0.30 (-1.10; 0.49)	-0.57 (-1.11; 0.04)	-0.08 (-0.91; 1.07)
Adjusted model	-0.33 (-0.85; 0.18)	-0.48 (-1.20; 0.24)	-0.27 (-1.00; 0.46)
HEMOG			
Unadjusted model	0.05 (-0.86; 0.77)	0.14 (-0.46; 0.74)	0.07 (-1.15; 1.02)
Adjusted model	0.26 (-0.48; 1.01)	0.40 (-0.48; 1.29)	0.12 (-1.20; 1.44)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 2 previous day-average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S17 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the long-term exposure scenario (1-year average PM_{10} concentrations) (**Article 3**).

	Total	**Females**	**Males**
	(n=2211)	**(n=1195)**	**(n=1016)**
RDW			
Unadjusted model	**4.33 (2.81; 5.86)**	**4.80 (2.91; 6.70)**	**3.84 (2.05; 5.62)**
Adjusted model	**2.82 (0.62; 5.02)**	2.88 (-0.61; 6.38)	**2.96 (0.80; 5.12)**
WBC			
Unadjusted model	4.54 (-0.15; 9.26)	3.56 (-5.75; 12.96)	**5.64 (1.03; 10.27)**
Adjusted model	2.43 (-1.82; 6.70)	-1.31 (-10.31; 7.78)	**5.78 (1.46; 10.13)**
PLAT			
Unadjusted model	2.12 (-0.96; 5.20)	1.41 (-2.87; 5.71)	3.11 (-0.98; 7.22)
Adjusted model	**3.31 (0.61; 6.01)**	3.34 (-1.73; 8.45)	2.96 (-0.39; 6.32)
RBC			
Unadjusted model	-0.24 (-2.17; 1.69)	-0.95 (-2.96; 1.06)	0.35 (-2.35; 3.05)
Adjusted model	-0.44 (-2.23; 1.34)	-0.17 (-2.38; 2.04)	-0.60 (-2.54; 1.33)
HEMOG			
Unadjusted model	-0,64 (-2,35; 1,07)	-1,18 (-2,95; 0,60)	-0,25 (-2,89; 2,39)
Adjusted model	0,61 (-1,18; 2,40)	0,27 (-2,01; 2,56)	0,84 (-1,04; 2,72)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 1-year average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S18 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the long-term exposure scenario (1-year average PM_{10} concentrations), after exclusion of participants with anaemia (**Article 3**).

	Total	**Females**	**Males**
	(n=2092)	**(n=1107)**	**(n=985)**
RDW			
Unadjusted model	**4.33 (2.88; 5.79)**	**4.88 (3.07; 6.70)**	**3.77 (1.96; 5.57)**
*Adjusted model	**2.99 (0.81; 5.18)**	3.18 (-0.37; 6.73)	**2.82 (0.69; 4.97)**
WBC			
Unadjusted model	**4.75 (0.06; 9.59)**	3.99 (-5.13; 13.20)	**5.62 (0.79; 10.48)**
*Adjusted model	2.52 (-1.78; 6.84)	-1.23 (-9.94; 7.56)	**5.67 (0.87; 10.49)**
PLAT			
Unadjusted model	2.29 (-0.63; 5.23)	0.76 (-3.36; 4.90)	**4.41 (0.50; 8.33)**
*Adjusted model	**4.01 (1.26; 6.76)**	4.16 (-0.97; 9.31)	**3.63 (0.44; 6.82)**
RBC			
Unadjusted model	-0.37 (-1.93; 1.18)	-1.03 (-2.87; 0.80)	0.01 (-1.98; 2.01)
*Adjusted model	-0.93 (-2.28; 0.42)	-0.72 (-2.63; 1.19)	-0.95 (-2.79; 0.90)
HEMOG			
Unadjusted model	-0.57 (-2.06; 0.93)	-0.94 (-2.68; 0.80)	-0.49 (-2.72; 1.74)
*Adjusted model	0.31 (-1.46; 2.08)	0.00 (-2.13; 2.14)	0.68 (-1.11; 2.46)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 1-year average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S19 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the long-term exposure scenario (1-year average PM_{10} concentrations), after restricting participants to those living within a 20km radius of at least one air quality monitoring station with available PM_{10} measurements (**Article 3**).

	Total	**Females**	**Males**
	(n=1683)	**(n=905)**	**(n=778)**
RDW			
Unadjusted model	**5.01 (3.25; 6.78)**	**5.64 (3.95; 7.33)**	**4.36 (2.24; 6.49)**
*Adjusted model	**3.08 (1.63; 4.53)**	**3.09 (0.18; 6.02)**	**3.38 (1.02; 5.76)**
WBC			
Unadjusted model	4.04 (-2.03; 10.15)	3.95 (-7.76; 4.55)	**4.15 (1.40; 6.90)**
*Adjusted model	2.91 (-3.21; 9.07)	0.44 (-11.32; 12.33)	4.92 (-1.63; 11.52)
PLAT			
Unadjusted model	0.21 (-3.77; 4.21)	-0.74 (-4.80; 3.34)	1.41 (-4.70; 7.57)
*Adjusted model	2.44 (-1.17; 6.06)	3.05 (-1.34; 7.46)	2.35 (-3.57; 8.31)
RBC			
Unadjusted model	-0.20 (-2.14; 1.74)	-0.67 (-2.74; 1.40)	0.17 (-2.67; 3.01)
*Adjusted model	0.00 (-2.10; 2.09)	0.58 (-1.88; 3.04)	-0.39 (-3.62; 2.84)
HEMOG			
Unadjusted model	-0.74 (-2.47; 0.98)	-0.68 (-2.41; 1.05)	-0.88 (-3.18; 1.42)
*Adjusted model	0.81 (-1.69; 3.32)	1.06 (-1.95; 4.08)	0.49 (-2.13; 3.11)

**adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 1-year average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S20 – Percent changes in WBC, PLAT, RBC, HEMOG and RDW per 10 µg/m^3 increment of PM_{10} according to sex in the long-term exposure scenario (1-year average PM10 concentrations), considering the PM_{10} obtained by the air quality modelling system (WRF-CAMx) (**Article 3**).

	Total	**Females**	**Males**
	(n=2211)	**(n=1195)**	**(n=1016)**
RDW			
Unadjusted model	**1.78 (0.56; 3.00)**	**1.81 (0.28; 3.35)**	**1.62 (0.17; 3.08)**
*Adjusted model	**1.59 (0.17; 3.01)**	1.71 (-0.25; 3.67)	**1.58 (0.10; 3.06)**
WBC			
Unadjusted model	3.60 (-0.51; 7.72)	3.50 (-3.21; 10.26)	3.61 (-1.86; 9.12)
*Adjusted model	4.42 (-1.36; 10.24)	3.62 (-4.55; 11.85)	5.33 (-1.33; 12.04)
PLAT			
Unadjusted model	1.22 (-2.41; 4.88)	0.15 (-4.62; 4.95)	1.26 (-4.48; 7.03)
*Adjusted model	0.56 (-3.25; 4.40)	0.26 (-3.71; 4.25)	1.68 (-4.99; 8.38)
RBC			
Unadjusted model	-0.76 (-2.54; 1.03)	-0.87 (-2.73; 1.00)	0.40 (-2.15; 2.96)
*Adjusted model	-0.31 (-2.48; 1.87)	-1.05 (-3.13; 1.04)	0.03 (-2.17; 2.24)
HEMOG			
Unadjusted model	-0.56 (-2.36; 1.24)	-0.39 (-2.26; 1.49)	0.52 (-2.06; 3.11)
*Adjusted model	0.23 (-2.25; 2.73)	-0.82 (-3.27; 1.63)	0.77 (-1.79; 3.33)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary and individual allocated 1-year average temperature. Abbreviations: WBC, White blood cells count; PLT, platelets count; RBC, Red blood cells count; HEMOG, Haemoglobin; RDW, red cell distribution width. Results in bold are those statistically significant *(p*<0.05).

Table S21. Comparison of the general characteristics of the included *versus* excluded INSEF mainland participants (**Article 4**).

Characteristics	**Included participants (n=2272)**	**Excluded participants (n=1195)**	**Total mainland INSEF participants (n=3467)**
Sex (n=3467) - %			
Males	47.2	48.7	47.5
Females	52.8	51.3	52.5
Age group (n=3467) - %			
25-49 years	53.0	50.8	52.5
50-74 years	47.0	49.2	47.5
[a] Level of Education (n=3464) - %			
Low education	57.8	63.3	58.9
Medium education	21.9	20.2	21.6
High education	20.3	16.5	19.5
[b] Occupation (n=3200) - %			
White-collar occupation	63.6	56.3	62.2
Blue-collar occupation	36.4	43.7	37.8
Lifestyles variables - %			
[c] Smokers (n=3465)	21.5	23.6	21.9
[d] Excessive alcohol consumption (n=3465)	35.8	36.9	36.1
[e] Unhealthy Diet (n=3463)	36.4	35.5	36.2
[f] Sedentary (n=3443)	45.0	43.6	44.7
[g] Hypertension (n=3466) - %	36.4	35.0	36.1
[h] Hypertensive medication (n=3467) - %	25.1	25.0	25.1

[a] Low education: levels 0–2 of the ISCED 2011; medium education: levels 3–4 of the ISCED 2011; high education: levels 5–8 of the ISCED 2011; [b] White-collar occupation: Managers, Professionals, Technicians and Associate Professional, Clerical Support Workers and Services and Sales Workers; Blue-collar occupation: Skilled Agricultural Workers, Craft and Related trades Workers, Plant and Machine Operators and Elementary occupations; [c] Smokers include current daily and occasional smokers; [d] 3 or more days/week of consumption of at least one of the following alcoholic beverages (wine, beer, brandy/spirits, port wine/martini/liqueur, whisky/gin/vodka); [e] No consumption of fruit and vegetables at least once a day; [f] Reading, watching TV or other sedentary activities declared as the best description of the leisure time activities during the last 12 months; [g] defined as defined as measured SBP ≥140 mmHg or measured DBP ≥90 mmHg or self-report of taking antihypertensive medication; [h] self-report of taking antihypertensive medication.

Table S22. Percent changes in DBP and SBP per 10 µg/m^3 increment of PM_{10} according to sex after restricting participants to those living within a 20km radius of at least one air quality monitoring station with available PM_{10} measurements (**Article 4**).

		% Change per 1 µg/m^3 of PM_{10} increment	
		DBP	SBP
Total (n=1740)			
	Unadjusted model	-0.71 (-2.03; 0.61)	-0.33 (-1.65; 0.99)
	*Adjusted model	0.51 (-1.35; 2.37)	0.75 (-1.02; 2.53)
Males (n=797)			
	Unadjusted model	-0.39 (-1.96; 1.17)	-0.06 (-1.53; 1.42)
	*Adjusted model	0.26 (-1.75; 2.27)	1.06 (-1.30; 3.43)
Females (n=943)			
	Unadjusted model	-0.84 (-3.29; 1.63)	-0.38 (-2.88; 2.13)
	*Adjusted model	0.61 (-2.30; 3.53)	0.41 (-2.54; 3.37)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

Table S23. Percent changes in DBP and SBP per 10 µg/m^3 increment of PM_{10} according to sex considering a different previous period of exposure (2 previous days' average PM_{10} concentrations instead of 3 previous days' average PM_{10} concentrations) (**Article 4**).

		% Change per 1 µg/m^3 of PM_{10} increment	
		DBP	SBP
Total (n=2272)			
	Unadjusted model	-0.85 (-1.86; 0.16)	-0.71 (-1.78; 0.37)
	*Adjusted model	-0.12 (-1.41; 1.18)	0.00 (-1.05; 1.06)
Males (n=1037)			
	Unadjusted model	-0.82 (-1.88; 0.23)	-0.76 (-1.87; 0.35)
	*Adjusted model	-0.63 (-1.55; 0.30)	0.05 (-1.16; 1.25)
Females (n=1235)			
	Unadjusted model	-0.85 (-2.66; 0.95)	-0.62 (-2.52; 1.28)
	*Adjusted model	0.25 (-1.82; 2.32)	-0.17 (-2.07; 1.74)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

Table S24. Percent changes in DBP and SBP per 10 µg/m^3 increment of PM_{10} according to sex considering a different previous period of time (5 previous days' average PM_{10} concentrations instead of 3 previous days' average PM_{10} concentrations) (**Article 4**).

		% Change per 1 µg/m^3 of PM_{10} increment	
		DBP	SBP
Total (n=2272)			
	Unadjusted model	-0.83 (-2.26; 0.60)	0.60 (-2.04; 0.84)
	*Adjusted model	0.59 (-0.61; 1.79)	0.53 (-0.86; 1.92)
Males (n=1037)			
	Unadjusted model	-0.55 (-2.08; 0.98)	0.53 (-2.11; 1.05)
	*Adjusted model	0.29 (-0.86; 1.44)	0.66 -0.65; 1.98)
Females (n=1235)			
	Unadjusted model	-1.04 (-3.12; 1.06)	0.58 (-2.76; 1.60)
	*Adjusted model	0.80 (-1.05; 2.65)	0.41 (-1.85; 2.68)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

Table S25. Percent changes in DBP and SBP per 10 µg/m^3 increment of PM_{10} according to sex when considering the PM_{10} obtained by the air quality modelling system (WRF-CAMx) (**Article 4**).

		% Change per 1 µg/m^3 of PM_{10} increment	
		DBP	SBP
Total (n=2272)			
	Unadjusted model	0.08 (-0.99; 1.16)	0.11 (-0.74; 0.95)
	*Adjusted model	0.33 (-0.97; 1.63)	0.44 (-0.54; 1.41)
Males (n=1037)			
	Unadjusted model	-0.13 (-1.01; 0.76)	0.03 (-0.75; 0.81)
	*Adjusted model	-0.16 (-1.44; 1.12)	0.22 (-0.90; 1.35)
Females (n=1235)			
	Unadjusted model	0.50 (-1.04; 2.04)	0.48 (-0.76; 1.72)
	*Adjusted model	0.74 (-1.04; 2.52)	0.54 (-0.75; 1.82)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

Table S26. Percent changes in DBP and SBP per 10 µg/m^3 increment of PM_{10} according to sex after exclusion of participants taking hypertensive medication (**Article 4**).

		% Change per 1 µg/m^3 of PM_{10} increment	
		DBP	SBP
Total (n=1688)			
	Unadjusted model	-0.97 (-2.22; 0.29)	-0.43 (-1.69; 0.83)
	*Adjusted model	0.05 (-1.41; 1.51)	0.43 (-0.88; 1.74)
Males (n=776)			
	Unadjusted model	-0.82 (-2.11; 0.48)	-0.33 (-1.49; 0.82)
	*Adjusted model	-0.23 (-1.93; 1.46)	0.76 (-0.45; 1.97)
Females (n=912)			
	Unadjusted model	-1.10 (-3.28; 1.08)	-0.51 (-2.75; 1.73)
	*Adjusted model	0.31 (-2.01; 2.64)	0.13 (-2.30; 2.56)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

Table S27. Percent changes in DBP and SBP per 10 µg/m^3 increment of PM_{10} according to sex after restricting participants to those having hypertension (defined as SBP ≥140 mmHg and/or DBP ≥90 mmHg or taking antihypertensive medication) (**Article 4**).

		% Change per 1 µg/m^3 of PM_{10} increment	
		DBP	SBP
Total (n=851)			
	Unadjusted model	-0.43 (-2.20; 1.34)	-0.44 (-2.21; 1.34)
	*Adjusted model	0.47 (-1.42; 1.52)	0.29 (-1.61; 2.20)
Males (n=440)			
	Unadjusted model	-0.42 (-2.83; 2.00)	-0.22 (-2.56; 2.11)
	*Adjusted model	-0.45 (-3.37; 2.49)	0.31 (-2.83; 3.53)
Females (n=411)			
	Unadjusted model	-0.31 (-2.83; 2.21)	-0.57 (-2.99; 1.86)
	*Adjusted model	0.68 (-1.89; 3.26)	0.04 (-2.71; 2.79)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

Table S28. Percent changes in DBP and SBP per 10 µg/m^3 increment of PM_{10} according to sex after restricting participants to those with abdominal obesity (Waist to height ratio >0.5) (**Article 4**).

		% Change per 1 µg/m^3 of PM_{10} increment	
		DBP	SBP
Total (n=1738)			
	Unadjusted model	-0.88 (-1.93; 0.18)	-0.62 (-1.87; 0.62)
	*Adjusted model	0.08 (-1.06; 1.23)	0.31 (-1.12; 1.74)
Males (n=829)			
	Unadjusted model	-0.61 (-1.80; 0.58)	-0.42 (-1.76; 0.92)
	*Adjusted model	-0.42 (-1.88; 1.04)	0.54 (-1.50; 2.58)
Females (n=909)			
	Unadjusted model	-1.14 (-2.85; 0.58)	-0.82 (-2.69; 1.05)
	*Adjusted model	0.40 (-1.43; 2.24)	0.95 (-2.06; 1.87)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

***Table S29**–Changes in DBP and SBP (mmHg) per 10 µg/m^3 increment of PM_{10} according to sex* (**Article 4**).

		mmHg change per 10 µg/m^3 of PM_{10} increment	
		DBP	SBP
Total (n=2272)			
	Unadjusted model	-0.53 (-1.41;0.35)	-0.74 (-2.34; 0.86)
	*Adjusted model	0.33 (-0,62;1.27)	0.57 (-1.10; 2.54)
Males (n=1037)			
	Unadjusted model	-0.46 (-1.48;0.55)	-0.64 (-2.42; 1.14)
	*Adjusted model	-0.02 (-0.83;0.79)	0.88 (-1.04; 2.80)
Females (n=1235)			
	Unadjusted model	-0.56 (-2.04; 0.90)	-0.77 (-3.31;1.76)
	*Adjusted model	0.58 (-0.96; 2.12)	0.19 (-2.36; 2.74)

*adjusted for age, sex, educational level, occupation, smoking status, excessive alcohol consumption, unhealthy diet, sedentary, individual allocated ambient temperature, individual allocated 1-year average of PM_{10} concentrations. Abbreviations: DBP, Diastolic blood pressure; SBP, Systolic blood pressure.

10. Annex I: Ethics committee approval

Comissão de Ética para a Saúde

Parecer sobre o Projeto: ***Association between air pollution exposure and cardiovascular parameters: Linkage between the first National Health Examination Survey and the Environmental data.***

Investigador Principal: **Vânia Isabel da Silva Gaio**

Orientador e co-orientador: Baltazar Nunes, Coordenador executivo do Departamento de Epidemiologia e Carlos Matias Dias, Coordenador do Departamento de Epidemiologia

Após análise e apreciação do projeto supracitado, na reunião da Comissão de Ética para a Saúde (CES) no passado dia 21/02/2018, envia-se abaixo o parecer emitido por esta Comissão.

I – DESCRIÇÃO DO PROJETO

As doenças cardiovasculares continuam a ser a principal causa de morte em Portugal e a poluição do ar é hoje, reconhecido, como um importante fator de risco para o desenvolvimento destas doenças. Neste sentido, é essencial produzir evidência científica relativamente à associação entre a exposição aos poluentes atmosféricos e a alteração dos parâmetros cardiovasculares. Por outro lado, tendo em conta que uma grande proporção da população continua a ser exposta a níveis de poluentes atmosféricos acima dos valores recomendados, é importante verificar se os níveis de poluição do ar em Portugal estão a ter impacto ao nível cardiovascular. Através dos resultados deste projeto, nomeadamente se for estabelecida a associação entre os níveis de poluentes e os valores dos parâmetros cardiovasculares, será reforçada a importância da redução dos níveis de poluentes, o que por sua vez, terá potencial para reduzir o burden das doenças cardiovasculares.

O estudo proposto pretende estimar a associação entre a exposição a poluentes ambientais (PM10, O3, NO2) e alguns parâmetros de risco cardiovascular, incluindo os valores da tensão arterial, do perfil lipídico (Colesterol total e triglicéridos) e de biomarcadores de inflamação (contagem de glóbulos brancos e plaquetas).

Como objetivos secundários, pretende-se: desenvolver um modelo conceptual da relação entre a exposição aos poluentes e os seus efeitos sobre os parâmetros cardiovasculares; estimar a associação entre a exposição a cada poluente e os valores dos parâmetros cardiovasculares, bem como entre a exposição combinada dos vários poluentes e os parâmetros cardiovasculares; estimar os níveis de PM10, 03 e N02 a partir dos quais existe maior probabilidade da pessoas terem os parâmetros cardiovasculares alterados; e avaliar o potencial efeito de modificação de alguns fatores como o consumo de tabaco, as comorbilidades e a medicação, sobre a associação anteriormente descrita

O presente estudo pretende confrontar os dados obtidos através do Inquérito nacional de saúde com Exame Físico (INSEF) com os dados disponibilizados publicamente pela Agência Portuguesa do Ambiente, através das Comissões de Coordenação regionais, sobre os níveis de poluição da qualidade do ar medidos em 68 estações de monitorização.

Estas estações estão classificadas de acordo com o tipo de área geodemográfica que representam (rural, urbano e suburbano) e as fontes de emissão dominantes nessa área (fundo, tráfego e industrial) seguindo os critérios propostos pelas Estações Europeias da Agência Ambiental.

Os dados obtidos através dos participantes no INSEF, através do código postal, serão correlacionados com os dados das estações de monitorização, de forma a averiguar a existência de associações entre poluentes do ar e cada parâmetro cardiovascular, avaliadas e medidas por modelos lineares generalizados após ajuste para um conjunto de possíveis fatores de confusão.

Os possíveis fatores de confusão e / ou modificadores de efeito que serão considerados desde o início são: idade, sexo, nível de escolaridade, situação profissional, atividade ocupacional, tabagismo, tabagismo passivo, consumo de álcool, índice de massa corporal, comorbidades (hipertensão, hipercolesterolemia, diabetes e doenças respiratórias) e medicamentos. As variáveis meteorológicas (temperatura e umidade) também serão consideradas como potenciais fatores de confusão. No entanto, através da construção do modelo conceitual, variáveis adicionais podem ser adicionadas a este conjunto descrito.

Para medir a associação entre os três poluentes combinados e os parâmetros cardiovasculares, serão introduzidos os modelos de interação para a associação entre poluentes. Quanto à estimativa dos níveis de PM10, O3 e NO2 dos quais existe maior probabilidade de aumentar os parâmetros cardiovasculares, serão utilizados métodos de curvas ROC.

Para avaliar o potencial efeito de modificação do estado de tabagismo, comorbidades (hipertensão, hipercolesterolemia e diabetes) e medicação sobre a associação entre exposição a poluentes do ar e parâmetros cardiovasculares, será feita uma análise estratificada. Alternativamente, a avaliação da modificação pode ser feita pela inclusão da interação entre a variável modificadora e os níveis de poluentes variáveis no modelo.

Considerando que o estudo é realizado através da análise secundária dos dados do INSEF, não está previsto o pedido de consentimento informado, nem a notificação do estudo à CNPD.

II - ANÁLISE

1. Instrução do pedido de parecer

O presente projeto foi remetido à CES INSA em 30 de janeiro de 2018, contendo os elementos instrutórios essenciais para a sua análise e para a formação de decisão.

2. Utilidade

Considera-se fundamentada a utilidade, tendo presente a necessidade de melhorar o conhecimento científico sobre os efeitos da poluição do ar na saúde, especificamente no que concerne ao aumento do risco de doença cardiovascular.

3. Princípio da beneficência/não maleficência

Face à metodologia adoptada, o estudo consiste basicamente numa análise estatística, com base em dados já recolhidos no âmbito do INSEF, pelo que não existem riscos de maleficência.

4. Consentimento Informado

Não está previsto e concorda-se que não será aplicável na medida em que foi devidamente utilizado para a recolha dos dados no âmbito do INSEF, sendo que a utilização dos dados ora recolhidos é anonimizada.

5. Parecer CNPD

Não está previsto e concorda-se que não será aplicável dada a anonimização dos dados.

6. Conflito de interesses

Nada a observar.

7. Financiamento

O estudo será financiado por uma Bolsa de Doutoramento da FCT (SFRH/BD/129426/2017), sendo referida a inexistência de qualquer ligação dos parte dos investigadores à esta instituição financiadora.

III - CONCLUSÃO

Pelo exposto, parece poder concluir-se:

i) O presente Estudo contém os elementos essenciais para enformar a decisão da CES;
ii) Considera-se fundamentada a utilidade.
iii) Não existem riscos de maleficência.
iv) Não se vê necessidade de uso de pedido de consentimento informado e da intervenção da CNPD, salvaguardada a manutenção da anonimização dos dados do INSEF utilizados.
v) Nada a observar em matéria de conflito de interesse ou financiamento.

A CES - INSA delibera positivamente quanto à realização do Estudo "Association between air pollution exposure and cardiovascular parameters: Linkage between the first National Health Examination Survey and the Environmental data", nos termos propostos.

Por último, solicita-se que, ao abrigo do disposto no nº23 da atual versão da Declaração de Helsínquia, dê igualmente conhecimento à CES INSA do relatório final com as conclusões do estudo, de eventuais alterações ao protocolo de investigação e demais informações tidas por relevantes.

Aproveitamos ainda para desejar o maior sucesso no desenvolvimento deste trabalho.
Com os melhores cumprimentos.

A Comissão de Ética para a Saúde do INSA, I.P.