Received: 27 July 2023 | Accepted: 23 September 2023

DOI: 10.1111/eci.14101

ORIGINAL ARTICLE

WILEY

Association between exposure to air pollution and blood lipids in the general population of Spain

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Funding information
Consejería de Salud y Bienestar Social, Junta de Andalucía, Grant/Award Number: RC-0006-2016; Instituto de Salud Carlos III, Grant/Award Number: CM21/00214, INT21/00037, PI17/02136 and PI20/01322

Abstract

Background and Aims: We aimed to assess the associations of exposure to air pollutants and standard and advanced lipoprotein measures, in a nationwide sample representative of the adult population of Spain.

Methods: We included 4647 adults (>18 years), participants in the national, cross-sectional, population-based di@bet.es study, conducted in 2008–2010. Standard lipid measurements were analysed on an Architect C8000 Analyzer (Abbott Laboratories SA). Lipoprotein analysis was made by an advanced ^{1}H-NMR lipoprotein test (Liposcale®). Participants were assigned air pollution concentrations for particulate matter <10 μm (PM_{10}), <2.5 μm ($PM_{2.5}$) and nitrogen dioxide (NO_2), corresponding to the health examination year, obtained by modelling combined with measurements taken at air quality stations (CHIMERE chemistry-transport model).

Results: In multivariate linear regression models, each IQR increase in PM_{10}, $PM_{2.5}$ and NO_2 was associated with 3.3%, 3.3% and 3% lower levels of HDL-c and 1.3%, 1.4% and 1.1% lower HDL particle (HDL-p) concentrations ($p < .001$ for all associations). In multivariate logistic regression, there was a significant association between PM_{10}, $PM_{2.5}$ and NO_2 concentrations and the odds of presenting low HDL-c (<40 mg/dL), low HDL-p (<p25) and higher LDL particle (LDL-p) concentrations (≥p75). In subgroup analyses there were stronger associations between PM_{10} and NO_2 and low HDL-p in men (p for interaction .008 and .034), and between NO_2 and low HDL-p in individuals with obesity (p for interaction .015).

Conclusions: Our study shows an association between the exposure to air pollutants and blood lipids in the general population of Spain, suggesting a link to atherosclerosis.

KEYWORDS
air pollution, cholesterol, HDL, lipids, lipoprotein, triglyceride

1 | BACKGROUND

The World Health Organization (WHO) has identified air pollution as the largest single environmental health risk worldwide, with outdoor air pollution accounting for more than 4.2 million deaths every year.[1] Nearly half of outdoor air pollution-related premature deaths are due to ischaemic heart disease and stroke.[1] Air pollution can impact the cardiovascular system through a number of mechanisms, including endothelial dysfunction, systemic and pulmonary oxidative stress and inflammation, autonomic nervous system dysfunction and epigenetic changes.[2–5] Alterations in lipid metabolism could be another potential pathway in the association between air pollution and arteriosclerosis. In this regard, several previous epidemiological studies have explored the relationships between the exposure to several ambient air pollutants and blood lipid levels and the presence of dyslipidaemia.[6–24] However, most of this evidence has relied on standard lipid measures. Standard lipid panels measure the cholesterol or triglyceride content (in concentration per decilitre) carried by each lipoprotein class, rather than the numbers of these particles. In contrast, NMR spectroscopy provides a direct assessment of the number and size of lipoprotein particles, providing additional information about cardiovascular disease (CVD) risk.[25–29]

Accordingly, in the present study, we aimed to assess the associations between the exposure to air pollutants [particles with an aerodynamic diameter of less than 10 microns (PM_{10}), particles with an aerodynamic diameter of less than 2.5 microns ($PM_{2.5}$) and nitrogen dioxide (NO_2)], with both the blood lipid levels of a standard lipid profile as well as the particle concentrations of lipoproteins, in a nationwide sample representative of the adult population of Spain.

2 | METHODS

2.1 | Study design, setting and population

The Di@bet.es study is a national, cross-sectional, population-based survey conducted in 2008–2010.[30] A cluster sampling design was used to select participants to form a representative random sample of the Spanish population. One hundred health centres or their equivalent from all around the country were selected at random, with a probability for selection proportional to their target population size, after which 100 individuals aged ≥18 years were randomly selected from each health centre. Of the eligible adults, 55.8% came for examination, of which 9.9% were excluded (institutionalized, severe disease, pregnancy or recent delivery), resulting in a final sample of 5072 individuals.

The present study focuses on 4647 individuals (92% of the study sample), in whom complete data on concentrations of air pollutants and blood lipids were available for analyses.

This research was carried out in accordance with the Declaration of Helsinki of the World Medical Association.[31] Written informed consent was obtained from all the participants. The study was approved by the Ethics and Clinical Investigation Committee of the Hospital Regional Universitario de Málaga (Málaga, Spain) in addition to other regional ethics and clinical investigation committees all over Spain.

2.2 | Variables and procedures

The participants were invited to attend a single examination visit at their health centre. Information was collected by means of an interviewer-administered structured questionnaire, followed by a physical examination and blood sampling. Information on age, gender, educational level (none/basic/high school/college), smoking habit (current, former or never smokers) and alcohol intake (<30/30–60/>60 servings per month), was obtained by questionnaire. Food consumption was determined by a food frequency questionnaire and adherence to the Mediterranean diet was estimated by an adaptation of a 14-item Mediterranean diet score (MedScore).[32] The level of daily physical activity was estimated by the short form of the International Physical Activity Questionnaire (IPAQ).[33] Weight and height were measured and the body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Medical history and medications were also recorded.

Blood samples, obtained in fasting conditions, were immediately centrifuged and the serum was frozen until analysis. Samples were managed by the biochemistry laboratory of the Hospital Regional Universitario de Málaga, the IBIMA Biobank and by the CIBERDEM Biorepository (IDIBAPS Biobank).

2.3 | Lipid measurements

Standard lipid measurements Serum levels (mg/dL) of triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) were measured on an Architect C8000 Analyzer (Abbott Laboratories SA). Low-density lipoprotein cholesterol (LDL-c) was estimated by the Friedewald formula. 'High total cholesterol' was defined as TC ≥ 240 mg/dL; 'high LDL-c' was defined as LDL-c ≥ 160 mg/dL; 'low HDL-c' was defined as HDL-c < 40 mg/dL; and hypertriglyceridaemia was defined as TG ≥ 200 mg/dL.[34]

Lipoprotein analysis Lipoprotein analysis was performed by using Liposcale® Test (CE, ISO 13.485 approved), an advanced lipoprotein test based on two-dimensional (2D) diffusion-ordered ^{1}H-NMR (Proton nuclear magnetic resonance) spectroscopy.[35] Before ^{1}H-NMR analysis, 200 μL of serum were diluted with 50 μL deuterated water and 300 μL of 50 mM phosphate buffer solution at pH 7.4. ^{1}H-NMR spectra were recorded at 306K on a Bruker Avance III 600 spectrometer operating at a proton frequency of 600.20 MHz. The methyl signal was deconvoluted by using Lorentzian functions to determine the lipid concentration of the main lipoprotein classes (VLDL, LDL and HDL), and their size associated diffusion coefficients. Then, the lipid concentrations were combined with their associated particle volume in order to quantify the number of particles required to transport the measured lipid concentration of each lipoprotein subclass calculating the particle concentrations of VLDL (VLDL-p), LDL (LDL-p) and HDL (HDL-p). The variation coefficients for particle number were between 2% and 4%, and for the particle sizes were lower than 0.3%. These analyses were performed at the Biosfer Teslab facilities. We classified participants as having normal or abnormal levels of LDL-p, VLDL-p and HDL-p, using the equivalents of the 75th (LDL-p and VLDL-p) and 25th (HDL-p) percentiles (p) of particle concentrations within our study population.

2.4 | Exposure assessment

Modelled mean annual PM_{10}, $PM_{2.5}$ and NO_2 concentrations in Spain for the period 2008–2010 were calculated with the CHIMERE chemistry-transport model.[36] This model calculates the concentration of gaseous species and both inorganic and organic aerosols of primary and

secondary origin, including primary particulate matter, mineral dust, sulphate, nitrate, ammonium, secondary organic species and water. This model has been broadly evaluated in Spain by comparison with measured air pollutants at a large set of monitoring sites.[37,38] The model was applied to a domain covering the Iberian Peninsula at a horizontal resolution of $0.1 \times 0.1°$. The modelled concentrations were corrected with observed values, by considering a methodology described by Martín et al.[39] in which (1) a bias is calculated with regard to the observations in the Spanish air quality network of monitoring sites, (2) these biases are spatially interpolated using a kriging methodology to obtain a gridded bias and (3) this gridded bias is applied to the modelled concentration grid. This methodology considers a different bias grid for rural and urban sites that are then combined and weighted by population density. We assigned the average annual exposure to air pollutants corresponding to the health examination year of each participant by interpolating the estimated concentrations to the centroid of their residential postal codes.

Data on mean annual temperature (°C) from each municipality of residence were obtained from the Spanish National Meteorological Agency.[40]

2.5 | Statistical analysis

We applied linear regression models to assess associations between air pollutant and lipid measurements, which were log transformed to normalize distributions and also to limit the influence of extreme values. Association estimates were presented as percent changes with corresponding 95% confidence intervals (calculated by $100 \times [\exp(b) - 1]$), per each interquartile range (IQR) increase in air pollutant concentrations which equated to 7.7 μg/m^3 PM_{10}, 4.8 μg/m^3 $PM_{2.5}$ and 12.1 μg/m^3 NO_2.

We also used logistic regression models to investigate the associations of ambient air pollutants with high total cholesterol (TC ≥ 240 mg/dL); high LDL-c (LDL-c ≥ 160 mg/dL); low HDL-c (HDL-c < 40 mg/dL); and hypertriglyceridaemia (TG ≥ 200 mg/dL) as defined by the standard lipid measurements, and with high LDL-p and VLDL-p concentrations (≥p75), and low HDL-p concentrations (<p25), measured by the Liposcale® test. These results are presented as odds ratios (ORs) with corresponding 95% CIs again per each IQR increase in air pollutants.

All these models were controlled for possible confounders such as age, sex, BMI, education level, smoking status, alcohol intake, municipality population, MedScore, IPAQ, mean ambient temperature of the municipality and lipid lowering medication.

In addition, we investigated potential effect modification of the associations by sex (male/female), age (<40/40–60 or ≥60 years) and BMI (<30 or ≥30 kg/m^2). Each potential modifier was examined in a separate model by adding an interaction term.

All the statistical analyses were performed with IBM SPSS statistics 23.0. Reported *p* values were based on two-sided tests with statistical significance set at .05. Bonferroni correction for multiple comparisons was applied at a level of alpha =.05/7 = .007.

3 | RESULTS

A total of 4647 individuals were included in the analysis (Table 1). The sample was composed of 1976 men (42.5%) and 2671 women (57.5%). Mean age of the population was 50.5 ± 17.0 years (range: 18–93 years). The characteristics of the study population followed a distribution as expected in the Spanish general population. Distributions of standard lipids and NMR particle concentrations are also displayed in the table.

Table 2 summarizes residential estimates of outdoor air pollution concentrations assigned to the study participants in the year of examination. The median (25th–75th percentile) PM_{10}, $PM_{2.5}$ and NO_2 exposure levels were 23.7 (19.6–27.3), 12.2 (10.5–15.3) and 16.6 (12.5–24.6) μg/m^3, respectively. Most values were within the current European Ambient Air Quality Directive target values (Directive 2008/50/EC).[41]

Table 3 shows the results of the linear correlations between air pollutant concentrations and standard lipid biomarkers (TC, LDL-c, HDL-c and TG) and NMR particle concentrations (LDL-p, VLDL-p and HDL-p), in crude, and multivariate adjusted linear regression models. Greater air pollutant exposures were associated with lower TC and LDL-c concentrations in crude models, but these associations were attenuated after multivariate adjustment. This trend was not observed by NMR analyses. In fact the association between air pollutants and LDL-p although not significant tended to be positive. The three pollutants tested showed a strong and highly significant negative association with HDL-c concentrations in both crude and multivariate models. In the fully adjusted model, an IQR increase in PM_{10}, $PM_{2.5}$ and NO_2 was associated with 3.3%, 3.3% and 3% lower levels of HDL-c, respectively. The same trend was observed regarding the association between contaminants and the concentration of HDL particles measured by NMR. In the multivariate analysis, each IQR increase in PM_{10}, $PM_{2.5}$ and NO_2 was associated with 1.3%, 1.4% and 1.1% lower HDL-p. $PM_{2.5}$ concentrations also showed a positive association with TG levels in multivariate analyses.

Table 4 shows the crude and multivariate-adjusted ORs for presenting lipid abnormalities in the standard lipid

TABLE 1 Clinical characteristics and lipid values of the study sample ($n = 4647$).

	%	Mean ± SD	Range
Age (years)		50.5 ± 17.0	18–93
Women	57.5		
Smoking			
Current	23.8		
Former	26.0		
Never	50.2		
Alcohol intake (servings-month)			
<30	73.9		
30–60	14.9		
>60	11.3		
Municipality population			
<10,000	18.4		
10,000–50,000	27.8		
>50,000	53.8		
Education level			
No studies	12.8		
Basic	47.6		
High school-college	39.6		
BMI (kg/m^2)		28.0 ± 5.2	12.2–61.3
Med diet score		7.8 ± 1.8	1–13
Physical activity (IPAQ)			
Low	42.0		
Medium	34.5		
High	23.5		
Lipid lowering medication	13.3		
Standard lipid biomarkers (mg/dL)			
TC		196 ± 40	58–395
LDL-C		105 ± 30	21–254
HDL-C		52 ± 13	7–14
TG		121 ± 88	17–2095
Particle concentration (^{1}H-NMR) (nmol/L)			
LDL-p		1392 ± 278	358–2702
VLDL-p		53 ± 40	12–619
HDL-p		28 ± 5	6–56

Abbreviations: HDL-C, high-density lipoprotein cholesterol; HDL-p, HDL particles; LDL-C, low-density lipoprotein cholesterol; LDL-p, LDL particles; TC, total cholesterol; TG: triglycerides; VLDL-p, VLDL particles; ^{1}H-NMR, proton nuclear magnetic resonance.

profile and NMR, per each IQR increase in air pollutant concentrations. Replicating what was seen in the linear model, there was a strong negative association between the three air pollutants tested, and HDL-c. In the fully adjusted model, the odds of presenting low HDL-c (<40 mg/dL), were 1.36 (95% CI 1.21–1.54) $p < .001$, 1.41 (95% CI 1.25–1.59) $p < .001$ and 1.31 (95% CI 1.18–1.44) $p < .001$ per each IQR increase in PM_{10}, $PM_{2.5}$ and NO_2. Again, this same trend was observed for the association between air pollutants and HDL-p, with multivariate ORs for presenting low HDL-p (<p25) of 1.23 (95% CI 1.09–1.37) $p < .001$, 1.23 (95% CI 1.10–1.37) $p < .001$ and 1.18 (95% CI 1.07–1.29) $p = .001$ per each IQR increase in PM_{10}, $PM_{2.5}$ and NO_2, respectively. In the logistic regression models, there was also a positive association between PM_{10} and $PM_{2.5}$ concentrations and the odds of presenting higher LDL-p, with multivariate ORs for presenting LDL-p concentrations ≥p75 of 1.24 (95% CI 1.11–1.39) $p < .001$ and 1.16 (95% CI 1.04–1.29) $p = .007$, per each IQR increase, respectively.

In the subgroup analysis, we found a significant interaction between sex and the association between PM_{10} and NO_2 and low HDL-p (p for interaction .008 and .034, respectively), with stronger associations in men. We also found a stronger association between NO_2 and low HDL-p in individuals with obesity (p for interaction .015) (Figure 1).

4 | DISCUSSION

In this nationwide sample representative of the adult population of Spain we found significant associations between exposure to various air pollutants and several standard and novel blood lipoprotein measures, pointing to a pro-atherogenic lipid profile in subjects exposed to a higher degree of pollution. In particular we found strong negative associations between PM_{10}, $PM_{2.5}$ and NO_2 exposures, and both HDL-c and HDL-p concentrations both in linear and in logistic multivariate regression models. Additionally, we found a significant association between exposure to PM_{10} and $PM_{2.5,}$ and

TABLE 2 Descriptive statistics for air pollutants concentrations (μg/m^3) in the study sample.

	Percentile								
Pollutant	5th	25th	50th	75th	95th	IQR	Mean	Minimum	Maximum
PM_{10}	14.4	19.6	23.7	27.3	33.1	7.7	23.2	4.0	42.3
$PM_{2.5}$	8.1	10.5	12.2	15.3	20.0	4.8	12.7	3.4	22.3
NO_2	6.6	12.5	16.6	24.6	50.3	12.1	20.2	3.6	51.4

Abbreviations: IQR, interquartile range; NO_2, nitrogen dioxide; PM_{10}, particles with an aerodynamic diameter of less than 10 microns; $PM_{2.5}$, particles with an aerodynamic diameter of less than 2.5 microns.

TABLE 3 Associations between air pollutants (per IQR concentrations increase) and blood lipid levels.

	Standard lipid biomarkers							
	TC		LDL-C		HDL-C		TG	
	% change (95% CI)	*p*	% change (95% CI)	*p*	% change (95% CI)	*p*	% change (95% CI)	*p*
PM_{10}								
Crude	**−1.7 (−2.5, −1.0)**	<.001	**−1.6 (−2.7, −0.5)**	.004	**−3.2 (−4,1, −2.3)**	<.001	0.4 (−1.4, 2.0)	.667
Multivariate	−1.1 (−1.9, −0.2)	.013	−0.3 (−1.6, 0.9)	.590	**−3.3 (−4.3, −2.3)**	<.001	2.0 (0.1, 3.9)	.037
$PM_{2.5}$								
Crude	−1.1 (−1.9, −0.3)	.01	−0.8 (−2.0, 0.4)	.190	**−3.0 (−4.0, −2.1)**	<.001	1.0 (−1.0, 2.9)	.323
Multivariate	−0.6 (−1.4, 0.3)	.215	−0.3 (−1.0, 1.5)	.628	**−3.3 (−4.3, −2.3)**	<.001	**2.8 (0.9, 4.7)**	.003
NO_2								
Crude	**−1.8 (−2.5, −1.2)**	<.001	**−1.8 (−2.7, −0.9)**	<.001	**−2.7 (−3.4, −1.9)**	<.001	−1.5 (−2.9, 0.0)	.053
Multivariate	**−1.4 (−2.2, −0.7)**	<.001	−0.9 (−1.9, 0.2)	.118	**−3.0 (−3.8, −2.2)**	<.001	−0.5 (−1.2, 2.1)	.570
	Particle concentration (^{1}H-NMR)							
	LDL-p		VLDL-p		HDL-p			
	% change (95% CI)	*p*	% change (95% CI)	*p*	% change (95% CI)	*p*		
PM_{10}								
Crude	0.2 (−0.6, 0.9)	.703	1.1 (−1.0, 3.2)	.324	−0.7 (−1.3, 0.0)	.049		
Multivariate	0.6 (−0.3, 1.4)	.205	1.9 (−0.3, 4.1)	.091	**−1.3 (−2.0, −0.6)**	<.001		
$PM_{2.5}$								
Crude	0.4 (−0.4, 1.2)	.364	1.0 (−1.3, 3.2)	.391	−0.8 (−1.5, −0.1)	.018		
Multivariate	0.6 (−0.2, 1.5)	.159	1.9 (−0.3, 4.1)	.089	**−1.4 (−2.1, −0.7)**	<.001		
NO_2								
Crude	0.6 (0.0, 1.2)	.068	0.3 (−1.3, 2.0)	.703	−0.5 (−1.1, 0.0)	.040		
Multivariate	0.5 (−0.2, 1.3)	.155	1.8 (−0.1, 3.6)	.059	**−1.1 (−1.6, −0.5)**	<.001		

Note: % changes and *p* values calculated by linear regression per interquartile range (IQR) increase in air pollutants concentrations (PM_{10}: 7.7 μg/m^3, $PM_{2.5}$: 4.8 μg/m^3, NO_2: 12.1 μg/m^3). Multivariate model: adjusted to age, sex, BMI, smoking status, alcohol intake, education level, MedScore, IPAQ, municipality population, ambient temperature and lipid lowering medication. In bold: % changes with *p* values <.007 (alpha .05 corrected by Bonferroni = .05/7).

Abbreviations: HDL-C, high-density lipoprotein cholesterol; HDL-p, HDL particles; LDL-C, low-density lipoprotein cholesterol; LDL-p, LDL particles; NO_2, nitrogen dioxide; PM_{10}, particles with an aerodynamic diameter of less than 10 microns; $PM_{2.5}$, particles with an aerodynamic diameter of less than 2.5 microns; TC, total cholesterol; TG, triglycerides; VLDL-p, VLDL particles; ^{1}H-NMR, proton nuclear magnetic resonance.

TABLE 4 Odd ratios (OR) for presenting lipid abnormalities per interquartile range (IQR) increase in air pollutants concentrations.

	Standard lipid biomarkers											
	TC ≥ 240 mg/dL			LDL-C ≥ 160 mg/dL			HDL-C < 40 mg/dL			Triglycerides ≥ 200 mg/dL		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
PM_{10}												
Crude	0.94	0.85–1.05	.282	0.99	0.82–1.20	.952	**1.29**	**1.17–1.43**	.000	0.96	0.85–1.08	.489
Multivariate	1.02	0.90–1.16	.718	1.10	0.87–1.37	.428	**1.36**	**1.21–1.54**	.000	0.97	0.83–1.12	.655
$PM_{2.5}$												
Crude	1.00	0.89–1.13	.958	1.07	0.87–1.31	.521	**1.33**	**1.20–1.47**	.000	0.94	0.82–1.07	.353
Multivariate	1.07	0.94–1.22	.314	1.16	0.92–1.46	.198	**1.41**	**1.25–1.59**	.000	0.95	0.82–1.11	.542
NO_2												
Crude	0.93	0.85–1.02	.139	0.97	0.83–1.14	.975	**1.23**	**1.14–1.32**	.000	0.91	0.82–1.01	.073
Multivariate	0.99	0.88–1.11	.853	1.11	0.91–1.36	.285	**1.31**	**1.18–1.44**	.000	0.96	0.84–1.10	.558
	Particle concentration (^{1}H-NMR)											
	LDL-p ≥ p75			VLDL-p ≥ p75			HDL-p < p25					
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*			
PM_{10}												
Crude	**1.15**	**1.06–1.26**	.002	1.03	0.94–1.12	.578	1.12	1.03–1.23	.011			
Multivariate	**1.24**	**1.11–1.39**	.000	1.08	0.96–1.21	.188	**1.23**	**1.09–1.37**	.000			
$PM_{2.5}$												
Crude	1.12	1.02–1.23	.016	1.02	0.93–1.12	.631	**1.13**	**1.03–1.24**	.007			
Multivariate	**1.16**	**1.04–1.29**	.007	1.09	0.97–1.21	.144	**1.23**	**1.10–1.37**	.000			
NO_2												
Crude	1.08	1.01–1.16	.023	0.99	0.92–1.06	.741	**1.10**	**1.03–1.17**	.007			
Multivariate	1.11	1.02–1.22	.018	1.05	0.95–1.15	.334	**1.18**	**1.07–1.29**	.001			

Note: ORs. 95% CI and *p* values were calculated by logistic regression per interquartile range (IQR) increase in air pollutants concentrations (PM_{10}: 7.7 μg/m^3, $PM_{2.5}$: 4.8 μg/m^3, NO_2: 12.1 μg/m^3). In bold: % changes with *p* values < .007 (alpha .05 corrected by Bonferroni = .05/7). Multivariate model: adjusted to age, sex, BMI, smoking status, alcohol intake, education level, MedScore, IPAQ, municipality population, ambient temperature and lipid lowering medication.

Abbreviations: HDL-C, high-density lipoprotein cholesterol; HDL-p, HDL particles; LDL-C, low-density lipoprotein cholesterol; LDL-p, LDL particles; NO_2, nitrogen dioxide; PM_{10}, particles with an aerodynamic diameter of less than 10 microns; $PM_{2.5}$, particles with an aerodynamic diameter of less than 2.5 microns; TC, total cholesterol; TG, triglycerides; VLDL-p, VLDL particles; ^{1}H-NMR, proton nuclear magnetic resonance.

higher LDL-p concentrations (≥p75), although the associations between air pollutants and the standard LDL-c measures were null.

These findings are in line with previous studies suggesting that air pollution might negatively impact blood lipids in the general population,[6–24] and may be a potential contributor to air pollution-related CVD risk. In fact, low HDL-c is a well-established atherosclerotic CVD risk factor,[34] whereas lower HDL and higher LDL particle numbers have also been associated with increasing cardiovascular events.[42–44]

Interestingly, although the relationship of the exposure to different air pollutants with standard lipid biomarkers has been widely studied, to the best our knowledge, only two previous studies have assessed associations with particle concentration measures. Bell et al.[9] examined the relationship between air pollution and both HDL-c and HDL-p, in 6654 men and women free of prevalent clinical CVD, participants in the Multi-Ethnic Study of Atherosclerosis Air Pollution study (MESA Air). A 5 μg/m^3 higher $PM_{2.5}$ was associated with lower HDL-p (−0.64 μmol/L [95% CI −1.01, −0.26]), but not HDL-c (−0.05 mg/dL [95% CI −0.82, 0.71]). McGuinn et al.[13] studied linear associations to estimate change in lipoprotein levels with each μg/m^3 increase in annual average $PM_{2.5}$ in 6587 patients who had a cardiac catheterization in Duke University between 2001 and 2010 (CATHGEN study). The percent change from the mean outcome level was 2.00% (95% CI 1.38%, 2.64%) for total LDL-p. However, the associations between air pollution and HDL particle concentrations in this study were inconsistent. This is in contrast to the findings

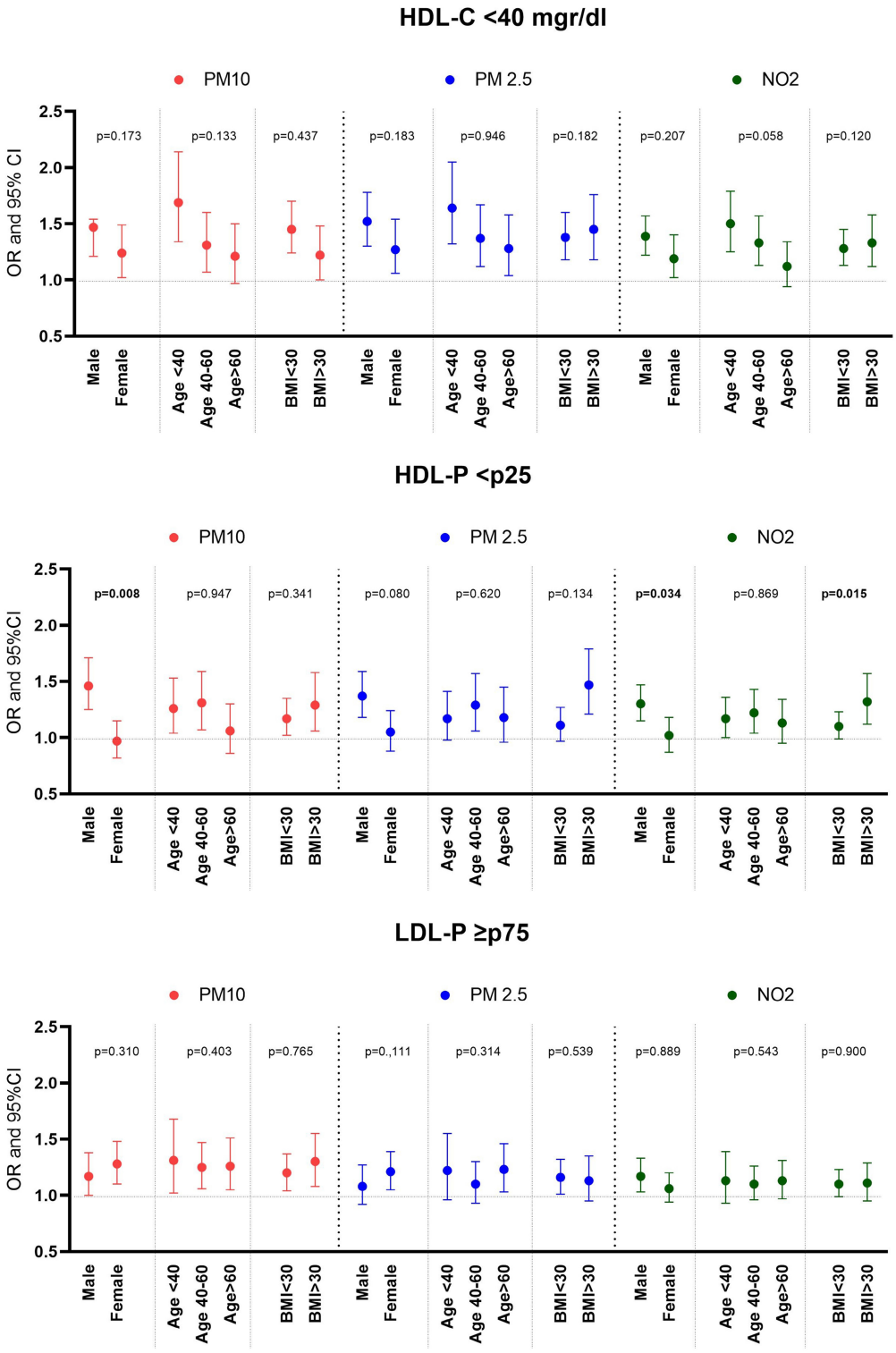

FIGURE 1 Logistic regression analyses between $PM_{2.5}$, PM_{10} and NO_2 exposures and HDL-c <40 mg/dL, HDL-p <p25 and LDL-p ≥p75 in different population subgroups.

reported by Bell et al. in the MESA Air study[9] and to our results in di@bet.es, which indicate strong negative associations between air pollutants and HDL markers. Of note, the CATHGEN study sample consisted of patients who underwent cardiac catheterization, who may represent a highly selective population, while the MESA Air and the di@bet.es study populations may be more generalizable to the general background population.

Although the observed differences in blood lipids found in our study may seem small, it is of note that, as

pointed out by Bell et al.,[9] the magnitude of the decrease of HDL-c and HDL-p we have observed (between −3.0% and −3.3% decrease in HDL-c and −1.1% to −1.4% decrease in HDL-p per IQR increase in air pollutants concentration), can be compared to the effect of smoking on these measurements as observed in smoking cessation studies (2.4 mg/dL and 1.0 μmol/L change on HDL-c and HDL-p, respectively).[45]

On the other hand, exposure to air pollution has also been shown to induce the development of dysfunctional HDL resulting in alteration of its atheroprotective capacities,[46,47] which may contribute to atherosclerosis progression beyond the total lipoprotein concentrations measured in our study.

The apparently counterintuitive negative association between air pollutants and TC observed is of small magnitude and could be explained by the contribution of HDL-c to the TC levels, which was significantly reduced when associated with higher exposures. In line with this, no associations with LDL-c levels in the multivariate analyses were found. This apparently benign effect of air pollutants in the standard LDL-c measures was however accompanied by a significant association between higher exposures to PM_{10} and $PM_{2.5}$, and higher LDL-p concentrations (≥p75). LDL-p has been found to be a better predictor of CVD than LDL-c, especially in individuals with LDL-p/LDL-c discordance.[28,29,48] So again, these associations could be regarded as pro-atherogenic. In subgroup analyses, we found stronger associations between PM_{10} and NO_2 and low HDL-p in men, and between NO_2 and low HDL-p in individuals with obesity, suggesting that these subgroups may be more susceptible. A stronger susceptibility in overweight–obese individuals is consistent with Sørensen et al.,[6] Yang et al.,[10] Kim et al.,[12] Mao et al.,[16] Zhang et al.[22] and Kim et al.[19] among others. Both air pollution exposure and overweight/obesity are associated with higher systemic inflammation[2,49] which may explain an interplay of these factors. The modification effects of sex in previous studies have been mixed and harder to interpret.

The underlying mechanisms explaining the effect of air pollutants on lipids have not been fully elucidated. The main hypothesis is that the oxidative stress and systemic inflammation caused by inhaled air pollution could induce adverse lipid metabolism and lipid oxidation.[50,51] Air pollutants have also been found to cause DNA methylation of genes related to lipid metabolism.[52] Further studies are warranted to clarify the full spectrum of these mechanisms.

Our study has several strengths, including the large population-based design and the inclusion of advanced lipoprotein measures, as well as other extensive individual-level data of clinical, demographic and lifestyle variables, which allowed us to perform a complete multivariate adjustment of the data. Our nationwide perspective, allows us to extrapolate our results more widely than local or regional studies, increasing the public health implications of the findings.

The limitations of our study include its observational cross-sectional nature; so that we cannot establish causal associations or exclude residual confounding in the relation between air pollutants and lipids. Also, we used ambient outdoor measurements modelled at the residential addresses of the participants as a proxy for exposure to air pollution, whereas no other relevant information such as time–activity patterns, proximity to main roads, occupational exposures or personal monitoring data was available. Exposure measurement error is possible when using modelled pollutant levels and this could, in fact, have attenuated our effect estimates. Finally, our exposure models were developed based on yearly exposures to air pollutants, whereas more refined measures to look at different lags were not available.

5 | CONCLUSIONS

In summary, our study, in keeping with previous data, suggests a deleterious effect of the exposure to air pollutants on blood lipids, in the general population of Spain. Our results reinforce the need for improving air quality as much as possible to decrease the risk of atherosclerosis in our population, as the lipid changes observed in our study may be a potential contributor to air pollution-related CVD risk.

AUTHOR CONTRIBUTIONS

Conception and design: G.R.M and J.R. Acquisition of epidemiological data: C.M.A., E.G.E., S.G.S., A.C.P., L.C., E.D., E.M., J.F.N., S.G., J.G., F.J.C., S.V. and G.R.M. tandard biochemical samples management: W.O.B. and G.R.M. Liposcale® samples management and analyses: J.R., N.A. and M.G. Air pollution modelling: M.G.V., J.L.G., M.T., V.G. and F.M.L. Creation of new software used in the work: J.L.G.G and G.A.V. Analysis and interpretation of data: S.V. J.C.V. and G.R.M. Drafting the article: S.V., V.K.D.G. and G.R.M. All authors revised and approved the final manuscript.

ACKNOWLEDGEMENTS

The di@bet.es project is a collaborative study with various phases and sub-projects in which a large number of researchers and technicians have collaborated, to whom we are indebted. Our profound appreciation goes to the primary care managers and personnel of the participating health centres, to all the fieldworkers, nurses and technicians and to the study participants for their altruistic participation.

FUNDING INFORMATION

CIBERDEM (Ministerio de Economía, Industria y Competitividad-ISCIII), Ministerio de Sanidad, Servicios Sociales e Igualdad-ISCIII, Instituto de Salud Carlos III (PI17/02136, PI20/01322), European Regional Development Fund (ERDF) 'A way to build Europe'. GRM belongs to the regional Nicolás Monardes research program of the Consejería de Salud (RC-0006-2016; Junta de Andalucía, Spain). VKDG is recipient of a 'Rio Hortega' research contract (CM21/00214, Instituto de Salud Carlos III). S.V.H. was supported by an intensification research program (INT21/00037, ISCIII, Spain; co-funded by the Fondo Europeo de Desarrollo Regional-FEDER). Funding for open access charge: Universidad de Málaga/CBUA.

CONFLICT OF INTEREST STATEMENT

N.A. is stock owner of Biosfer Teslab and has a patent on the method for lipoprotein profiling described in the present manuscript. The other authors declare that they have no competing financial interests related to this manuscript.

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How to cite this article: Valdés S, Doulatram-Gamgaram V, Maldonado-Araque C, et al. Association between exposure to air pollution and blood lipids in the general population of Spain. *Eur J Clin Invest*. 2023;00:e14101. doi:10.1111/eci.14101