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INFLUENCE OF AIR POLLUTION ON LIPOPROTEINS IN THE GENERAL POPULATION

FINAL DEGREE RESEARCH WORK

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1. ABSTRACT

Introduction: Since cardiovascular diseases are the main cause of death in the world, we consider that studying their risk factors is of crucial importance. Among them, lipoproteins and their characteristics have proven to be one of the most influential. Other studies show that pollution and its particles favor cardiovascular diseases. That is why we want to study the relationship between pollution and lipoproteins.

Objective: Demonstrate that air pollutants have a qualitative and quantitative effect on lipoprotein characteristics in the general population.

Methods: This study consists of 4,464 people with a mean age of 50 years and a BMI of 28.99 kg/m2. The samples and data were obtained from the population-based cohort study Di@bet.es, carried out between 2008-2010. The Di@bet.es study sample consisted of 5,072 subjects over 18 years of age, randomly selected. This study was approved by the Ethics and Clinical Research Committee of the Hospital Regional Universitario de Málaga. The information was collected through a structured questionnaire administered by the interviewer, followed by a physical examination and a blood sample. Lipoprotein analysis was performed using the Liposcale® test. The atmospheric levels of CO, NO2, O3, SO2, particles smaller than 10μm (PM10) and particles smaller than 2.5μm (PM2.5) were obtained through modeling combined with measurements at air quality stations. Work carried out in the Atmospheric Pollution Division of the CIEMAT.

Results: Total cholesterol and LDL cholesterol significantly decrease with increasing concentrations of pollutants, except SO2, which increases it.

Increased CO is accompanied by a significant increase in LDL particles. On the contrary, O3 is associated with a lower concentration of LDL particles.

SO2, PM10, PM2.5 and PCAF1 significantly increase triglyceride values, except for O3, which decreases them. All of them with a modest effect. This effect on TG concentrations does no affect the concentration of VLDL particles. Except for SO2, all pollution particles showed a very significant association with HDL cholesterol and a relationship was also seen with circulating HDL particles.

Conclusions:

- Air pollutants have a qualitative and quantitative effect on lipoprotein characteristics in the general population.
- Air pollutants produce an apparently protective effect against cardiovascular events since they reduce total cholesterol.
- These could also be considered cardiovascular risk factors because they increase triglycerides and HDL particles.
- CO considerably increases LDL particles.

1. RESUMEN

Introducción: Dado que las enfermedades cardiovasculares son la principal causa de muerte en el mundo, consideramos de crucial importancia el estudio de sus factores de riesgo. Entre ellos, las lipoproteínas y sus características han demostrado ser uno de los más influyentes. Otros estudios demuestran que la contaminación y sus partículas favorecen las enfermedades cardiovasculares. Por eso queremos estudiar la relación entre la contaminación y las lipoproteínas.

Objetivo: Demostrar que los contaminantes del aire tienen un efecto cualitativo y cuantitativo sobre las características de las lipoproteínas en la población general.

Métodos: Este estudio consta de 4.464 personas con una edad media de 50 años y un IMC de 28,99 kg/m2. Las muestras y datos se obtuvieron del estudio de cohortes de base poblacional Di@bet.es, realizado entre 2008-2010. La muestra del estudio Di@bet.es estuvo formada por 5.072 sujetos mayores de 18 años, seleccionados aleatoriamente. Este estudio fue aprobado por el Comité de Ética e Investigación Clínica del Hospital Regional Universitario de Málaga. La información se recolectó a través de un cuestionario estructurado administrado por el entrevistador, seguido de un examen físico y una muestra de sangre. El análisis de lipoproteínas se realizó mediante el test Liposcale®. Los niveles atmosféricos de CO, NO2, O3, SO2, partículas menores a 10μm (PM10) y partículas menores a 2.5μm (PM2.5) se obtuvieron mediante modelación combinada con mediciones en estaciones de calidad del aire. Trabajo realizado en la División de Contaminación Atmosférica del CIEMAT.

Resultados: El colesterol total y el colesterol LDL disminuyen significativamente al aumentar las concentraciones de contaminantes, excepto el SO2 que lo aumenta.

El aumento de CO se acompaña de un aumento significativo de las partículas LDL. Por el contrario, el O3 se asocia con una menor concentración de partículas LDL.

SO2, PM10, PM2.5 y PCAF1 aumentan significativamente los valores de triglicéridos, excepto el O3 que los disminuye. Todos ellos con un efecto modesto. Este efecto sobre las concentraciones de TG no afecta la concentración de partículas VLDL. A excepción del SO2, todas las partículas de contaminación mostraron una asociación muy significativa con el colesterol HDL y también se observó una relación con las partículas de HDL circulantes.

Conclusiones:

- Los contaminantes del aire tienen un efecto cualitativo y cuantitativo sobre las características de las lipoproteínas en la población general.
- Los contaminantes del aire producen un efecto aparentemente protector frente a eventos cardiovasculares ya que reducen el colesterol total.
- Estos también podrían considerarse factores de riesgo cardiovascular porque aumentan los triglicéridos y las partículas HDL.
- El CO aumenta considerablemente las partículas LDL.

1. RESUM

Introducció: Atès que les malalties cardiovasculars són la principal causa de mort al món, considerem que l'estudi dels seus factors de risc és de cabdal importància. Entre elles, les lipoproteïnes i les seves característiques han demostrat ser una de les més influents. Altres estudis demostren que la contaminació i les seves partícules afavoreixen les malalties cardiovasculars. És per això que volem estudiar la relació entre la contaminació i les lipoproteïnes.

Objectiu: Demostrar que els contaminants atmosfèrics tenen un efecte qualitatiu i quantitatiu sobre les característiques de les lipoproteïnes en la població general.

Mètodes: Aquest estudi està format per 4.464 persones amb una edat mitjana de 50 anys i un IMC de 28,99 kg/m2. Les mostres i dades s'han obtingut de l'estudi de cohorts poblacional Di@bet.es, realitzat entre 2008-2010. La mostra de l'estudi Di@bet.es va estar formada per 5.072 subjectes majors de 18 anys, seleccionats aleatòriament. Aquest estudi ha estat aprovat pel Comitè d'Ètica i Investigació Clínica de l'Hospital Regional Universitari de Màlaga. La informació es va recollir mitjançant un qüestionari estructurat administrat per l'entrevistador, seguit d'un examen físic i una mostra de sang. L'anàlisi de lipoproteïnes es va realitzar mitjançant la prova Liposcale®. Els nivells atmosfèrics de CO, NO2, O3, SO2, partícules menors de 10μm (PM10) i partícules menors de 2,5μm (PM2.5) es van obtenir mitjançant modelització combinada amb mesures en estacions de qualitat de l'aire. Treball realitzat a la Divisió de Contaminació Atmosfèrica del CIEMAT.

Resultats: El colesterol total i el colesterol LDL disminueixen significativament amb l'augment de les concentracions de contaminants, excepte el SO2, que l'augmenta

L'augment de CO s'acompanya d'un augment significatiu de partícules de LDL. Per contra, l'O3 s'associa a una menor concentració de partícules LDL.
SO2, PM10, PM2,5 i PCAF1 augmenten significativament els valors de triglicèrids, excepte l'O3, que els disminueix. Tots ells amb un efecte modest. Aquest efecte sobre les concentracions de TG no afecta la concentració de partícules VLDL. Excepte el SO2, totes les partícules de contaminació van mostrar una associació molt significativa amb el colesterol HDL i també es va observar una relació amb les partícules HDL circulants.

Conclusions:

- Els contaminants atmosfèrics tenen un efecte qualitatiu i quantitatiu sobre les característiques de les lipoproteïnes de la població general.
- Els contaminants atmosfèrics produeixen un efecte aparentment protector contra els esdeveniments cardiovasculars ja que redueixen el colesterol total.
- Aquests també es podrien considerar factors de risc cardiovascular perquè augmenten els triglicèrids i les partícules de HDL.
- El CO augmenta considerablement les partícules de LDL.

2. INTRODUCTION

Nowadays, cardiovascular diseases are the main cause of death in the world. According to WHO, 17.9 million people had died in 2019 by this reason, representing 32% of total deaths.[1]

Most common cardiovascular risk factors include smoking, unhealthy diets, sedentary lifestyle, alcohol abuse, high blood pressure, high glucose, dyslipidemia and obesity.[1]

The main mechanism involved in cardiovascular diseases is atherosclerosis, consisting of thickening and loss of elasticity of artery walls due to accumulation of lipids, inflammatory cells, smooth muscle cells and connective tissue matrix, forming the so-called atherosclerotic plaque. Previous damage in the endothelium, known as endothelial dysfunction can be caused by multiple factors.[2]

According to current WHO recommendations, to prevent atherosclerotic plaque formation, maintaining normal lipoprotein levels is one of the main strategies, that is why it is important to assess their levels and understand all factors that can modify lipid concentrations.[3]

Different studies have shown that air pollution causes endothelial dysfunction mainly through reactive oxygen species (ROS). These determine the half-life of nitric oxide (NO). Studies confirm that the association of NO plus a superoxide causes vasoconstriction. This also leads to cytotoxicity, as it damages the protective enzymes of the endothelium. [3]

Other studies show that endothelial dysfunction is a chronic damage and there is no minimum exposure from which an onset of damage has been demonstrated. Chronic exposure to air pollution promotes insulin resistance, increased risk of obesity and thrombosis. These are all

factors affecting atherosclerosis, however, data on how air pollutants affect lipoprotein concentrations from a quantitative and qualitative point of view is scarce. [4]

We hypothesize that air pollutants affect quantitative and qualitative lipoprotein characteristics.

3. MATERIAL AND METHODS

Study design, setting and population. Samples and data were based on the population-based, cohort study Di@bet.es.

The initial cross-sectional Di@bet.es study was undertaken in 2008–2010 from a random cluster sampling of the Spanish population. The Di@bet.es study sample consisted of 5072 subjects more than 18 years old, randomly selected from National Health System registries distributed into 100 clusters. Subjects with severe disease such as cancer or hepatitis were excluded by protocol. Detailed information on the methodology of the Di@bet.es cohort study has been previously described. [5]

The research was carried out in accordance with the Declaration of Helsinki (WHO 2011) of the Word Medical Association. 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.

Data collection and laboratory measurements. Participants were invited to attend an examination visit at their health center with a nurse specially trained for this project. Information was collected using an interviewer administered structured questionnaire, followed by a physical examination and blood sampling.

For the present study the anthropometric and sociodemographic variables considered were: age, sex, weight, waist, family history of T2DM; educational level (classified as unlettered, attendance to primary or high school, and university); alcohol consumption (never: no alcohol consumption, low: < 1 serving/week, moderate: between 1 serving/week and 2 servings/day for men and 1 serving/day for women, and high: > 2 servings/day for men and over 1 serving/day for women);

adherence to the Mediterranean diet (a 14-point Mediterranean diet score was calculated and the cut-off for considering adherence was over 8 points in the score); physical activity (classified as low, moderate and high levels according to the IPAQ questionnaire); smoking habits (current smokers vs former/never been smokers). The use of teratogenic medications (amiodarone, methotrexate, tamoxifen, fluoxetine, valproic acid, acetylsalicylic acid or nonsteroidal anti-inflammatory drugs) has been also considered.

Also, clinical variables such as blood pressure levels, fasting levels of glucose, insulin and lipid profile (total cholesterol, high-density lipoprotein, low-density lipoprotein and triglycerides (TAG)) and transaminases (GGT, aspartate transaminase (AST) and alanine transaminase (ALT) were considered.

BMI was calculated. Insulin resistance was estimated by the homeostasis model assessment (HOMA), and the HOMA 75th percentile of our population excluding subjects with T2DM was calculated as the insulin resistance risk category (HOMA-IR).

Lipoprotein analysis. Before 1H-NMR analysis, 200 µl of serum were diluted with 50 µl deuterated water and 300 µl of 50 mM phosphate buffer solution (PBS) at pH 7.4. 1H-NMR spectra were recorded at 306 K on a Bruker Avance III 600 spectrometer operating at a proton frequency of 600.20 MHz.

Lipoprotein analysis was made by using Liposcale® Test, a novel advanced lipoprotein test based on 2D diffusion-ordered 1H-NMR spectroscopy. The methyl signal was deconvoluted by using 9 Lorentzian functions to determine the lipid concentration of the large, medium and small subclasses of the main lipoprotein classes (VLDL, LDL and HDL), and their size associated diffusion coefficients. Then, we combined the lipid concentration with their associated particle volume in order to quantify the number of particles required to transport the measured lipid concentration of each lipoprotein subclass. Finally, weighted average VLDL, LDL and HDL particle sizes were calculated from various subclass concentrations by summing the known diameter

of each subclass multiplied by its relative percentage of subclass particle number. The variation coefficients for particle number were between 2% and 4%, and for the particle sizes were lower than 0.3%.

Air pollutants. As indicators of air quality, the average annual values from 2008 to 2016 of the atmospheric levels of CO, NO2, O3, SO2, particles smaller than 10um (PM10) and particles smaller than 2.5um (PM2.5) obtained through modeling combined with measurements at air quality stations work carried out in the CIEMAT Atmospheric Pollution Division. The data was obtained for the postal code of the address of each subject participating in the study, and ad hoc programs had to be designed to be able to join them to the general base of the study.

Given that these variables are highly correlated, by means of factorial analysis by principal components, a factor has been extracted from the atmospheric variables plus the average temperature which strongly correlates with all the pollutants except O3.

Statistics. Statistical software SPSS, version 23 was used to analyze all data. Continuous variables normally distributed are presented as the mean and standard deviation (SD) and continuous variables non-normally distributed are presented as the median and interquartile range (IQR). Categorical variables are presented as the percentage and the number of individuals. For categorical variables, the differences between the proportions were analyzed using the chi-Squared test.

To estimate the association between the different contaminant variables and the different lipid variables, we used linear regression models with multivariate variables added. We initially selected known confounders variables and their interaction terms for inclusion in the multivariable regression models. The interaction terms were considered significant when significantly improved the variability of the dependent variable explained by the model. We adjusted one model for each lipid and contaminant variables. Tolerance was measured to avoid collinearity between contaminant variables. The R-squared (R2) statistic was used

to provide an estimate of the percentage of the response variable variability that was explained by the linear models and by each of the predictors added in the models. p-value of <0.05 was considered statistically significant.

4. RESULTS

The final sample was of 4464 people. Among them, there were 2559 women and 1905 men. The age of the patients who have participated in the study was between 18 and 93 years old, with an average of 50. As for the BMI, there was an average of 27.99 kg/m2,

Regarding cholesterol, its average was 5.05 mmol/L. The mean values of HDL were 1.332 and those of LDL were 2.709. Lastly, that of triglycerides was 1.364.

The average of CO was 0.32 units, that of NO2 was 19.12 and that of O3 was 112.68. The average value of SO2 was 4.48, that of PM10 was 20.46 and that of PM2.5 was 11.10.(Table 1)

Table 1. Baseline characteristics of the studied population.

	MINIMUM	MAXIMUM	MEDIUM	SD
AGE (years)	18	93	50.48	16.994
BMI (kg/m2)	12.160	61.260	27.998	5.123
CHOLESTEROL (mmol/L)	1.490	10.220	5.057	1.027
HDL CHOLESTEROL (mmol/L)	0.180	2.960	1.332	0.329
LDL CHOLESTEROL (mmol/L)	0.550	6.450	2.709	0.768
TRIGLYCERIDES (mmol/L)	0.190	23.650	1.364	1.005
CO (mg/m3)	0.170	1.370	0.325	0.146
NO2 (µg/m3)	4.420	49.840	19.115	11.205
O3 (µg/m3)	88.230	125.700	112.688	6.907
SO2 (µg/m3)	1.700	12.040	4.480	2.046

PM10 (µg/m3)	11.860	30.180	20.460	4.142
PM2.5 (µg/m3)	5.940	16.490	11.101	2.267

According to geographical location, the south of the country is where there have been the most, with a total of 1306. This is followed by the center with 1154. The rest of the areas are, with 842 the northeast, the Levante with 584 and the North with 578.(Table 2)

Table 2. Frequency of the population studied by areas.

	FREQUENCY	%
SOUTH	1306	29.3
NORTH	578	12.9
HUB	1154	25.9
NORTHEAST	842	18.8
EAST	584	13.1

During the study we have analyzed different pollutant particles such as Carbon Monoxide (CO), Nitrous Oxide (NO2), Ozone (O3), Sulfur Dioxide (SO2), particles with molecular weight of 10 (PM10) and 2.5 (PM2.5), and the composite PCAF1 to assess its association with the lipid and lipoprotein profile. From the conventional lipid profile we have obtained the concentration of total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol. By means of 1H-NMR (LipoScale®) we have also analyzed the size (large, medium and small) and concentration of LDL, HDL, VLDL particles and according to their respective diameters.

Below we will describe how the individual pollutants affect the following lipid parameters: total cholesterol and LDL cholesterol, triglycerides and HDL cholesterol. For each lipid parameter, plasma concentration and lipoprotein size and concentration are studied.,

Total cholesterol and LDL cholesterol significantly decrease with increasing concentrations of these pollutants, except for sulfur dioxide, which is associated with an increase of both of them.(Table 3 and 4)

Table 3. Influence of air pollutants on cholesterol.

CHOLESTEROL	P VALUE	CHANGE (B)	95% CONFIDENCE INTERVAL	
M_CO (mg/m3)	0.006	-0.286	-0.490	-0.082
NO2 (µg/m3)	<0.001	-0.007	-0.009	-0.004
O3 (µg/m3)	<0.001	-0.008	-0.012	-0.003
SO2 (µg/m3)	0.044	0.015	0.000	0.029
PM10 (µg/m3)	<0.001	-0.016	-0.023	-0.009
PM2.5 (µg/m3)	0.001	-0.022	-0.035	-0.008
PCA F1	<0.001	-0.068	-0.098	-0.039

Table 4. Influence of air pollutants on LDL cholesterol.

LDL	P VALUE	CHANGE (B)	95% CONFIDENCE INTERVAL	
M_CO (mg/m3)	0.289	-0.083	-0.235	0.070
NO2 (µg/m3)	0.003	-0.003	-0.005	-0.001
O3 (µg/m3)	<0.001	-0.006	-0.010	-0.003
SO2 (µg/m3)	<0.001	0.018	0.007	0.029
PM10 (µg/m3)	0.39	-0.006	-0.011	0.000
PM2.5 (µg/m3)	0.134	-0.008	-0.017	0.002
PCA F1	0.022	-0.026	-0.048	-0.004

The decrease in total and LDL cholesterol associated with M-CO is accompanied by a significant increase in total LDL particles. A similar, although non-significant trend was observed for NO2. On the contrary, O3 cholesterol-lowering association resulted in a significant decrease in total LDL particles(Table 5). The largest effect on plasma cholesterol and LDL particles is that of M-CO. Although the effect of O3 on LDL particles does not depend on the size of the LDL particle.

Table 5. Influence of air pollutants on LDL particles.

LDL PARTICLES	P VALUE	CHANGE (B)	95% CONFIDENCE INTERVAL	
M_CO (mg/m3)	0.048	54.543	0.415	108.672
NO2 (µg/m3)	0.057	0.688	-0.021	1.397
O3 (µg/m3)	0.021	-1.373	-2.535	-0.212
SO2 (µg/m3)	0.512	-1.291	-5.151	2.569
PM10 (µg/m3)	0.998	-0.002	-1.917	1.912
PM2.5 (µg/m3)	0.491	1.234	-2.279	4,.48

We have studied the effect of air pollution on plasma triglycerides and its main transporter in circulation, VLDL particles.

Ozone (O3), Sulfur Dioxide (SO2), particles with molecular weight of 10 (PM10) and 2.5 (PM2.5), and the composite PCAF1 show a significant effect on plasma TG. All pollutants associated with increased TG except in the case of O3. In all cases, the magnitude of the association was modest. (Table 6)

Table 6. Influence of air pollutants on TG.

TRIGLYCERIDES (LogTRIS)	P VALUE	CHANGE (B)	95% CONFIDENCE INTERVAL	
M_CO (mg/m3)	0.233	0.025	-0.016	0.067
NO2 (µg/m3)	0.059	0.001	0.000	0.001
O3 (µg/m3)	0.045	-0.001	-0.002	0.000
SO2 (µg/m3)	0.006	0.004	0.001	0.007
PM10 (µg/m3)	0.023	0.002	0.000	0.003
PM2.5 (µg/m3)	0.001	0.005	0.002	0.008
PCA F1 (µg/m3)	0.035	0.007	0.000	0.013

We have checked whether the effect of air pollutants on circulating TG also affects TG-rich transporting particles. The results shown in Table 7 demonstrate that air pollutants have no significant effect on the number of circulating VLDL particles and only affect their TG content. (Table 7)

Table 7. Influence of air pollutants on VLDL particles.

VLDL PARTICLES	P VALUE	CHANGE (B)	95% CONFIDENCE INTERVAL	
M_CO (mg/m3)	0.176	5.151	-2.306	12.609
NO2(µg/m3)	0.091	0.084	-0.013	0.182
O3(µg/m3)	0.534	-0.051	-0.211	0.109
SO2(µg/m3)	0.063	0.505	-0.027	1.036

PM10 (µg/m3)	0.100	0.222	-0.042	0.485
PM2.5 (µg/m3)	0.052	0.479	-0.004	0.963

Except for SO2, all studied air pollutants showed a highly significant association with decreased concentrations of HDL cholesterol, being the strongest effect linked to M-CO. Contrary to what was observed in relation to TG, the decrease in HDLc was also accompanied by a decrease in the number of circulating HDL particles.(Table 8)

Table 8. Influence of air pollutants on HDL cholesterol.

HDL	P VALUE	CHANGE (B)	95% CONFIDENCE INTERVAL	
M_CO (mg/m3)	0.001	-0.107	-0.173	-0.041
NO2 (µg/m3)	<0.001	-0.003	-0.003	-0.002
O3 (µg/m3)	0.003	-0.002	-0.003	-0.001
SO2 (µg/m3)	0.141	0.004	-0.001	0.008
PM10 (µg/m3)	<0.001	-0.008	-0.010	-0.005
PM2.5 (µg/m3)	<0.001	-0.012	-0.017	-0.008
PCA F1(µg/m3)	<0.001	-0.029	-0.039	-0.019

When we analyze the HDL particles according to size, we see that the same thing happens in large and medium-sized particles, that is, some air pollutant is statistically significant in terms of influence on the units of these particles in the blood, but we see that it does not really increase or decrease with relevance in this type of patient.(Table 9)

Table 9. Influence of air pollutants on HDL particles.

HDL PARTICLES	P VALUE	CHANGE (B)	95% CONFIDENCE INTERVAL	
M_CO (mg/m3)	0.098	-0.770	-1.684	0.143
NO2 (µg/m3)	0.004	-0.018	-0.030	-0.006
O3 (µg/m3)	0.006	0.027	0.008	0.047
SO2 (µg/m3)	0.639	-0.016	-0.081	0.050
PM10 (µg/m3)	0.016	-0.040	-0.072	-0.008
PM2.5 (µg/m3)	0.003	-0.089	-0.148	-0.030

We would now like to point out, albeit slightly, that there is one component of air pollution that does have a significant effect on small HDL particle units. This is carbon monoxide (CO) and it causes this type of lipid particle to decrease by 1.190 units for each unit of CO exposure. This same air pollutant is again important, with less magnitude, in these small HDL particles. Bearing in mind that most of the repercussions are minimal when the p-value is statistically significant, in this case there is a greater relevance in terms of numbers in these patients. In fact, it is as much as 100-fold and 1000-fold greater than the other airborne pollutants in terms of magnitude. And once again, carbon monoxide is the protagonist.

It should be noted in this section of the statistics that the tendency of air pollution to affect small HDL particles is to decrease the units of these particles for each unit of exposure to these pollutants. Therefore, although we see that carbon monoxide is the main component that decreases these particles, the overall tendency of all the dependent variables is to produce a decrease in the lipid profile.

5. DISCUSSION

The aim of our study was to test the effect of air pollutants on lipoproteins both qualitatively and quantitatively. Our results show that air pollutants have a significant effect on total and LDL cholesterol, triglycerides and HDL cholesterol.

Quite to our surprise, air pollutants are associated with a significant decrease in total and LDL cholesterol (except SO2) although the magnitude of such association is modest. On the contrary, the number of LDL particles significantly increases, particularly in relation to M-CO. These data suggest that the apparently benign effect of some pollutants decreasing circulating cholesterol may, in fact, result in an increased number of LDL particles. From an atherosclerotic point of view, this could be regarded as a pro-atherogenic effect. In fact, more than half of cases of acute myocardial infarction occur in individuals with normal cholesterol concentrations delivered by smaller and more abundant LDL particles [(6)]. Comparison with existing data is difficult as previous similar studies were done in animals with quite different conditions, mostly related to O3. Lovati M et al report that O3 exposure in rats acutely increases cholesterol and HDL levels. [(7)] In short, exposure to pollutants causes an increase in LDL levels and a decrease in O3. The only study coincident with our results is Zhang K et al, The association between ambient air pollution and blood lipids, China (2021), but it only looks at PM10 and PM2.5 exposure.[(8)]

Four out of the six pollutants studied have a significant effect on TG by increasing plasma concentrations. As for the effect on cholesterol, the magnitude of these associations is modest. Contrary to what we observed regarding cholesterol transport, the increase in TG concentrations is not associated with an increase in the number of circulating VLDL particles. This suggests an effect on impaired TG

hydrolysis rather that an increased VLDL hepatic synthesis. Our results are in line with a study reporting that PM10 and PM2.5 increase triglycerides[7]. However, other studies in rats report a decrease in triglycerides associated with O3 exposure [8,9]

Regarding HDL, all pollutants except SO2 were significantly associated with lower HDL cholesterol concentrations, a well-known cardiovascular risk factor. Consistent with the observations made on cholesterol and TG, the magnitude of these associations was modest. The effect of these pollutants on the concentration of HDL particles is diverse although it tends to be associated with lower numbers of circulating HDL particles. Existing literature reports an increase in HDL on O3 exposure,[7] alterations in the functionality of HDL[10] and a decrease in HDL and increase of LDL and TG when exposed to PM2.5 and PM10. [7], consistent with our results. Of note, the last article has a population of 73,117 studied over 10 years with more than 600,000 samples taken.

The best-known relationships between air pollutants and their effect on our organisms are as follows: CO produces dizziness, headaches and can be lethal at high doses; NO2 is related to respiratory infections; PM10 and PM2.5 damage the respiratory and cardiovascular systems; O3 has been shown to inflame the lungs and damage the liver and SO2 promotes asthma exacerbations. Although the magnitude of the effects is limited, we should not rule-out a significant influence on human health as these are life-time stimuli. For future studies, we believe it is important to know the origin of the contaminants in order to apply preventive measures. CO and NO2 are products of vehicle combustion, O3 is the product of chemical reactions of VOCs and NOx with sunlight, SO2 comes from the use of liquid fuels derived from petroleum and volcanic eruptions, and PM10 and PM2.5 from the burning of firewood and/or coal, constructions and unpaved roads. Ways to reduce these pollutants could be to use public transport, share

a vehicle, use recycled products, avoid burning garbage, take care of green areas... [11,12,13,14]

We consider that the strongest point of our work is the large population studied, which provides more reliability to the results and greater statistical power. On the other side, we have as a limitation the heterogeneity of contamination. Pollution measurements vary greatly between different places, change quite a bit over time, and even vary with climate.

In conclusion, our study demonstrates that contamination and air pollutants have deleterious effects on lipid and lipoprotein metabolism. These produce a protective effect against cardiovascular events since they lower total cholesterol. We could also consider them cardiovascular risk factors because they increase triglycerides and HDL particles.
CO considerably increases LDL particles and O3 reduces them but to a lesser extent. Further studies will be necessary to understand in more detail the impact on public health of these changes.

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