Association between Long-Term Exposure to Traffic-Related Air Pollution and Subclinical Atherosclerosis: The REGICOR Study

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BACKGROUND: Epidemiological evidence of the effects of long-term exposure to air pollution on the chronic processes of atherogenesis is limited.

OBJECTIVE: We investigated the association of long-term exposure to traffic-related air pollution with subclinical atherosclerosis, measured by carotid intima media thickness (IMT) and ankle–brachial index (ABI).

METHODS: We performed a cross-sectional analysis using data collected during the reexamination (2007–2010) of 2,780 participants in the REGICOR (Registre Gironí del Cor: the Gerona Heart Register) study, a population-based prospective cohort in Girona, Spain. Long-term exposure across residences was calculated as the last 10 years’ time-weighted average of residential nitrogen dioxide (NO2) estimates (based on a local-scale land-use regression model), traffic intensity in the nearest street, and traffic intensity in a 100 m buffer. Associations with IMT and ABI were estimated using linear regression and multinomial logistic regression, respectively, controlling for sex, age, smoking status, education, marital status, and several other potential confounders or intermediates.

RESULTS: Exposure contrasts between the 5th and 95th percentiles for NO2 (25 μg/m3), traffic intensity in the nearest street (15,000 vehicles/day), and traffic load within 100 m (7,200,000 vehicles/m-day) were associated with differences of 0.56% (95% CI: –1.5, 2.6%), 2.32% (95% CI: 0.48, 4.17%), and 1.91% (95% CI: –0.24, 4.06) percent difference in IMT, respectively. Exposures were positively associated with an ABI of > 1.3, but not an ABI of < 0.9. Stronger associations were observed among those with a high level of education and in men ≥ 60 years of age.

CONCLUSIONS: Long-term traffic-related exposures were associated with subclinical markers of atherosclerosis. Prospective studies are needed to confirm associations and further examine differences among population subgroups.


Air pollution from traffic and other sources is an established cause of premature mortality (Brook et al. 2010). A relevant part of this environmental burden of disease relates to cardiovascular diseases (CVDs), which were responsible for 10% of total disability-adjusted life years in 2004 and the leading causes of death worldwide in 2008 (World Health Organization 2008). A common feature of this group of diseases is atherosclerosis, a chronic and degenerative process that mainly occurs in large and medium-sized arteries and is characterized by asymmetric focal thickenings of the innermost layer of the artery, the intima. The development of atherosclerosis is the result of the total cumulative exposure to atherogenic risk factors such as hypertension, high cholesterol, diabetes, obesity, smoking, physical inactivity, and other lifestyle factors and their interactions with genetic susceptibility (Lusis 2000). Acute events such as myocardial infarction or stroke can be triggered by short-term exposure to air pollution (Brook et al. 2010). However, whether and how ambient air pollution contributes to atherogenesis is a subject of debate. Although experimental studies on animals provide strong evidence for a causal atherogenic role of air pollution, particularly in obese mice (Sun et al. 2005), evidence from epidemiological studies is limited.

The long subclinical phase of atherosclerosis makes it possible to investigate the determinants of the vascular pathology long before its clinical manifestation. The association between air pollution and intima media thickness (IMT), an established marker of subclinical atherosclerosis, was reported for the first time in volunteers participating in two clinical trials in California (Künzli et al. 2005). Two population-based cross-sectional analyses, namely the Heinz Nixdorf Recall study in Germany (Bauer et al. 2010) and the Multi-Ethnic Study of Atherosclerosis (MESA) cohort in the United States (Diez Roux et al. 2008), also reported associations between air pollution and IMT, although a study of young adults in the Netherlands found no association (Lenters et al. 2010). So far, only one longitudinal study has been published, and it was based on heterogeneous samples of volunteers participating in five clinical trials (Künzli et al. 2010), including the two trials of the first cross-sectional study published on this topic (Künzli et al. 2005). Findings from the study suggested a possible role of ambient air pollutants, indicated by particulate matter (PM) ≥ 2.5 μm in diameter (PM2.5) and living close to busy highways, in the progression of IMT (Künzli et al. 2010).

Most previous studies have reported that associations between air pollution and IMT differ among population subgroups (Künzli et al. 2011). However, subgroup patterns have not been consistent; thus a clear understanding of susceptibility factors is still lacking. Human studies have not investigated whether diet modifies the effects of air pollution—a plausible hypothesis given evidence from animal studies (Sun et al. 2005) and the effects of diet on oxidative stress and systemic inflammation that are likely to contribute to the systemic effects of ambient air pollution (Brook et al. 2010).

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The Mediterranean region of Girona, and Spain in general, has one of the lowest cardiovascular mortality rates in Europe, despite very high prevalence of conventional cardiovascular risk factors (Masiá et al. 1998), a paradox that may be explained in part by protective effects of the Mediterranean diet (Martínez-González et al. 2011). We aimed to investigate the association between long-term exposure to traffic-related air pollution and subclinical atherosclerosis in Spain, and modification of this association by diet and other factors identified in previous studies (Künzl et al. 2011). We investigated this in the follow-up of participants of three population-based cohorts of the REGICOR (Registre Gironí del Cor: the Girona Heart Register) study (Grau et al. 2007). Subclinical atherosclerosis was measured by carotid IMT, a validated marker of atherosclerosis (Coll and Feinstein 2008). In addition, we measured the ankle–brachial index (ABI), a marker of the presence and severity of peripheral artery disease. Both IMT and ABI are associated with cardiovascular events and mortality (Ankle Brachial Index Collaboration 2008; Lorenz et al. 2007).

In the absence of PM measurements in this region, we used estimates of 10-year average home outdoor nitrogen dioxide (NO\textsubscript{2}) concentrations and residential traffic intensity as markers of exposure to local traffic-related air pollutants.

**Methods**

REGICOR-Air is a cross-sectional study nested in the REGICOR cohort study (Grau et al. 2007) evaluating the association between air pollution and cardiovascular diseases. We used data from the follow-up of three population-based cohorts originally enrolled in 1995, 2000, and 2005 (with response rates of 72.4, 70.0, and 73.8%, respectively) from 12 towns that represent the geographic diversity of the Girona Province and have large contrasts in ambient air pollution levels [see map in Supplemental Material, Figure S1 (http://dx.doi.org/10.1289/ehp.1205146)]. During 2007–2010, the participants residing in these towns, who were alive and not institutionalized, were invited to participate in REGICOR-Air, and the response rate was approximately 82%. In addition to extensive health status reassessment, IMT and ABI were measured for the first time in this population. Address histories for the past 10 years were collected by questionnaire, and each address was geocoded at the front-door level. This study was approved by the Hospital del Mar Research Institute ethics committee, and participants gave written informed consent.

IMT was measured by three trained, certified sonographers using ultrasound examinations of the left and right carotid arteries. Standardized scan and image analyses protocols were used. The scan protocol entailed the right and left carotid arteries. In each artery, the following predefined longitudinal segments were imaged: (a) the common carotid 1 cm proximal to the dilation, (b) the carotid bulb, and (c) the internal carotid 1 cm distal to the flow divider. For imaging, an Acuson Aspen ultrasound instrument (Acuson-Siemens, Erlangen, Germany) equipped with an L7 5-12MHz transducer and dedicated REGICOR application scan protocol (AMC IMAGELAB, University of Amsterdam, Amsterdam, and Technical University Eindhoven, Eindhoven, the Netherlands) were used. A still image of each arterial segment was saved as a DICOM (digital information and communication in medicine) file. These source files were locally stored and securely transferred to IMAGELAB, where trained and certified sonographers analyzed the images using validated “eTrack REGICOR” software (Department of Physiology and Vascular Medicine Academic Medical Centre, Amsterdam, the Netherlands) (de Groot et al. 2008). IMT was defined as the average distance between the lumen-intima and media-adventitia interfaces in a given 1 cm segment of the artery far wall. The main outcomes were (a) the mean of the IMTs for the left and right common carotid arteries (IMT\textsubscript{cca}), and (b) the mean of the IMTs for the left and right common carotid arteries, internal carotid arteries, and carotid bulbs (IMT\textsubscript{fseg}). These two outcomes were assessed separately because of differences in the segments’ cellular constituents—with more foam cells in the common carotid artery and more cholesterol-rich plaques in the carotid bulb and the internal carotid artery (Gállego Pérez-Larraya et al. 2012)—and in risk factors for IMT (Polak et al. 2010), which suggest that etiology may differ according to location. Between-sonographer and between-visit variability were evaluated based on repeated IMT measurements conducted at two visits, 2 weeks apart, by up to three sonographers, in 42 participants.

Systolic blood pressure was measured in a supine position after a 5-min rest in the brachial artery of both arms and the posterior tibial and dorsalis pedis arteries of both legs, using a continuous Doppler device. Right and left ABI were calculated as the ratio of the highest leg pressure to the highest brachial pressure in the corresponding arm, and the lowest of the two ABI ratios were categorized as low (<0.9), normal (0.9–1.3), or high (>1.3) for analysis (Ankle Brachial Index Collaboration 2008; McDermott et al. 2005).

Adherence to the Mediterranean diet was measured by a 10-point index based on sex-specific intake tertiles of seven beneficial (cereals, fruits, vegetables, legumes, seafood, nuts, moderate red wine) and two detrimental (meats, dairy products) food groups, and categorized in quartiles. Modifications such as excluding low-fat dairy products or white meats from the detrimental foods, or incorporating additional unhealthy food groups (e.g., soft drinks, salty snacks, pastries) as detrimental components, had no meaningful impact on findings in sensitivity analyses (data not shown). A detailed description of the methodology used to measure adherence to the Mediterranean diet in the REGICOR study is given by Schröder et al. (2004). The plausibility of reported dietary intakes was assessed based on disparities between reported energy intakes and estimated energy requirements (Mendez et al. 2011).

Participants with a clinical history of CVD (myocardial infarction, stroke, angina, catheterization, angioplasty, bypass surgery, or amputation due to circulatory problems) were excluded (n=227) because medication use or altered health behaviors among these participants may have influenced IMT/ABI measures obtained for this study.

**Exposure assessment.** We estimated the 10-year time-weighted average of the home outdoor concentrations of NO\textsubscript{2} for each participant using land use regression (LUR) models. In the absence of air quality data in most of these towns, we conducted an extensive monitoring campaign using NO\textsubscript{2} passive samplers to validate model estimates. We measured NO\textsubscript{2} in the balcony of 562 participants’ homes for 1 month in the spring and again in the fall between June 2007 and July 2009. Homes were selected to cover a broad range of traffic-related pollution and urban settings (e.g., low- and high-building–density areas), to be representative of the residential locations of the cohort participants and to be well distributed across the towns. We adjusted for temporal variability using monthly mean NO\textsubscript{2} concentrations collected at a fixed location in each town for at least 1 year. NO\textsubscript{2} annual means were derived by multiplying the monthly means at each location by the ratio of the annual mean to the same month mean NO\textsubscript{2} at the town’s fixed location.

To predict NO\textsubscript{2} at each participant’s residence we used LUR models based on NO\textsubscript{2} annual means and data on traffic intensity, bus routes and stops, distance to traffic, land cover, building density, and other geographic information system (GIS)-derived variables. Given the geographic diversity of the study area and differences in the availability of GIS data among towns, we divided the study area into seven subareas, i.e., groups of adjacent towns. LUR models were derived for each group by supervised forward linear regression according to the methodology described by Rivera et al. (2012). The models explained between 33% and 63% of measured NO\textsubscript{2} (cross-validation \(R^2\) 0.32 and 0.61, respectively) [see Supplemental Material, Table S1 and Figure S2 (http://dx.doi.org/10.1289/ehp.1205146)].
We estimated the outdoor annual mean NO₂ at each residential location by applying the LUR models to the address geocode. NO₂ concentrations were back-extrapolated to the time period of residence in each address using daily means at a fixed urban background monitoring location. Finally, for each participant we calculated the time-weighted average of NO₂ estimates across all residences in the 10 years preceding the IMT measurement (10-year NO₂ exposure). Time periods when participants lived at addresses that were geo-coded with low precision or were outside of the study area were excluded when deriving the 10-year NO₂ exposure. Participants who lived in the study towns for < 6 years or had high-precision geocodes (i.e., at the front-door level) for < 6 years (n = 365) were excluded from the main analyses.

We also used traffic proximity markers as surrogates of air pollution exposure in independent analyses. Traffic intensity, available from local registries and the Urban Mobility Plan for Girona (2007), was assigned to the central road network used within ESCAPE (European Study of Cohorts for Air Pollution Effects) (Hoek et al., 2012). Traffic counts were conducted at approximately 670 streets with missing traffic information to complete the traffic inventory. The traffic intensity assessment is described in detail elsewhere (Rivera et al., 2012). For each address, we calculated the traffic intensity at the nearest street and the traffic load (sum of traffic intensity multiplied by length of road segment in all segments) in a 100 m buffer and derived 10-year average values for each participant.

All GIS calculations were performed using ArcGIS 9.2 (ESRI, Redlands, CA, USA).

**Statistical analysis.** Crude and adjusted associations of IMT (which were natural log-transformed to reduce heteroscedasticity) with individually assigned air pollution exposures were estimated using linear regression models. Linear models were compared with additive models with smoothing splines using the generalized additive model (GAM) function in the MGCV R package (http://www.R-project.org) and linearity was confirmed. We analyzed ABI using multinomial logistic regression (Hosmer and Lemeshow, 2004). We initially adjusted our models by age and sex. Age was a main determinant of both outcome and exposure. Thus, in a second step, we analyzed the association of the potential confounders with both outcome and exposure variables, adjusting by age and sex. Variables associated with both, namely, smoking status, education level (as a proxy of socioeconomic status), and marital status were included in a minimal adjustment set. The association between exposure and IMT was strongly confounded by age. To determine whether age was an effect modifier, the interaction term age × exposure was introduced in the model, and it was not statistically significant (p > 0.05). We also evaluated whether the effect of age differed for men and women. This was the case, so we added the sex × age interaction to our minimal adjustment set (model 1). We further adjusted by other potential confounders [physical activity (weekly energy expenditure in leisure time based on the Minnesota questionnaires), diet [adherence to Mediterranean diet and plausibility of reported diet], SES at the census-tract level [percentage of residents with < secondary school education in the tract where participants resided the longest, based on the 2001 census], and occupational status], and results (not shown) did not differ from those of model 1. Finally, in addition to the variables listed above, we also included potential intermediates [body mass index (BMI), high-density lipoprotein level (HDL), waist circumference, systolic and diastolic blood pressure, and medication treatment (lipid-lowering, antihypertensives, antiplatelets, or anticoagulants, based on self-report and medical records)] to explore the effects of air pollution that do not follow these pathways (model 2). Adjustment variables were entered in the models either as continuous variables or dummy variables for all categories according to Table 1. [Also see Supplemental Material, Table S2 (http://dx.doi.org/10.1289/ ehp.1205146).]

We did not adjust the exposure–outcome associations for area of residence in the main analyses because the study area was relatively small (65 × 70 km), data collection in every town was done by the same team using the exact same procedures and because doing so would have partially removed the exposure contrast corresponding to between-town variability. However, we explored the sensitivity of the results to the inclusion of area of residence (corresponding to the address of longest residence) as a random effect variable. The trend of the associations remained the same, but the estimates were closer to the null and less precise (data not shown). This is consistent with a decrease in the exposure contrast, which makes the detection of an effect more difficult. Residual confounding by area is less likely because the estimates were adjusted by a large array of confounders, including area-level confounders such as education at the census-tract level.

Because living near busy roads is also associated with traffic-related noise, we adjusted for road traffic noise in a subsample of participants with available noise data (n = 1,084) (Foraster et al., 2011). However, results were not sensitive to adjustment for noise (data not shown).

### Table 1. Descriptive statistics [n (%), unless otherwise indicated] of the study population characteristics included in main analyses (n = 2,780).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMTcca [mm (median ± IQR)]</td>
<td>0.68 ± 0.19</td>
</tr>
<tr>
<td>IMT6seg [mm (median ± IQR)]</td>
<td>0.67 ± 0.18</td>
</tr>
<tr>
<td>ABI (lower of left and right)</td>
<td>1.21 (4.2)</td>
</tr>
<tr>
<td>ABI &lt; 0.9</td>
<td>56 (2.0)</td>
</tr>
<tr>
<td>ABI &gt; 1.3</td>
<td>116 (4.2)</td>
</tr>
<tr>
<td>Age [years (median ± IQR)]</td>
<td>58 ± 18</td>
</tr>
<tr>
<td>Sex [women]</td>
<td>1,491 (53.6)</td>
</tr>
<tr>
<td>Education level</td>
<td>1,476 (53.1)</td>
</tr>
<tr>
<td>Secondary school</td>
<td>758 (27.3)</td>
</tr>
<tr>
<td>Education level</td>
<td>526 (18.9)</td>
</tr>
<tr>
<td>Occupation status</td>
<td>1,447 (53.1)</td>
</tr>
<tr>
<td>Employed</td>
<td>358 (12.9)</td>
</tr>
<tr>
<td>Retired</td>
<td>852 (30.7)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>68 (2.5)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>1,202 (54.5)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>628 (28.5)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>377 (17.1)</td>
</tr>
<tr>
<td>Marital status</td>
<td>165 (16.9)</td>
</tr>
<tr>
<td>Single</td>
<td>2,178 (78.4)</td>
</tr>
<tr>
<td>Married/living together</td>
<td>171 (8.2)</td>
</tr>
<tr>
<td>Widow</td>
<td>247 (8.9)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (0.3)</td>
</tr>
<tr>
<td>BMI [kg/m² (median ± IQR)]</td>
<td>26.6 ± 5.5</td>
</tr>
<tr>
<td>Waist circumference [cm (median ± IQR)]</td>
<td>93 ± 17</td>
</tr>
<tr>
<td>HDL [mg/dL (median ± IQR)]</td>
<td>52.9 ± 15.6</td>
</tr>
<tr>
<td>Any cardiovascular or antihypertensive medication treatment</td>
<td>1,137 (40.9)</td>
</tr>
<tr>
<td>Mediterranean diet index (median ± IQR)</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Energy expenditure in leisure time [MET-min/week (median ± IQR)]</td>
<td>1,515 ± 1,937</td>
</tr>
<tr>
<td>People with low education in the census tract [% (median ± IQR)]</td>
<td>11 ± 12.2</td>
</tr>
<tr>
<td>Living at the same address for 10 years before IMT measurement</td>
<td>2,252 (81)</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; MET, metabolic equivalent.
Results are expressed as the cross-sectional percent difference in IMT, and the relative risk ratio (RRR) of high and low ABI, associated with a 10-year exposure contrast corresponding to the difference between the 5th and 95th percentiles in the study population. The RRR of high ABI is the prevalence of high ABI relative to normal ABI in exposed participants divided by the prevalence of high ABI relative to normal ABI in nonexposed participants.

We tested effect modification by diet, and factors identified as potential effect modifiers in previous studies, namely, age, sex, education level, smoking, diabetes, menopause, and medication treatment, using stratified analysis. We report the effect estimates of percent differences in IMT by strata of the potential effect modifiers.

To assess the sensitivity of the results to the NO\textsubscript{2} LUR models, we used the annual mean NO\textsubscript{2} at the closest monitor both within 100 m and within 200 m of the residence, instead of the 10-year modeled NO\textsubscript{2} in two separate sensitivity analyses. Median distance to the closest NO\textsubscript{2} monitoring site was 90 m. The sample size was restricted in these analyses because only those whose address of longest residence was within 100 m and within 200 m of a monitor were kept (e.g., 2,265 and 1,778 persons lived within 200 m of a monitor with data available on IMT\textsubscript{cca} and IMT\textsubscript{6seg}, respectively).

Analyses were performed using Stata version 10.1 (StataCorp, College Station, TX, USA) and R version 2.12 (http://www.R-project.org). The alpha level for statistical significance was set at 0.05.

### Results

Information on IMT\textsubscript{cca}, IMT\textsubscript{6seg}, and ABI was available for 2,780, 2,188, and 2,738 participants, respectively. Fewer participants had IMT\textsubscript{6seg} measurements because of difficulties in analyzing the images to obtain measurements from the internal carotid artery and carotid bulb (Gállego Pérez-Larraya et al. 2012). The characteristics of the study populations are summarized in Table 1. Participants were 32–86 years of age. Percentages of participants with low, medium, and high education levels are consistent with Spain as a whole (Instituto Nacional de Estadística 2012). Participants included in the analyses did not differ from those excluded (participants who lived in the study towns for < 6 years or with clinical history of CVD) in terms of exposure levels (data not shown). The median IMT\textsubscript{cca} was 0.68 mm (range, 0.40–2.05 mm). The repeatability study showed intra-class correlation coefficients for sonographers and visits of 0.83 for the IMT\textsubscript{cca} and of 0.77 for the IMT\textsubscript{6seg}. ABI was on average 1.10 (range, 0.5–1.75) with 2.0% of the study population classified as having low ABI (< 0.9), and 4.2% with high ABI (> 1.3) (Table 1). Participants with low ABI were on average 9 years older than participants with normal ABI, whereas those with high ABI did not differ in age from participants with normal ABI (data not shown). Inter- and intra-operator variability of ABI measurements were low, with intra-class correlation coefficients of 0.92 and 0.94, respectively.

The 10-year average home outdoor NO\textsubscript{2} concentrations varied from 5 to 48 µg/m\textsuperscript{3} [see Supplemental Material, Table S3 (http://dx.doi.org/10.1289/ehp.1205146)], and its correlation with NO\textsubscript{2} at the address of longest residence was > 0.99 over all participants (see Supplemental Material, Table S4) and 0.96 among those who moved at least once during the 10 years (data not shown). Ranges of 10-year time-weighted average values for traffic in the nearest street and traffic within 100 m indicated substantial variability among participants overall (see Supplemental Material, Table S3) and according to town of residence (see Supplemental Material, Table S5). Traffic intensity showed higher spatial variability than NO\textsubscript{2} [(75th percentile – 25th percentile)/50th percentile was 2.51 for 10-year traffic in the nearest street, 1.56 for 10-year traffic load in 100 m, and 0.73 for 10-year NO\textsubscript{2}]. NO\textsubscript{2} and traffic exposure variables were moderately correlated (Pearson correlation coefficients, r = 0.52–0.72), and the two traffic variables had a correlation of 0.58 (see Supplemental Material, Table S4).

In unadjusted models, NO\textsubscript{2}, traffic in the nearest street, and traffic in the 100 m buffer were strongly associated with the intima media thickness (both IMT\textsubscript{cca} and IMT\textsubscript{6seg}) (Table 2). Associations decreased after adjusting for age, and in general, further adjustment (model 1 and model 2) provided similar estimates. Associations between a 25 µg/m\textsuperscript{3} increase in NO\textsubscript{2} (corresponding

### Table 2. Estimated percent difference in IMT associated with a 10-year average exposure contrast between the 5th and 95th percentiles.

<table>
<thead>
<tr>
<th>Exposure (exposure contrast)</th>
<th>IMT\textsubscript{cca}</th>
<th>IMT\textsubscript{6seg}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textsuperscript{a}NO\textsubscript{2} (25 µg/m\textsuperscript{3})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>2.780</td>
<td>2.188</td>
</tr>
<tr>
<td>Adjusted for sex</td>
<td>2.780</td>
<td>2.188</td>
</tr>
<tr>
<td>Adjusted for age &amp; sex</td>
<td>2.780</td>
<td>2.188</td>
</tr>
<tr>
<td>Model 1\textsuperscript{b}</td>
<td>2.738</td>
<td>2.155</td>
</tr>
<tr>
<td>Model 2\textsuperscript{b}</td>
<td>2.632</td>
<td>2.074</td>
</tr>
<tr>
<td>Traffic load in a 100 m buffer\textsuperscript{c} (7,200,000 veh-m/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>2.780</td>
<td>2.188</td>
</tr>
<tr>
<td>Adjusted for sex</td>
<td>2.780</td>
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</tr>
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<td>2.188</td>
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<td>2.155</td>
</tr>
<tr>
<td>Model 2\textsuperscript{d}</td>
<td>2.632</td>
<td>2.074</td>
</tr>
<tr>
<td>Traffic intensity in nearest street (15,000 veh/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>2.780</td>
<td>2.188</td>
</tr>
<tr>
<td>Adjusted for sex</td>
<td>2.780</td>
<td>2.188</td>
</tr>
<tr>
<td>Adjusted for age &amp; sex</td>
<td>2.780</td>
<td>2.188</td>
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<tr>
<td>Model 1\textsuperscript{e}</td>
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</tr>
<tr>
<td>Model 2\textsuperscript{e}</td>
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</tbody>
</table>

\textsuperscript{a}Estimates adjusted by model 1: sex, age, sex–age interaction, smoking status, education, and marital status. \textsuperscript{b}Estimates adjusted by model 2: model 1 plus BMI, HDL, waist circumference, systolic and diastolic blood pressure, weekly energy expenditure in physical activity during leisure time (tortillas), adherence to Mediterranean diet, plausibility of reported diet, medication treatment, and percentage of persons with low education at the census-tract level. \textsuperscript{c}Models for traffic load were additionally adjusted for occupational status.

### Table 3. RRRs (95% CIs) for low or high ABI (< 0.9 or > 1.3 versus ABI = 0.9 to 1.3, respectively) associated with 10-year average exposure contrasts between the 5th and 95th percentiles.

<table>
<thead>
<tr>
<th>Exposure (exposure contrast)</th>
<th>ABI &lt; 0.9</th>
<th>ABI &gt; 1.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO\textsubscript{2} (25 µg/m\textsuperscript{3})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1\textsuperscript{f}</td>
<td>2.698</td>
<td>1.91</td>
</tr>
<tr>
<td>Model 2\textsuperscript{f}</td>
<td>2.600</td>
<td>1.98</td>
</tr>
<tr>
<td>Traffic load in a 100 m buffer\textsuperscript{g} (7,200,000 veh-m/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1\textsuperscript{h}</td>
<td>2.698</td>
<td>1.99</td>
</tr>
<tr>
<td>Model 2\textsuperscript{h}</td>
<td>2.600</td>
<td>1.98</td>
</tr>
<tr>
<td>Traffic intensity in nearest street (15,000 veh/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1\textsuperscript{i}</td>
<td>2.698</td>
<td>1.96</td>
</tr>
<tr>
<td>Model 2\textsuperscript{i}</td>
<td>2.600</td>
<td>1.97</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Estimates adjusted by model 1: sex, age, sex–age interaction, smoking status, education, and marital status. \textsuperscript{b}Estimates adjusted by model 2: sex, age, sex–age interaction, smoking status, education, marital status, BMI, HDL, waist circumference, systolic and diastolic blood pressure, weekly energy expenditure in physical activity during leisure time (tortillas), adherence to Mediterranean diet, plausibility of reported diet, medication treatment, and percentage of persons with low education at the census-tract level. \textsuperscript{c}Models for traffic load were additionally adjusted for occupational status.
to the difference between the 5th and 95th percentiles of exposure) and both IMTcca and IMTseg were positive, but small and non-significant after adjustment. Fully adjusted models—including potential intermediates (model 2)—indicated that a corresponding exposure contrast for traffic load within 100 m (7,200,000 vehicle-m/day) was associated with a 1.91% difference in IMTcca (95% CI: −0.24, 4.06) and a 2.06% difference in IMTseg (95% CI: −0.09, 4.21). An increase of 15,000 vehicles/day on the nearest street was associated with a 2.32% difference in IMTcca (95% CI: 0.48, 4.17) and a 1.80% difference in IMTseg (95% CI: 0.01, 3.59).

Ten-year NO2 and both residential traffic indicators were associated with higher prevalence of high ABI (Table 3). Adjusted RRRs for high versus normal ABI were 1.98 (95% CI: 1.09, 3.60) for a 25 µg/m³ increase in NO2; 1.89 (95% CI: 1.07, 3.34) for a 7,200,000 vehicle-m/day increase in traffic load within 100 m; and 1.70 (95% CI: 1.13, 2.57) for an increase of 15,000 vehicles/day on the nearest street. The RRR of low ABI compared to normal ABI were consistent with the null hypothesis for all exposures.

**Effect modification.** The association of all exposure markers with IMT differed across education level (Figure 1). In persons with a higher level of education, the association of 10-year exposure to air pollution with IMT was stronger (model 2 with NO2 and IMTcca). Increases of 25 µg/m³ in NO2, 7,200,000 vehicle-m/day in traffic load in 100 m, and 15,000 vehicles/day on the nearest street were associated with 4.6% (95% CI: 0.4, 8.9) 4.8% (95% CI: 0.7, 8.9), and 3.3% (95% CI: −0.02, 6.7) differences in IMTcca, respectively, among persons with a high education level; −1.5% (95% CI: −5.1, 2.1), 1.3% (95% CI: −2.4, 5.0), 1.3% (95% CI: −1.8, 4.4) among persons with a secondary school-level education; and 0.6% (95% CI: −2.8, 2.9), 0.5% (95% CI: −2.7, 3.8), 2.4% (95% CI: −0.6, 5.6) among persons with a primary school-level education. Similar patterns were observed for the associations of exposure to air pollutants with IMTseg. Effects estimates were also stronger in men ≥ 60 years of age compared to younger men, showing significant associations of NO2 and traffic load in 100 m with IMT (percent difference in IMTcca for NO2, traffic load, and traffic intensity were 4.3% (95% CI: 0.2, 8.4), 5.9% (95% CI: 1.6, 10.3), and 3.4% (95% CI: −0.07, 7.0), respectively, among men ≥ 60 years of age, and −1.5% (95% CI: −5.7, 2.7), −1.5% (95% CI: −5.7, 2.6), and 1.2% (95% CI: −2.7, 5.0), respectively, among men < 60 years of age. No evidence of effect modification by smoking, medication treatment (Figure 1), menopause, diabetes (results not shown), or Mediterranean diet [see Supplemental Material, Figure S3 (http://dx.doi.org/10.1289/ehp.1205146)] was observed.

**Discussion**

Our multivariate models revealed positive associations between three markers of long-term exposure to traffic-related air pollution,
namely traffic load within 100 m of the resi-
dence, traffic intensity in the nearest street, and
defined concentration of home outdoor
NO\textsubscript{2} and cardio- subclinical atherosclerosis in
a random healthy (no history or current signs
of CVD) population sample of the Spanish
Mediterranean region. However, associations
with NO\textsubscript{2} were weak and reached statistical
significance only in the subgroups of per-
sons with a high education level (IMT\textsubscript{cca}
and IMT\textsubscript{aseg}) and men ≥ 60 years of age
(IMT\textsubscript{cca}). Both indicators of residential traf-

cic mass as a marker of pollution. However, our effect
estimates are within the same order of magnitude
as seen in previous studies.

In contrast, the association between polli-


only the 5th–95th percentile exposure contrast


expressing our results in terms of fic intensities of 50,000–100,000 vehicles/day


frequent observations in European cities.


who have been associated with more subclinical disease among persons with
greater exposure” (Diez Roux et al. 2008). On
the other hand, a study in Germany reported
that living 50 m from a major road compared to
living > 200 m away was associated with an
odds ratio (OR) of 1.77 (95% CI: 1.01, 2.1) for
peripheral arterial disease (ABI of < 0.9
or history of treatment for peripheral artery
disease), whereas no associations were found
with annual residential PM\textsubscript{2.5} (Hoffmann
et al. 2009b).

The predictive power of an ABI of > 1.3 for


have been associated with calcification of the arterial
wall, higher levels of many CVD risk factors
(McDermott et al. 2005), and higher risk of
all-cause mortality and foot ulcers; it has been
weakly associated with heart failure and stroke
(Allison et al. 2008). Thus, there is evidence of
higher cardiovascular risk both at low and high
levels of ABI. We found that traffic-related
exposure was associated with high ABI. To
our knowledge, the association of air pollu-
tion with high ABI has not been investigated
previously. ABI is considered to be a marker
for medial sclerosis, which is a specific form
of arterial disease distinct from atherosclerosis
(Alzamora et al. 2012). Because ABI is a ratio
of systolic blood pressures, high ABI could
result from low brachial pressure, or high ankle
pressure, or both (Allison et al. 2008). Thus, if
air pollution affects the upper and lower vascu-
lar beds differently, for example, by affecting
brachial pressure at an earlier stage than ankle
pressure, it could be associated with both high
and low ABI. This hypothesis is consistent with
our finding that high (vs. normal) ABI was not
associated with IMT (adjusted RRR of 1.4;
95% CI: 0.3, 6.3 per 1 mm increase in
IMT\textsubscript{cca}), whereas low (vs. normal) ABI was
associated with thicker artery walls (adjusted
RRR of 7.7; 95% CI: 1.6, 36.8, \(p = 0.01\) per
1 mm increase in IMT\textsubscript{cca}).

In contrast to our findings, 20-year expo-
sures to PM\textsubscript{2.5} and PM\textsubscript{10}, in the MESA
study were weakly associated with carotid
IMT (1–3% differences in IMT for 12.5 and
21 \(\mu\text{g/m}^3\) increases in 20-year average PM\textsubscript{2.5}
and PM\textsubscript{10}, respectively) but not with ABI
(Diez Roux 2008). In Los Angeles, a 10 \(\mu\text{g/m}^3\) increase
in PM\textsubscript{2.5} at the current address was
associated with a 4.2% (95% CI: -0.2, 8.9
increase in IMT (Künzli et al. 2005). In a lon-
gitudinal study that included the population
of Künzli et al. (2005), a 10 \(\mu\text{g/m}^3\) increase
in PM\textsubscript{2.5} and living within 100 m of a highway
were associated with 2.5 \(\mu\text{m/year} (95\% \text{CI}:
-0.31, 5.38) and 5.5 \(\mu\text{m/year} (95\% \text{CI}:
0.13, 10.8) increases in IMT, respectively
(Künzli et al. 2010). In the Heinz Nixdorf
Recall study, a 4.2 \(\mu\text{g/m}^3\) increase in 1-year
average PM\textsubscript{2.5} and a 1.939 m increase in dis-
tance from a major road were associated with
a 4.3% (95% CI: 1.9, 6.7%) and a 1.2%
(95% CI: -0.2, 2.6%) difference in IMT, respec-
tively (Bauer et al. 2010). Distance to
traffic was used as a marker of traffic-related
exposure; thus a negative association with IMT
was expected. A 5.2 \(\mu\text{g/m}^3\) increase in 1-year
average PM\textsubscript{2.5} was significantly associated with
IMT in a study of > 2,000 civil servants in
London (Tonne et al. 2012). Positive, though
not significant, associations of IMT with mea-


In addition to the role of specific constituents of air pollu-
tion on atherogenesis. In the study of young adults
in Utrecht, associations of NO\textsubscript{2} black smoke,
PM\textsubscript{2.5}, and sulfur dioxide (SO\textsubscript{2}) with
IMT, pulse wave velocity, and augmentation index
were investigated, but significant associations
were found only for NO\textsubscript{2} with pulse wave
velocity and augmentation index and for SO\textsubscript{2}
with augmentation index (Lenters et al. 2010).
The evidence on the health effects of NO\textsubscript{2} var-
ies across studies. It has been reported to be
more strongly associated with cardiovascular
mortality than PM\textsubscript{2.5} black smoke, and SO\textsubscript{2}
(Beelen et al. 2008) and with cardiopulmonary
mortality than PM\textsubscript{10}, total suspended particles,
black smoke, and SO\textsubscript{2} (Gehring et al. 2006),
but Pope et al. (2002) found no association
between NO\textsubscript{2} and cardiopulmonary mortality.
The different associations observed between
NO\textsubscript{2} and cardiopulmonary and cardio-
vascular outcomes across studies might reflect
the suitability of using NO\textsubscript{2} as a marker of
traffic-related pollution. Although the general
suitability of NO\textsubscript{2} has been questioned before,
the possibility of its being site dependent has
not been studied. It has been reported that
the correlation of NO$_2$ with traffic intensity
varies across locations (Raaschou-Nielsen et al.
2000). The correlation of NO$_2$ with the com-
ponents of the traffic emissions cocktail that
promote (assuming the association was causal)
or are truly associated with cardiovascular out-
comes might also vary across locations.

Our exposure estimates (both NO$_2$ and traffic)
were based on data collected at partici-
pants’ residences, instead of data from air qual-
ity monitoring stations as far away as 10 km or
more (Diez Roux et al. 2008). In addition, our
traffic markers were based not only on proxim-
ity and road type classification (Allen et al. 2009; Hoffmann et al. 2009a), but also
on actual traffic intensity derived from a dense
traffic-count network. However, $R^2$s for our
NO$_2$ models indicate some exposure measure-
ment error (Basagaña et al. 2012). To evaluate
the potential influence of measurement error
(both bias and misclassification) introduced by
the exposure models, we compared asso-
ciations with our model-based estimates of 10-year average NO$_2$, with associations based
on annual mean NO$_2$ at the closest monitor.
Results (not shown) remained unchanged,
suggesting that measurement error introduced
by the model was negligible or comparable to
the error associated with measures taken at the
closest monitor.

Stronger positive associations in persons
with a high education level were consistent
across IMT measurements and across all mark-
ers of pollution. Larger estimated effects of
traffic-related exposure on IMT were observed
in men ≥ 60 years of age in all models. We
did not find evidence of effect modification
by Mediterranean diet, established risk fac-
tors for CVDs, or subclinical disease (indicated
by medication treatment). Other studies have
found heterogeneous associations across sub-
groups of age, sex, BMI, smoking status, socio-
economic status, town of residence, and other
cardiovascular risks factors (Bauer et al. 2010;
Künzli et al. 2005; Lenters et al. 2010). The
detection of interactions in epidemiologic stud-
ies is often underpowered, and testing many
interactions can lead to a multiple comparison
problem. However, our subgroup analysis was
designed a priori, and although our results may
be subject to the aforementioned problems, the
accumulated evidence over different studies
will help to identify susceptible subgroups.

In Girona, exposure to traffic-related pollut-
ants was higher for persons with a high educa-
tion level. Higher NO$_2$ concentrations were
also found at the most privileged census tract
locations [see Supplemental Material, Figure S4
(http://dx.doi.org/10.1289/ehp.1205146)].
This has been reported before for southern
European cities (Cesaroni et al. 2010), where
wealthy persons live in downtown areas that
are more polluted, in sharp contrast to what has
been observed in North America (Gunier et al.
2003) and northern Europe (Chai et al. 2006),
where the most deprived bear the highest air
pollution concentrations.

The persons with higher levels of education
were also younger, had lower blood pressure,
BMI, glucose levels, and low-density lipopro-
tein levels; a higher percent had quit smoking;
and a lower percent had never smoked compared
to persons with low and medium education levels. Stronger associations between
air pollution and IMT among those with high
versus low education levels were not explained
by age interactions. This may indicate higher
susceptibility but, more likely, a better detect-
ability among persons with fewer competing
risks for atherosclerosis (due to less confound-
ing). It is difficult to evaluate whether this is
supported by previous studies given that
results are very heterogeneous. No effect mod-
ification by education level has been observed
before for the association of PM$_{2.5}$ with IMT,
ABI, or coronary artery calcification (Diez
Roux et al. 2008), nor for the association of
NO$_2$ or PM$_{2.5}$ with IMT and arterial stiffness
(Lenters et al. 2010). Whereas increasing
effects of roadway proximity on aortic artery
calcification have been reported for increasing
income ($p_{trend} < 0.01$) (Allen et al. 2009).

Associations of PM$_{2.5}$ with systemic inflamma-
tion markers have been reported to
be stronger in men, and more specifically in
highly educated men, in Germany (Hoffmann
2009a). Slightly stronger associations with annual PM$_{10}$ were estimated among men com-
pared to women in the study of civil servants
in London (Tonne et al. 2012). Contrary to our
results for men ≥ 60 years of age, stronger associations between PM$_{2.5}$ and IMT were esti-
mated for women ≥ 60 years of age in the study
in Los Angeles (Künzli et al. 2005). The differ-
ence in augmentation index associated with
increased levels of NO$_2$ and PM$_{2.5}$ was also
higher for young women in Utrecht (Lenters
et al. 2010).

Associations with NO$_2$ or traffic load or
intensity at the current address (at the time of
examination) were weaker and less pre-
cise (data not shown) than associations with 10-year weighted averages across addresses,
indicating exposure misclassification. Similar
results were observed in the London study in
which exposure at current address gave less
precise estimates compared to exposure aver-
gaged during 1 year before examination (Tonne
et al. 2012). This highlights the relevance of
using long-term exposure when studying effects
on the IMT. On the other hand, our
results remained unchanged when using expo-
sure at the address of longest residence com-
pared with 10-year averaged exposure (data
not shown). Thus, for settings similar to the
Spanish Mediterranean region, in terms of
patterns of spatial distribution of NO$_2$ and
low residential mobility (i.e., 80% of persons
did not change address in the 10 years),
the exposure at the address of longest residence
may be a good proxy for long-term exposure.

Limitations of our study include the cross-
sectional design and the possibility of unmea-
sured confounding, including confounding
related to environmental tobacco smoke, and
the lack of information on daily activity patterns
to assess time spent at home. Strengths include
the large population-based sample size and the
availability of data on health and potential con-
founders, the 10-year address histories used
to estimate long-term NO$_2$ and traffic exposure
markers, and the dense NO$_2$ and traffic moni-
toring network data used to estimate exposures
at each participant’s residence.

Conclusions

We found evidence supporting an associa-
tion between long-term exposure to traffic
and subclinical carotid atherosclerosis and
high ABI levels in our study population, with
stronger associations in persons with a high
level of education and in men ≥ 60 years of
age. Longitudinal studies are needed to con-
firm whether air pollution contributes to the
chronic processes of atherosclerosis.

REFERENCES

ankle-brachial index is associated with increased cardio-
vascular disease morbidity and lower quality of life. J Am
Cardiol 51:1292–1298.
Amato F, Pandolfi M, Viana M, Lepore E, Sferracastella A, Moreno T. 2009. Spatial and chemical patterns of PM$_{2.5}$ in road dust
deposited in urban environment. Atmos Environ 43:1650–1659.
Ankle Brachial Index Collaboration. 2008. Ankle brachial index
combined with Framingham risk score to predict cardio-
vascular events and mortality. JAMA 300:197–208.
2012. Effect of the number of measurement sites on land use
regression models in estimating local air pollution. Atmos
Environ 54:634–642.
Bauer M, Moebus S, Mohlenkamp S, Drangano N, Nonnemacher M,
Fuchsberger M, et al. 2010. Urban particulate matter air
pollution is associated with subclinical atherosclerosis: results from
the HNuN (Heinz Nuschke Recall) study. J Am Coll Cardiol
56:1803–1808.
Beelen R, Hoek G, van den Brandt PA, Goldbohm RA, Fischer P,
pollution on mortality in a Dutch cohort (NLCS-AIR study).
Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A,
Diez-Roux AV, et al. 2010. Particulate matter air pollution and
cardiopulmonary disease: an update to the scientific
statement from the American Heart Association. Circulation
121:2341–2378.
Cesaroni G, Badaloni C, Romano V, Donato E, Perucci C,
Forastiere F. 2010. Socioeconomic position and health status of
people who live near busy roads: the Rome Longitudinal
Study (RLoLS). Environmental Health 9:41; doi:10.1186/1476-
069X-9-41 [Online 21 July 2010].
Chai B, Gustafsson S, Jøreskog KE, Kristersson HK, Lithman T,
Boah Å, et al. 2006. Children’s exposure to nitrogen dioxide
in Sweden: investigating environmental injustice in an egaliti-

Environmental Health Perspectives • VOLUME 121 • NUMBER 2 • February 2013 229
to particulate matter air pollution and systemic inflammatory markers. Environ Health Perspect. 117:1322–1328.


