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Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children

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ABSTRACT

Exposure to severe air pollution produces neuroinflammation and structural brain alterations in children. We tested whether patterns of brain growth, cognitive deficits and white matter hyperintensities (WMH) are associated with exposures to severe air pollution. Baseline and 1 year follow-up measurements of global and regional brain MRI volumes, cognitive abilities (Wechsler Intelligence Scale for Children-Revised, WISC-R), and serum inflammatory mediators were collected in 20 Mexico City (MC) children (10 with white matter hyperintensities, WMH^+, and 10 without, WMH^-) and 10 matched controls (CTL) from a low polluted city. There were significant differences in white matter volumes between CTL and MC children – both WMH^+ and WMH^- – in right parietal and bilateral temporal areas. Both WMH^- and WMH^+ MC children showed progressive deficits, compared to CTL children, on the WISC-R Vocabulary and Digit Span subtests. The cognitive deficits in highly exposed children match the localization of the volumetric differences detected over the 1 year follow-up, since the deficits observed are consistent with impairment of parietal and temporal lobe functions. Regardless of the presence of prefrontal WMH, Mexico City children performed more poorly across a variety of cognitive tests, compared to CTL children, thus WMH^+ is likely only partially identifying underlying white matter pathology. Together these findings reveal that exposure to air pollution may perturb the trajectory of cerebral development and result in cognitive deficits during childhood.

1. Introduction

Environmental pollutants, chemicals, metals and drugs have a negative impact upon the developing central nervous system in children (Danzer, 2008; Hwang, 2007; Stein, Schettler, Wallinga, & Valenti, 2002; Wigle, Arbuckle, Walke, Liu, & Krewski, 2007). There is mounting evidence that exposure to air pollution can cause stroke-related sickness and death (Hong, Lee, Kim, & Kwon, 2002; Maheswaran, Pearson, Campbell, et al., 2006), as well as brain damage, neuroinflammation, and neurodegeneration (Block & Calderón-Garcidueñas, 2009; Calderón-Garcidueñas, Azzarelli, Acuña, et al., 2002; Calderón-Garcidueñas, D'Angiulli, Kulesza, et al., 2011; Calderón-Garcidueñas, Franco-Lira, Henriquez-Roldan, et al., 2009; Calderón-Garcidueñas, Kavanaugh, Block, et al., 2011; Calderón-Garcidueñas, Reed, Maronpot, et al., 2004; Calderón-Garcidueñas, Solt, Franco-Lira, et al., 2008; Dorado-Martinez, Paredes-Carbajal, Mascher, et al., 2001; Peters, Veronesi, Calderón-Garcidueñas, et al., 2006; Villarreal-Calderon, Torres-Jardon, Osnaya, et al., 2010). Children are a population at risk since childhood and adolescence are crucial periods of brain development associated with dynamic behavioral, cognitive and emotional changes.

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There is an important knowledge gap regarding the impact that chronic exposure to concentrations of air pollutants above the current standards have on brain structural and volumetric changes in healthy children. Studies from our laboratory have shown that long-term exposure to severe air pollution causes neuroinflammation, and Alzheimer's like pathology in adult megacity residents (average age 54.7 ± 4.8 years) (Calderón-Garcidueñas et al., 2004). Mexico City (MC) highly exposed children and young adults (average age 25.1 ± 1.5 years) exhibited significant up-regulation of cyclooxygenase 2 (COX2), interleukin 1β (IL1β) and CD14 in olfactory bulb, frontal cortex, substantia nigrae and vagus nerves, disruption of the blood–brain-barrier, endothelial activation, and inflammatory cell trafficking (Calderón-Garcidueñas, Solt, et al., 2008). Children living in MC exhibit evidence of chronic inflammation of the upper and lower respiratory tracts, increased serum inflammatory mediators, and breakdown of the nasal respiratory epithelial barrier (Calderón-Garcidueñas, Franco-Lira, Torres-Jardón, et al., 2007; Calderón-Garcidueñas, Mora-Tiscareño, Fordham, et al., 2003; Calderón-Garcidueñas, Valencia-Salazar, Rodríguez-Alcaraz, et al., 2001; Calderón-Garcidueñas, Vincent, Mora-Tiscareño, et al., 2007; Calderón-Garcidueñas et al., 2007c; Calderón-Garcidueñas, Macias-Parra, Hoffman, et al., 2009; Calderón-Garcidueñas, Villarreal-Calderon, Valencia-Salazar, et al., 2008). These children also have elevated concentrations of plasma endothelin-1 (Calderón-Garcidueñas, Vincent, et al., 2007; Calderón-Garcidueñas, Villarreal-Calderon, et al., 2008), a potent vasoconstrictor peptide involved in the homeostatic regulation of the cerebral microcirculation and up-regulated after exposure to air pollutants including particulate matter (Chauhan, Breznan, Thomson, Karthikeyan, & Vincent, 2005; McCarron, Chen, Tomori, et al., 2006; Thomson, Kumarathasan, Calderón-Garcidueñas, & Vincent, 2007). Fifty-six percent of clinically healthy Mexico City children exhibited MRI prefrontal white matter hyperintensities compared to 7.6% in age matched children residents in a low polluted area (Calderón-Garcidueñas, Mora-Tiscareño, Ontiveros, et al., 2008). Importantly, the Mexico City children also showed lower performance on IQ subtests and subscales (i.e., Verbal IQ and Full Scale IQ) as compared to their control counterparts (Calderón-Garcidueñas, Mora-Tiscareño, et al., 2008).

The primary purpose of this work was to evaluate volumetric brain alterations as detected by MRI in 7–8 year old healthy children with a life time residency in two significantly different urban environments, one with high concentrations of air pollutants (Mexico City), and the other (Polotitlán, Mexico State) with levels well below the current USA National Ambient Air Quality Standards (NAAQS). In the present study, we considered two distinct cohorts of children residents in Mexico City, one characterized by persisting MRI prefrontal white matter hyperintensities (WMH^+) and the other by absence of these lesions (WMH^-). We compared the latter two groups with a control group of children matched for age/socioeconomic status/maternal education who were residing in a clean air environment and showing no structural brain alterations. We aimed at identifying possible associations between the emergence of cognitive deficits (as reflected by IQ subtests and subscales), volumetric white matter regional changes, systemic inflammatory markers and residency in the distinctly different polluted environments. Because white matter facilitates rapid transmission of information from posterior to frontal brain regions, allowing for intact performance on complex cognitive tasks (Jung & Haier, 2007), we expected to find, in Mexico City children, evidence of disruption in some of the brain regions implicated in the temporo-parieto-frontal integration supporting performance on structured intelligence tests. Accordingly, we tested whether or not the deficits associated with severe exposure to pollution in Mexico City would be uniquely associated only with the presence of white matter hyperintensities. In addition, because the effects of pollutants are cumulative we tested whether in these young children there would be significant rapid changes after just 1 year of exposure.

2. Procedure

2.1. Study areas

This prospective protocol was approved by the review boards and ethics committees at involved institutions, written consent was obtained from parents and verbal consent from children. The geographic areas selected for this study were: Southwest Mexico City (SWMC) and Polotitlán in the Mexico state. Mexico City (MC) is an example of extreme urban growth and accompanying environmental pollution (Bravo-Alvarez & Torres-Jardón, 2002; Molina, Madronich, Gaffney, et al., 2010; Molina et al., 2007). The metropolitan area of over 2000 square kilometres lies in an elevated basin 2240 m above sea level surrounded on three sides by mountain ridges with a broad opening to the north and a narrower gap to the south–southwest. The MC's nearly 20 million inhabitants, over 40,000 industries and 4 million vehicles consume more than 40 million litres of petroleum fuels per day and produce an estimated annual emission of 2.6 tons of pollutants including fine particulate matter, oxidant gaseous pollutants, polycyclic aromatic hydrocarbons, and lipopolysaccharides (Bravo-Alvarez & Torres-Jardón, 2002; Dzepina, Arey, Marr, et al., 2007; Molina et al., 2010; SMA, 2010). Lipopolysaccharides detected in the coarse fraction of PM_{10} samples from south Mexico City show the highest endotoxin concentrations at 59 EU/mg PM_{10} (Bonner, Rice, Lindroos, et al., 1998; Calderón-Segura, Gómez-Arroyo, Villalobos-Pietrini, et al., 2004; Osornio-Vargas, Bonner, Alfaro-Moreno, et al., 2003; Rosas-Pérez, Serrano, Alfaro-Moreno, et al., 2007). Significant sources of environmental endotoxins in Mexico City include open field waste areas, wastewater treatment plants, and daily outdoor deposits of more than 500 metric tons of animal and human fecal material (Estrada-Garcia, Cerna, Thompson, et al., 2002). Ozone concentrations peak towards the downwind southwest area in the afternoon as a result of the typical diurnal wind transport of air polluted masses coming from the urban area. The higher 8-h O_3 and $PM_{2.5}$ concentrations coincide with the times children are outdoors during the school recess and physical education periods and when they play outdoors at home (Villarreal-Calderon, Acuña, Villarreal-Calderón, et al., 2002).

The selection of children from Southwest Mexico City (SWMC) was based on three factors: (i) the SW location of the base Mexico City pediatric hospital, (ii) children living and attending school close to the hospital, and (iii) the location of the two closest atmospheric monitoring stations: Pedregal and Coyoacán. In contrast, in the control (CTL) city Polotitlán all criteria pollutants (O_3, PM_{10}, SO_2, NO_2, CO and Pb) levels are below the current US standards, due to the fortunate combination of relatively few contributing emission sources from industry and cars and good ventilation conditions due to regional winds. Polotitlán is located 114 km NW of MC at 2300 m above sea level, and its selection as a control city was based on five key factors: (i) concentrations for all major air pollutants below the current USA standards, (ii) access to a children's healthy population, (iii) previous clinical studies with the Polotitlán cohort that indicated that children had no evidence of air pollution-related health issues, (iv) an altitude above sea level similar to that of MC, and (v) relative proximity to MC to facilitate the follow-up of the control cohorts (Calderón-Garcidueñas et al., 2003; Calderón-Garcidueñas, Vincent, et al., 2007; Calderón-Garcidueñas, Franco-Lira, et al., 2007; Calderón-Garcidueñas, Villarreal-Calderon, et al., 2008; Calderón-Garcidueñas, Macias-Parra, et al., 2009). More importantly, because PM contributions in Polotitlán are mainly from resuspension dust, most of the fine particulates have a crustal

origin with just a small portion of carbonaceous species and secondary aerosols compared to those in MC (Gobierno del Estado de México, 2008; Querol, Pey, Minguillón, Pérez, et al., 2008).

2.2. Participants

This work includes data from 20 children from Mexico City (*Mean age* = 7.1 years, *SD* = 0.69) and 10 children from Polotitlán (*Mean age* = 6.8 years, *SD* = 0.66). Children's clinical inclusion criteria were: negative smoking history and environmental tobacco exposure, lifelong residency in MC or in the control city, residency within 5 miles of the city monitoring stations, full term birth, unremarkable clinical histories, including no hearing or visual impairments. Cohorts were matched by age and socioeconomic status. Participants were from middle class families living in single-family homes with no indoor pets, used natural gas for cooking and kitchens were separated from the living and sleeping areas. Outdoor daily exposures were recorded, including the transit time to and from school, the time spent in recess and physical education during school, the outdoor time while playing and engaging in other activities. Since children's activities change during the weekend, the outdoor times were averaged for 7 days.

2.3. Pediatric and Otoneurological examination

Children were followed for 2 years, they had initial complete clinical histories and physical examinations, followed by two annual check-up visits to the pediatrician. The initial pediatric examination was followed by a complete neurological examination, otoscopy, and audiometry done by the Pediatric Otoneurologist. Audiometry was carried out using Interacoustics Diagnostic Audiometer AD229 with a peltor H7A headphone in a sound isolated room. Fasting blood samples were collected between March 2 and 31, 2008. All included children were clinically healthy and actively engaged in outdoor activities.

2.4. Peripheral blood analysis

Blood samples were taken for a complete blood count (CBC) with differential and custom made human multi-analyte Elisa cytokine arrays. We followed the manufacturer's instructions for Custom Human Multi-Analyte ELISArray Kits, SABiosciences, Frederick, MD 21703, USA. The following analytes were included in our panel: Tumor necrosis alpha (TNF α), Granulocyte Monocyte chemoattractant protein-1 (MCP-1) and C reactive protein (CRP).

2.5. Wechsler Intelligence Scale for Children-Revised (WISC-R)

We used the Spanish version of the Wechsler Intelligence Scale for Children-Revised WISC-R (Wechsler, 1974) on baseline (2007) and year 1 (2008) of follow-up.

2.6. Brain magnetic resonance imaging (MRI)

All 30 children had a brain MRI in the Summer of the baseline year (2007) and year 1 (2008). The 3D MRI for all subjects was acquired on a 1.5 Tesla 5T Signa Excite HD MR (General Electric, Milwaukee WI, USA) with an eight Channel Brain Array. White matter hyperintensities (WMH) were defined as hyperintense focal images observed in two different sequences: T1 and T2 weighted with fluid-attenuated inversion recovery (FLAIR). A pediatric radiologist and two neuroradiologists reviewed the studies independently, having access only to age and gender information in each case. The goal of the selected acquisition protocol was to allow (a) for brain structure assessment via global and regional brain volumes, and (b) for white matter hyperintensity visual assessment. High-resolution T1 weighted anatomical images (3D SPGR, voxel dimensions $0.9375 \times 0.9375 \times 1.5$ mm, 256×256 voxels, 124 slices, axial, 15 min) were acquired, as well as T2 weighted images using a 2D multi-slice dual fast spin echo sequence (FSE, voxel dimensions $0.9375 \times 0.9375 \times 2$ mm, axial, 10 min) and fluid attenuated inversion recovery images (Calderón-Garcidueñas, Mora-Tiscareño, et al., 2008). All sequences covered the entire surface of the brain. The total scanning time was approximately 45 min. For image processing, every individual structural MRI dataset, probabilistic tissue segmentations of white matter, gray matter and cerebrospinal fluid were computed in automatic fashion by our atlas-based tissue classification (Prastawa, Gilmore, Lin, & Gerig, 2005; Styner, Charles, Park, Lieberman, & Gerig, 2002; Styner, Gerig, Brechbuhler, & Szekely, 2000). Skull stripping, intensity inhomogeneity correction (Styner et al., 2000) and intensity calibration were additionally performed in this step. In the next step, a prior brain atlas, derived as an unbiased, symmetric average image in as separate study, is deformed to match each MRI image via non-linear high-dimensional fluid deformation (Joshi, Davis, Jomier, & Gerig, 2004). The atlas's structural probabilistic ROI definition of the lobar parcellations are propagated to each subject's image using the computed deformation field (Gouttard, Styner, Prastawa, et al., 2008). Propagated ROIs and parcellations were reviewed for accuracy and edited only if they deviated significantly from critical, well defined boundaries. The parcellation for each subject was then combined with that subject's tissue classification to obtain white matter, gray matter and cerebral–spinal fluid volumes for each lobe and region. The corresponding volumetric measurements for ROIs and parcellations were automatically computed. Both whole ROI and parcellation computation methodology has been validated and evaluated for stability. Repeatability studies show coefficients of variance less than 1.5% for all measurements.

2.7. Data analysis

Both years of the volume data, the subcortical data, and the WISC-R data were combined into one (for each type) data set for analysis. The WISC-R data was analyzed using generalized estimating equations (GEE) with an independent working-correlation matrix, in order to adjust for the within-individual correlation in responses. The Poisson distribution was assumed because of the count nature of the data. Covariates for each model in this set of analyses included year and group, including an interaction to allow for different estimated means for each group in each of the 2 years. The volume data was analyzed similarly to the WISC-R data using GEE with independent working-correlation and the Poisson distribution. The same covariates were used as in the WISC analyses, and total cranial volume (TVC) was included. The subcortical data was analyzed using the same methods as the volume data, assuming a Gaussian distribution. The three values in the blood data that were analyzed (2 cytokines and CRP) were compared amongst the groups using a Gaussian generalized linear model, and in addition to group, white blood cell count (WBC) was included as a covariate. For each set of analyses, Wald tests of linear contrasts were used to first test for the overall equality of means between the three groups (H_0: Mexico City with white matter hyperintensities (WMH^+) = Controls (CTL) = Mexico City without white matter hyperintensities (WMH^-) versus the alternative that at least one group was different in at least one of the 2 years. In addition to unadjusted *p*-values for each comparison, *q*-values were estimated for each set of data (each set of analyses was treated as one family of tests for the multiple testing adjustment). The *q*-value gives the minimum false discovery rate (FDR) at which the null hypothesis

would be rejected. In addition, adjusted *p*-values were obtained using the Simes procedure (Simes, 1986), this procedure expresses the *q*-values in terms of corresponding *p*-values. For tests with *q*-values of less than 0.1, further testing was done to compare groups pairwise at each year and *q*-values were computed for each of these further comparisons (within each family/group of tests).Statistical analyses were performed using R version 2.10 (http://www.r-project.org/). The clinical and laboratory data were also analyzed using ANOVA with an *F*-test for differences in means and Tukey HSD Pairwise Comparisons with adjusted *p*-values. Univariate descriptive measurements were summarized as mean values ±SD. Significance was assumed at $p < 0.05$.However, the assumed significance level actually corresponded to an adjusted $p < .03$ in terms of Simes adjustment for multiple comparisons. The latter was the significance threshold consistently applied for all GEE post-hoc multiple comparisons reported.

3. Results

3.1. Air pollution levels

Mexico City children in this study have been exposed to significant concentrations of O_3, and particulate matter (PM) for their entire life (Bravo-Alvarez & Torres-Jardón, 2002; Calderón-Garcidueñas, Franco-Lira, et al., 2007; Calderón-Garcidueñas, Solt, et al., 2008; Calderón-Garcidueñas, Villarreal-Calderon, et al., 2008; Calderón-Garcidueñas, Mora-Tiscareño, et al., 2008; Dzepina et al., 2007; Molina et al., 2010). The climatic conditions in MC are relatively stable thus pollutant concentrations are consistent year after year. During 2007–2009 that includes the study period, $PM_{2.5}$ annual concentrations in SWMC were 35.89 ± 0.93 μm/m^3 (Annual Standard below 15 μg/m^3). In the control city (Polotitlán) O_3, PM_{10}, SO_2, NO_2, and CO levels are below the current US standards (Calderón-Garcidueñas, Franco-Lira, et al., 2007; Calderón-Garcidueñas, Solt, et al., 2008; Calderón-Garcidueñas, Villarreal-Calderon, et al., 2008; Gobierno del Estado de México, 2008).

3.2. Demographic data and physical exams

Table 1 summarizes the characteristics of the matched samples. There were 10 children from Polotitlán (6 females) and 20 children from Mexico City (11 females). Vital signs and the physical examination results were unremarkable in all participant children. No overweight, obese or undernourished children were included.

3.3. Laboratory findings

Table 2 summarizes the comparison in peripheral blood endpoints for the year 2 (2008) between CTL and MC WMH$^+$ and WMH$^-$

Table 1
Characteristics of the groups of children studied.

	Groups				Tukey *a*		
	MC WMH$^+$	MC WMH$^-$	CTL	*F*(2,27)	MC WMH$^-$ vs. CTL	MC WMH$^+$ vs. CTL	MC WMH$^+$ vs. WMH$^-$
Weight (kg)	25.7 ± 4.3	26.6 ± 4.0	22.5 ± 1.9	3.59*	3.61*	2.81	−0.79
Height (m)	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.31	1.20			
BMI	17.1 ± 1.6	17.7 ± 2.4	15.8 ± 1.6	2.54			
Outdoor hours	3.9 ± 0.6	3.6 ± 0.2	4.5 ± 0.4	8.98**	−5.94**	−3.69*	2.25
Gender	4m/6f	4f/6m	6f/4m				

Note: MC WMH$^-$ = Mexico City children with no white matter hyperintensities. MC WMH$^+$ = Mexico City children with white matter hyperintensities. CTL = controls. The numerical values shown in the table represent means (±1 standard deviation) as well as ANOVA *F*-test values for difference among means. For all three groups $n = 10$. Pairwise comparisons for significant ANOVA results were conducted using Tukey HSD procedure to correct for multiple comparisons and adjust *p*-values.
* $p < 0.05$.
** $p < 0.001$.

Table 2
Peripheral blood endpoints and comparisons in Control and Mexico City children with and without white matter hyperintensities.

	Groups				Tukey *a*		
	MCWMH$^+$	MC WMH$^-$	CTL	*F*(2,27)	MC WMH$^-$ vs. CTL	MC WMH$^+$ vs. CTL	MC WMH$^+$ vs. WMH$^-$
Hemoglobin (g/dL)	15.0 ± 0.8	14.6 ± 0.6	13.9 ± 0.5	7.10**	3.25	5.28**	2.04
Hematocrit (%)	44.7 ± 2.1	43.1 ± 1.7	40.5 ± 1.7	12.76**	4.49*	7.06**	2.57
White blood cells ($\times 10^3$/μL)	7340.0 ± 1793.3	6350.0 ± 835.7	8370.0 ± 1866.7	4.14*	−4.07*	−2.07	1.99
Neutrophils (%)	47.5 ± 12.8	43.9 ± 8.3	53.5 ± 7.4	2.46			
Lymphocytes (%)	42.8 ± 11.1	44.9 ± 8.5	38.1 ± 7.8	1.42			
Monocytes (%)	6.5 ± 1.2	6.9 ± 1.6	5.6 ± 1.0	2.41			
Eosinophils (%)	2.7 ± 2.9	3.4 ± 2.8	2.3 ± 1.7	0.50			
Neutrophils total	3630.0 ± 1767.6	2790.0 ± 653.9	4540.0 ± 1274.7	4.44*	−4.21*	−2.19	2.02
Lymphocytes total	3020.0 ± 659.6	2870.0 ± 668.4	3120.0 ± 719.26	0.34			
Monocytes total	480.0 ± 103.3	420 ± 91.9	480.0 ± 175.1	0.72			
Platelets	318100.0 ± 70296.9	327300.0 ± 58359.1	381900.0 ± 59078.9	3.01			
C-reactive protein (ng/ml)	1052.2 ± 1806.1	1047.3 ± 1015.8	340 ± 0.9	0.87			
Granulocyte monocyte chemoattractant protein-1 pg/ml	105.0 ± 41.5	127.6 ± 51	21.3 ± 4.8	4.76**	4.34**	2.55	−1.79
Tumor necrosis factor α (pg/ml)	8.7 ± 1.7	10.5 ± 1.8	8.6 ± 2.0	3.39*	3.24†	0.10	−3.14†

Note: MC WMH$^-$ = Mexico City children with no white matter hyperintensities. MC WMH$^+$ = Mexico City children with white matter hyperintensities. CTL = controls. The numerical values shown in the table represent means (±1 standard deviation) as well as ANOVA *F*-test values for difference among means. For all three groups $n = 10$. Pairwise comparisons for significant ANOVA results were conducted using Tukey HSD procedure to correct for multiple comparisons and adjust *p*-values.
† $p < 0.10$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

children. MC WMH$^+$ children had higher values of hemoglobin and hematocrit relative to CTL, while WMH$^-$MC children had significantly lower total WBC, and total neutrophils compared to CTL. MCP-1 concentrations were significantly higher in MC WMH$^-$ children as compared to CTL, while marginally significant (corresponding to significant q-values (<0.035) were recorded for TNF α between CTL and MC WMH$^-$, as well as between MC WMH$^-$ and MC WMH$^+$. There were no statistically significant differences in total WBC, total neutrophils, CRP and MCP1 between Mexico City WMH$^+$ and WMH$^-$ children. The comparisons among the three groups of blood endpoint measures most critical to inflammation are additionally shown side by side to the other measures in the right panels of Fig. 1A–C.

3.4. MRI results

Hyperintense areas were localized predominantly in prefrontal white matter. Tests for equality of means were conducted on the adjusted GEE-estimated MRI means for all areas examined, by group and at each year, also adjusting for the mean total cranial volume (TCV) (The results on all means are reported in Supplementary Table 1). These overall tests between the three groups showed selective significant differences for white matter volume bilaterally in temporal and on the right side parietal areas. Therefore, these areas were submitted to further post-hoc multiple comparisons between the groups separately for 2007 and 2008. Post-hoc GEE analysis revealed significant differences only in the white matter

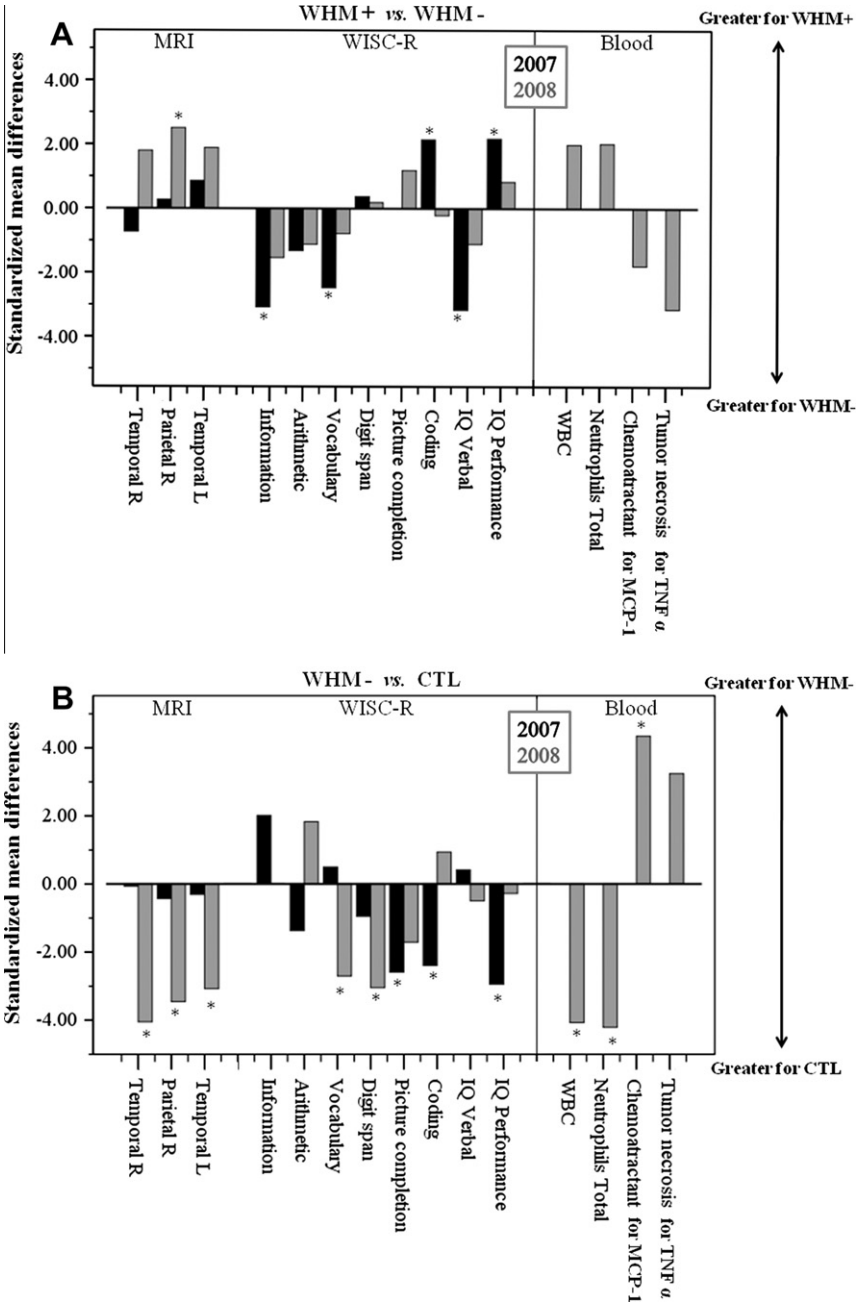

Fig. 1. Mean estimated difference scores and multiple comparisons in MRI volume, WISC-R measures and blood endpoints. The reported brain areas, WISC-R measures and serum inflammation markers were selected after significant overall tests (see text). The solid reference line separating the blood endpoints measurements indicates that the standardization of the mean differences was obtained using a different procedure than the procedure used for the other types of variables. Panel A: Comparisons Mexico Children with and without white matter hyperintensities. Panel B: Comparisons between Mexico Children without white matter hyperintensities and control children. Panel C: Comparisons between Mexico Children with white matter hyperintensities and control children.

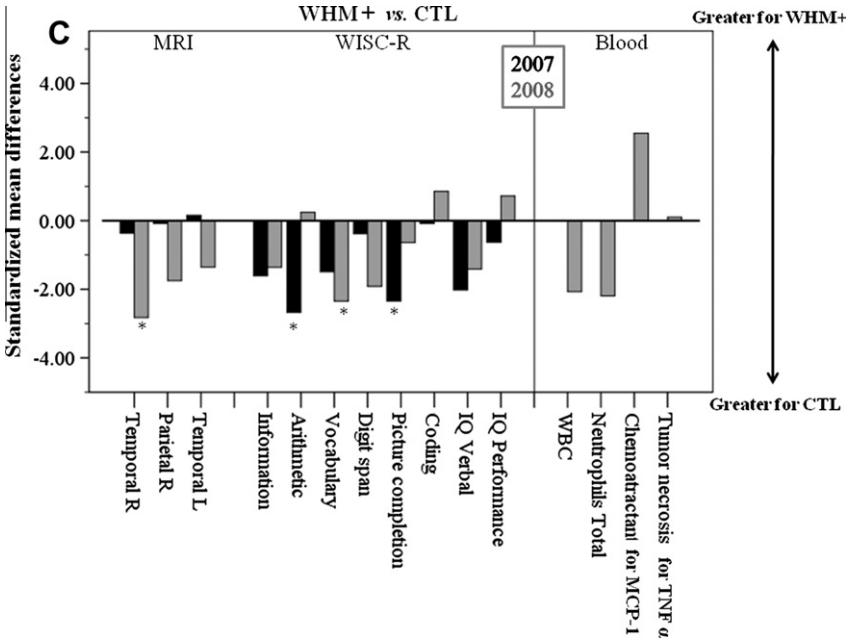

Fig. 1 (*continued*)

volumes measured in 2008 (shown in Table 3), specifically, for right temporal area between WMH^- and CTL, and between WMH^+ and CTL; this analysis also showed significant differences for white matter left temporal volume between WMH^- and CTL, and differences for white matter right parietal volume between CTL and WMH^-, as well as WMH^+ and WMH^-.

The left panels of Fig. 1A–C illustrate graphically the differences in the white matter for right temporal, right parietal and left temporal area. In these figures, the bars represent the estimated differences as relative advantage of either of the two groups contrasted. In keeping with the GEE data, CTL had significantly more white matter volume than WMH^- in the right temporal and parietal areas and in the left temporal areas (Fig. 1B, left panel).While CTL had significantly more white matter volume than WMH^+ in the right temporal area (Fig. 1C, left panel). When comparing the MC groups, WMH^+ children had more white matter volume than WHM^- in the right parietal white matter (Fig. 1A, left panel). Sub-cortical bilateral volumes including hippocampus, caudate, putamen, globus pallidus and amygdala were not significantly different across the three groups.

Table 3
Results of post-hoc analysis comparing MRI brain White Matter volumes in controls (CTL) and Mexico City children with white matter hyperintensities (WMH^+) and without white matter hyperintensities (WMH^-).

Group comparisons	Estimated difference	*SE*	$\chi(1)^2$
Temporal lobe, right side			
WMH^+ vs. WMH^-	0.02	0.01	3.25†
WMH^- vs. CTL	−0.07	0.02	16.33***
WMH^+ vs. CTL	−0.05	0.02	7.98**
Parietal lobe, right side			
WMH^+ vs. WMH^-	0.04	0.02	6.29*
WMH^- vs. CTL	−0.09	0.03	11.89**
WMH^+ vs. CTL	−0.04	0.02	3.05†
Temporal lobe, left side			
WMH^+ vs. WMH^-	0.04	0.02	3.57†
WMH^- vs. CTL	−0.07	0.02	9.44**
WMH^+ vs. CTL	−0.03	0.02	1.84

† $0.05 < p < 0.10$.
* $p < 0.03$.
** $p < 0.005$.
*** $p < 0.000025$.

3.5. WISC-R results

Table 4 shows the WISC-R means for each group at each year, whereas the differences between the means compared among the three groups are shown graphically in the central panels of Fig. 1A–C next to the MRI data and the blood endpoints.

The tests for the overall equality of means between the three groups showed selective significant differences in the following subscales: Information, Arithmetic, Vocabulary, Digit Span, Picture Completion, and Coding. In addition, overall differences were found for Verbal and Performance IQ subtests. Consequently, all

Table 4
WISC-R subscales and IQ verbal, performance and global normalized values by group and at each year.

WISC-R	Group					
	MC WMH^-		MC WMH^+		Controls	
	2007	2008	2007	2008	2007	2008
Subscales						
Information	*10.9*	*9.7*	*7.1*	*8.2*	*9.1*	*9.7*
Similarities	10.7	10.3	9.7	9.5	10.2	10.1
Arithmetic	*10.4*	*11.9*	*9.2*	*10.6*	*11.8*	*10.3*
Vocabulary	*10.5*	*9.1*	*8.6*	*8.5*	*10*	*10.8*
Comprehension	10.7	9.2	9.9	10.2	10.7	10.7
Digit Span	*7.8*	*7.1*	*8.2*	*7.3*	*8.6*	*9.4*
Picture Completion	*9.5*	*9.7*	*9.5*	*10.8*	*11.4*	*11.4*
Picture Arrangement	8.3	9.5	9	10.9	9.5	10.1
Block design	8.3	9.5	10.4	10.3	9.8	10.9
ObjectAss	9.5	10.2	10.2	10.3	10.1	9.1
Coding	*9.2*	*10.9*	*11.7*	*10.6*	*11.8*	*9.6*
Mazes	8.9	9.3	11	9.6	9	9.8
IQ						
Verbal	*103.6*	*99.9*	*92.8*	*95.9*	*101.8*	*101.7*
Performance	*92.3*	*99.4*	*100.5*	*103.5*	*103.1*	*100.8*
Full scale	98	99.4	96	99.1	102.5	101.2

Note: Italicized values indicate significance for tests of the overall equality of means between the three groups ($\chi(2)^2 > 9.21$; $p < 0.01$; $q < 0.10$).

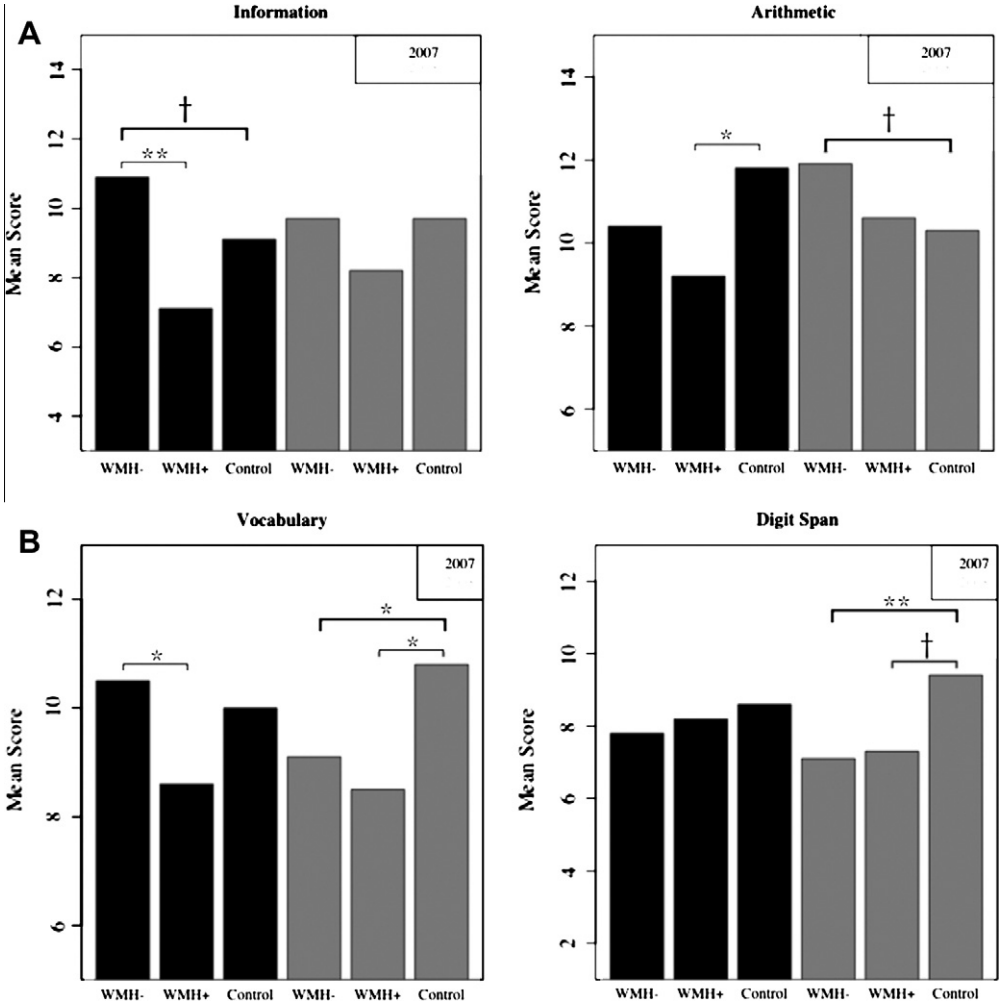

Fig. 2. Multiple post-hoc comparisons on WISC-R mean scores between Mexico Children with and without white matter hyperintensities and control children. The reported WISC-R measures were selected after significant overall tests. Panel A: Comparisons for Information and Arithmetic mean scores. Panel B: Comparisons for Vocabulary and Digit Span mean scores. Panel C: Comparisons for Picture Completion and Coding mean scores. Panel D: Comparisons for mean Verbal and Performance IQ scores.

these variables were submitted to further post-hoc multiple comparisons between the groups separately for 2007 and 2008. The post-hoc GEE analysis (reported in full in Supplementary Table 2) showed significant differences for five WISC-R subtests: Information, Arithmetic, Vocabulary, Digit Span, and Picture Completion. Fig. 2A–D illustrate the comparisons between the three groups in both years for Information, Arithmetic, Vocabulary, Digit Span, Picture Completion, Coding, IQ verbal and IQ Performance. MC WMH^- children outperformed their WMH^+ counterparts in Information, Arithmetic, and Vocabulary measures in 2007; except for Arithmetic, these differences disappeared in 2008. In addition, in 2007 and 2008, WMH^+ outperformed WMH^- in Picture Completion (2008), Coding (2007), and marginally in IQ Performance (2007). Importantly, when comparing CTL to the other two groups, both WMH^-WMH^+ children showed consistent and progressive deficits in Vocabulary and Digit Span (Fig. 2B).

4. Discussion

Children who *appeared* to be healthy and had no risk factors for neurological or cognitive deficits, but who are residents in a highly polluted atmosphere, exhibited selective impairment in IQ subscales tapping on attention, short term memory and learning abilities. Regardless of the presence of prefrontal WMH, Mexico City children performed more poorly, across a variety of cognition tests, compared to CTL children. Children with prefrontal white matter hyperintensities showed pronounced cognitive deficits, while children with no white matter lesions exhibited an additional deficit in a subscale implicating visual memory (picture completion). Thus, the cognitive deficits through reduced brain connectivity (Gläscher, Rudrauf, Colom, et al., 2010; Gläscher, Tranel, Paul, et al., 2009;Woolgar, Parr, Cusack, et al., 2010) in highly exposed children match the localization of the volumetric white matter differences detected over the 1 year follow-up, since the deficits observed are consistent with impairments of parietal and temporal lobe functions.

As compared to CTL, Mexico City children with no WMH exhibited the most significant systemic inflammation as evidenced by the highest serum concentrations of MCP-1 and TNF α, and the lowest numbers of peripheral neutrophils reflecting their attachment to activated endothelial cells in capillary beds (evidence of endothelial activation and dysfunction). White blood counts and inflammatory markers between WMH^+ and WMH^- Mexico City cohorts showed no significant differences.

Our study reveals interesting associative relationships between white matter hyperintensities and volumetric changes that in turn go hand in hand with cognition. First of all, in our previous Brain & Cognition paper we mentioned that white matter hyperintensities represent "*the extreme of vascular lesions with a breakdown of the blood–brain-barrier and that the vascular pathology is likely of a diffuse nature as seen in young dogs*" (Calderón-Garcidueñas,

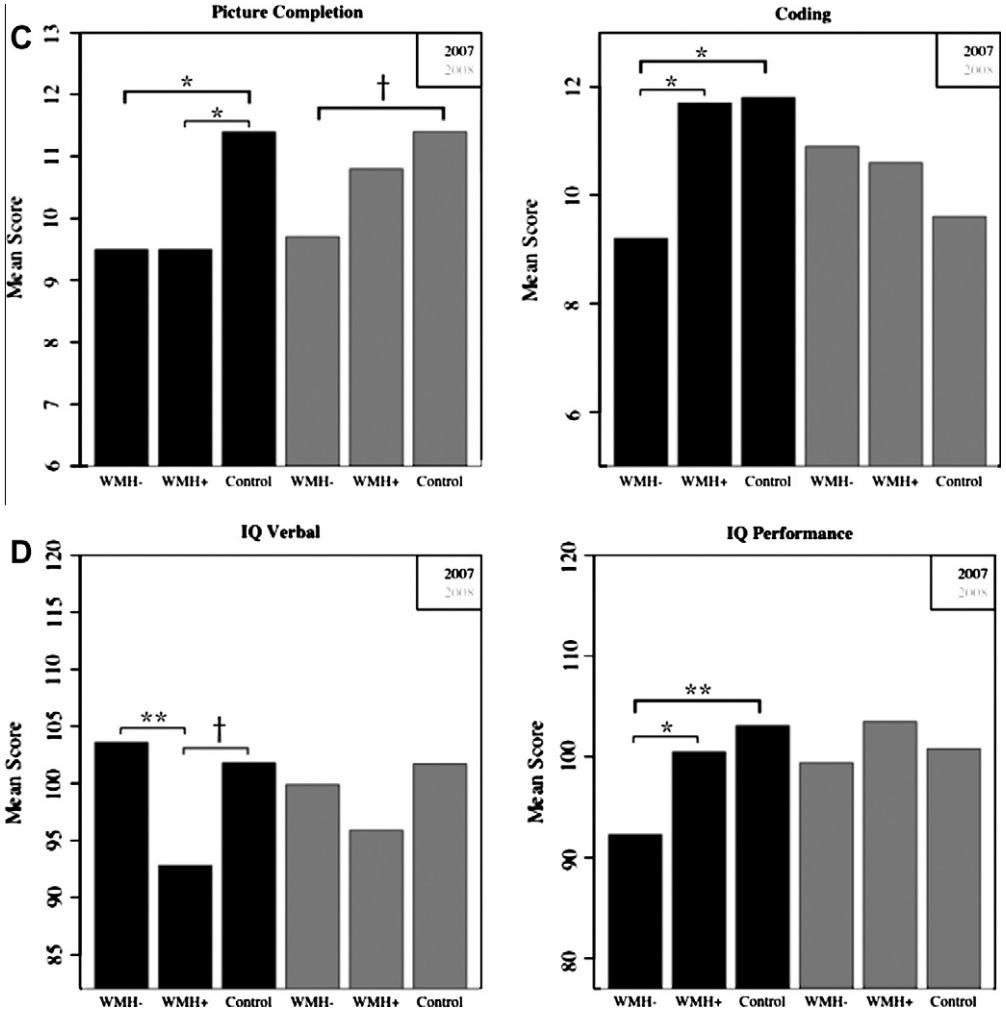

Fig. 2 (*continued*)

Mora-Tiscareño, et al., 2008). Mexico City children poor cognitive performance regardless of the presence of prefrontal WMH supports our previous statement. In support of the diffuse white matter damage the work by Maillard et al., using fractional anisotropy shows the significant white matter injury in the vicinity of WMH (Maillard, Fletcher, Harvey, et al., 2011). The concept of *white matter hyperintensity penumbra* is very relevant to our children and warrants fractional anisotropy maps for the white matter evaluation in our future studies (Maillard et al., 2011). Considering that WMH^{+} children showed in some areas increased white matter volumes, as compared to WMH^{-}, one biologically plausible interpretation is that since WMH are associated with regional cerebral blood flow alterations (Kraut, Beason-Held, Elkins, & Resnick, 2008), young brains could exhibit compensatory responses which would be also correlated with better than expected cognitive outcomes (i.e., same level as controls). Indeed, Kraut et al., described cortical compensation mechanisms with increases in regional cerebral blood flow in elderly subjects without dementia with progressive WMH in the Baltimore Longitudinal Study of Ageing (Kraut et al., 2008). Moreover, we know that endothelial cells play a key role in maintaining cerebral blood flow (McCarron et al., 2006) and Mexico City children have high concentrations of endothelin-1 (Calderón-Garcidueñas, Vincent, et al., 2007) thus endothelial dysfunction and potent vasoconstrictor effects (Huang, Zhang, Lin, et al., 2010; Moldes, Sobrino, Millan, et al., 2008; Salonia, Empey, Poloyac, et al., 2010) could be playing a role in the pathogenesis of the white matter lesions observed in these children. At this point, however, WMH^{+} impact in white matter volumes must be taken with caution. Therefore, although our study does give suggestive evidence as to the nature of the associations of interest, these need to be further examined in a larger study.

Several other possibilities arise when considering the relationships between volumetric changes and cognition deficits in children exposed to air pollution and lacking risk factors for cognitive or neurological deficits. Neuroinflammation as a diffuse process involving supra and infratentorial regions is present in brains of MC exposed children and young adults (Calderón-Garcidueñas, D'Angiulli, Kulesza, et al., 2011; Calderón-Garcidueñas, Solt, et al., 2008; Calderón-Garcidueñas, Franco-Lira, et al., 2009; Calderón-Garcidueñas et al., 2011). As part of the neuroinflammatory process, there is breakdown of the brain–blood-barrier (BBB) as evidenced by the abnormal tight junctions, endothelial activation, and inflammatory cell perivascular trafficking (Calderón-Garcidueñas, Solt, et al., 2008). Hypoperfusion could be the end result of the prevailing neuroinflammation. Thus, the possibility of chronic white matter hypoperfusion and disruption of the BBB, leading to chronic leakage of plasma into the white matter (Debette & Markus, 2010) has to be entertained.

We found no volumetric differences in subcortical structures at 1 year follow-up, suggesting that the disruptions in highly exposed 7 year olds occurred selectively just for certain higher cortical functions. The cognitive deficits present in these second graders

follow the cognitive deficient profile of a similar but older cohort (9.85 ± 2.15 years) we previously reported (Calderón-Garcidueñas, Mora-Tiscareño, et al., 2008). Older children had cognitive deficits in areas of fluid cognition, memory, and executive functions, with 56% of MC children exhibiting WMH. Thus, it seems reasonable to conclude that cognitive deficits are already present early and persist in older cohorts.

General intelligence (g) reflects the performance variance shared across cognitive tasks (Gläscher et al., 2010). Statistically significant associations have been found between g and damage to a frontal and parietal cortex network, critically including white matter association tracts and frontopolar cortex (Gläscher et al., 2010). In correlating with WAIS, the authors showed a modest significant effect on the Picture Arrangement, Block Design and Picture Completion subtests, indicating that visuospatial skills are vulnerable to damage in much larger areas of the right hemisphere. While working memory and verbal skills correlated substantially with the left hemisphere, most notably Arithmetic and Similarities.

In our study, Information, Arithmetic, Vocabulary, Digit Span, and Picture Completion exhibited q values below 0.05, while significant q values were recorded bilaterally for temporal white matter and for the right parietal white matter. Thus, subtests that rely heavily on the capacity for complex reasoning and integration of diverse forms of knowledge, in addition to basic verbal and working memory skills, i.e., Arithmetic, seem to be affected in highly exposed children. Jung and Haier reported that variations in the parietal–frontal/temporal occipital network with involvement of white matter regions, predict individual differences found on intelligence and reasoning tasks (Jung & Haier, 2007). White matter integrity of the target regions is key for a correlation to performance on the Wechsler Intelligence Scales, both in young and adult cohorts (Haier, Jung, Yeo, et al., 2004; Schmithorst, Wilke, Dardzinski, & Holland, 2005).

The systemic up-regulation of MCP-1 is of particular interest in view of the influence of MCP-1 on permeability of the BBB (Yadav, Saini, & Arora, 2010). Specifically, MCP-1 is involved in the recruitment of both monocytes/macrophages and activated lymphocytes into the CNS and induces an increase in brain endothelial permeability. Since an intact BBB is key for proper functioning of neuronal circuits, and synaptic transmission, the breakdown of the BBB in highly exposed children could account for regional hypoxic conditions (Zlokovic, 2008). Likewise, increased serum concentrations of TNF α relate to the systemic inflammation observed repeatedly in highly exposed children (Calderón-Garcidueñas, Franco-Lira, et al., 2007; Calderón-Garcidueñas, Solt, et al., 2008; Calderón-Garcidueñas, Macias-Parra, et al., 2009), but more importantly point to the role of TNF as a marker of brain disease (Clark, Alleva, & Vissel, 2010). In adult populations, inflammatory markers including TNF are associated with decreases in total brain volume (Jefferson, Massaro, Wolf, et al., 2007). Intra-carotid administration of TNF α produces astrocytic endfeet swelling and breakdown of the BBB in rats (Farkas, Süle, Tóth-Szűki, et al., 2006). Chronic expression of high levels of TNF α induced progressive neuronal loss in the nigrostriatal dopaminergic circuit of adult mice, in keeping with the view that is the level and time of expression of pro-inflammatory cytokines that determine their final effect in the CNS (Chertoff, Di Paolo, Schoeneberg, et al., 2011). Thus, the up-regulation of TNF α and MCP-1 in Mexico City children without WMH exhibiting additional deficits in visual memory opens the possibility of the differential regional brain effects of inflammatory cytokines in exposed children.

Notably, since pollution levels in MC have been sustained or worsened in the last 20 years (Bravo-Alvarez & Torres-Jardón, 2002), exposures of today's children and teenagers are truly lifelong and include significant exposures of their mothers during pregnancy. Longitudinal follow-up is required that examines the transition from childhood to adolescent stages on a range of cognitive and structural brain markers in relation to long term mental health outcomes, including cognitive impairment. From a lifespan perspective, our findings suggest that cognitive deficits may have not only structural CNS basis but also a systemic inflammatory component (Calderón-Garcidueñas, Mora-Tiscareño, et al., 2008). From a healthcare perspective this underlines the view that population-based neuroinflammation preventive strategies should start early in childhood.

In conclusion, we assessed a number of brain MRI measurements to characterize volumetric changes of target regions and established an association with specific cognitive deficits determined by residency effect in cohorts of clinically healthy 7 year olds.

We argue that although prefrontal WMH characterized highly exposed children and translated in cognitive deficits, hyperintensities likely only partially identified underlying white matter pathology. Moreover, systemic inflammation and endothelial activation likely play a key role in the detrimental structural and cognitive effects in keeping with the literature suggesting that up-regulation of serum inflammatory markers, microglial and endothelial cell activation and BBB abnormalities are key CNS pathology factors associated with air pollution (Block & Calderón-Garcidueñas, 2009; Calderón-Garcidueñas et al., 2011; Gerlofs-Nijland, van Berlo, Casee, et al., 2010; Levesque, Surace, McDonald, & Block, 2011; Villarreal-Calderon et al., 2010). Thus, as shown here, measures of brain volume changes in key areas, particularly in the temporal and parietal lobes, as well as target inflammatory cytokines may serve as very specific outcome measures for future longitudinal studies following the impact of air pollution from early childhood to adolescence.

If cognitive abilities are reduced during the critical childhood developmental years as a result of air pollution, it will have enormous detrimental effects on society. The issue of air pollution causing cognitive impairment and brain damage in children and teens is of major public importance.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bandc.2011.09.006.

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