Scientific paper

EXPOSURE TO URBAN AIR POLLUTION AND BONE HEALTH IN CLINICALLY HEALTHY SIX-YEAR-OLD-CHILDREN

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Air pollution induces systemic inflammation, as well as respiratory, myocardial and brain inflammation in children. Peak bone mass is influenced by environmental factors. We tested the hypothesis that six-year-olds with lifetime exposures to urban air pollution will have alterations in inflammatory markers and bone mineral density (BMD) as opposed to low-polluted city residents when matched for BMI, breast feeding history, skin phototype, age, sex and socioeconomic status. This pilot study included 20 children from Mexico City (MC) (6.17 years ± 0.63 years) and 15 controls (6.27 years ± 0.76 years). We performed full paediatric examinations, a history of outdoor exposures, seven-day dietary recalls, serum inflammatory markers and dual-energy X-ray absorptiometry (DXA). Children in MC had significantly higher concentrations of IL-6 (p=0.001), marked reductions in total blood neutrophils (p= 0.0002) and an increase in monocytes (p=0.005). MC children also had an insufficient Vitamin D intake and spent less time outdoors than controls (p<0.001) in an environment characterized by decreased UV light, with ozone and fine particulates concentrations above standard values. There were no significant differences between the cohorts in DXA Z scores. The impact of systemic inflammation, vitamin D insufficiency, air pollution, urban violence and poverty may have long-term bone detrimental outcomes in exposed paediatric populations as they grow older, increasing the risk of low bone mass and osteoporosis. The selection of reference populations for DXA must take into account air pollution exposures.

KEY WORDS: *DXA, particulate matter, systemic inflammation, UV light, vitamin D insufficiency*

Environmental pollutants, chemicals and metals have a negative health impact on children (1, 2). There is evidence that exposure to air pollution can result in long-lasting health problems (3-10). Vitamin D deficiency and rickets are associated with air pollution, indoor confinement during the day, residency at higher altitudes and darker skin pigmentation (11, 12). Systemic inflammation likely plays a critical role in bone metabolism through interleukin 6 (IL-6), a multifunctional cytokine that participates in

inflammation, regulates immune responses and has osteoclastic effects with its increased bone-resorption (13). Several studies involving elderly populations have shown that cumulative air pollution concentrations are inversely associated with total body bone mineral density (BMD) (14).

Since childhood and adolescence are crucial periods for skeletal and biochemical bone change, children are a population at risk. Given that nutritional vulnerability is a key aspect in children's growth, our pilot study focused on the evaluation of the impact of air pollutant exposures in two cohorts of six-year-old children residents of two environments with distinctly different levels of pollution.

The primary purpose of this work was to evaluate the bone mineral density as measured by the dual-energy X-ray absorptiometry (DXA) in six-year-olds with a lifetime residency in a highly polluted city (Mexico City) versus a control group from a city with levels well below the current USA National Ambient Air Quality Standards (NAAQS) (Polotitlán, Mexico State). We aimed to identify possible associations between blood inflammatory markers, bone mineral density and nutritional intake in the selected cohorts. Due to the fact that Mexico City children spend less time outdoors and live in a UV blocking environment caused by smog, we expected to find alterations in DXA measurements and inflammatory markers.

Air pollution

The Mexico City metropolitan area is the largest urban centre of North America and an example of extreme urban growth as well as of the accompanying environmental pollution (15). This metropolitan area of over 2000 square kilometres lies in an elevated basin 2240 m above mean sea level, while its urbanized area is surrounded on three sides by mountain ridges with a broad opening to the north and a narrow gap to the south-southwest. In this geographical setting, 20 million inhabitants (including 9 million children), over 40,000 industries and 4 million vehicles consume more than 40 million litres of petroleum fuels per day, producing an estimated annual emission of 2.6 tons of pollutants including coarse and fine particulate matter, gaseous pollutants, polycyclic aromatic hydrocarbons, and lipopolysaccharides (15). As a result of high emissions of secondary precursors (nitrogen oxides and volatile organic compounds), ozone concentrations peak in the downwind southwest area in the afternoon, in accordance with the typical diurnal wind transport of air polluted masses. The

higher short-term (i.e., hourly averages) fine particulate matter (particles with a diameter less than 2.5 μm, $PM_{2.5}$) concentrations coincide with periods when children are outdoors during school recess and physical education, whereas maximal O_3 levels coincide with when they play outdoors at home (16).

Due to high traffic density and increased energy usage, MC suffers from high levels of particulate black carbon (BC) pollution (15). The dominant light-absorbing aerosol species in the region is likely to be BC, commonly known as carbon soot, as well as the associated organic carbon, both of which are produced by diesel engines within the urban area and by the incomplete combustion of fossil fuel biomass burning in the surrounding rural areas. The absorption of solar light by freshly emitted BC aerosols is broadband, although they are more efficient in the UVB range (280 nm to 315 nm). The presence of highly absorbing fine mode aerosols in MC is expected to reduce the UV flux at ground level and therefore lessen the photochemical production of oxidants such as ozone (17). However, under certain meteorological circumstances, the presence of fine mode scattering aerosols in the boundary layer of the Mexico City air basin that are similar in size as the wavelength of the incoming UV radiation may also increase in the UV-B flux at ground level. This is due to their ability to strongly scatter light in the forward direction. In turn, this increase in UV-B flux leads to an increase in photochemical pollution (18). Nevertheless, the net reduction effect in UV-B radiation is predominant (18).

The selection of children from Southwest Mexico City (SW MC) was based on three factors: i. the SW location of the paediatric hospital, ii. children living and attending school close to the hospital, and iii. the location of the MC air pollution monitoring stations: Pedregal and Coyoacán. The control city Polotitlán is a small town 2300 m above mean sea level and 73 miles Northwest of Mexico City with an estimated population of 3000 residents. Its main activity is agriculture along with a few small dairy plants. In contrast to Mexico City, in Polotitlán all criteria pollutants (O_3, PM_{10}, SO_2, NO_2, CO and Pb) are below the current US standards due to the fortunate combination of a relatively small number of contributing industrial and traffic emission sources and good ventilation conditions due to regional winds.

PROCEDURES

Study areas and children's cohorts

This prospective pilot protocol was approved by the review boards and ethics committee at the Hospital Central Militar, and performed according to the Declaration of Helsinki. Written consent was obtained from parents and verbal consent from the children. The geographic areas selected for this study were Southwest Mexico City and Polotitlán. The children belonged to the same socioeconomic status and attended public schools with identical academic and physical education curricula.

Participants

This study includes data from 20 children from Mexico City (*Mean age* = 6.17 years, *SD* = 0.63 years) and 15 control children (*Mean age* = 6.27 years, *SD* = 0.76 years). The criteria for clinical inclusion were: a 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, breastfeeding period of ≥ 6 to ≤ 12 months, and unremarkable clinical histories. The exclusion criteria included: i. height or weight below the third percentile or above the 97 percentile ii. premature birth, iii. a history of any type of fracture, iv. any systemic disease, v. intake of any prescribed or other medications including vitamins and dietary supplements vi. a first degree relative with primary osteoporosis. Outdoor daily exposures in hours per day were recorded by the mother during seven days, including the transit time to and from school, the time spent on recess and physical education during school, and outdoor time while playing and engaging in other activities.

Paediatric and dermatological examination

Complete clinical histories were performed and the children underwent physical examinations. The dermatologist examined the children to establish the type of skin according to the Fitzpatrick Classification Scale (19). Fasting blood samples were collected between 7:00 and 9:00 am. Blood samples in the MC cohort were collected the same week as in the control cohort.

Evaluation of nutritional intake

A paediatric nutritionist interviewed the child and asked the mother to keep a detailed written seven-day food intake record, including the weekend.

Peripheral blood analysis

Blood samples were taken for a Comprehensive Metabolic Panel (CMP) and custom made human Multiplexing Laser Bead Technology (Eve Technologies Corporation, Calgary, Alberta, Canada) for the quantification of the following markers: epidermal growth factor EGF, Eotaxin, fibroblast growth factor-2 FGF-2, Fit-3L, Fractalkine, Granulocyte colony stimulating factor G-CSF, Granulocyte/ macrophage colony stimulating factor GM-CSF, Growth-related oncogene GRO, Interferon alpha 2 IFNα2, Interferon gamma IFN γ, Interleukin 1 alpha IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, Inducible protein 10 IP-10, Monocyte chemotactic protein MCP-1, MCP-3, Macrophage inflammatory protein 1 alpha MIP-1α, MIP-1β, Platelet-derived growth factor PDGF-AA, PDGF-AB/BB, Regulated upon Activation, Normal T-cell Expressed, and Secreted protein RANTES, Soluble CD40 ligand sCD40K, Membrane bound/ soluble IL-2 receptor alpha sIL-2Rα, Transforming growth factor alpha TGFα, Tumour necrosis factor alpha TNF α, TNF β, and Vascular endothelial growth factor VEGF.

TLR4 Genotyping

DNA was isolated from peripheral blood and genotyped for the presence of the Asp299Gly Toll-like receptor-4 (TLR-4) polymorphism, using an allelic discrimination assay protocol according to Applied Biosystems (Life Technologies Corporation, Carlsbad, CA).

Dual energy X-ray absorptiometry (DXA)

The entire body, spine, bilateral forearm and femur scans were acquired by a Lunar Prodigy Advance Encore 2004, General Electric using the Lunar version 8.80 software. Given that our main focus was exposure to distinct polluted environments, and considering that the selected children were healthy, we used Z scores based on the low-pollution matched population and compared them to the exposed MC children.

Measurement of bone age

A single X-ray of the left wrist, hand and fingers was taken for measurement of bone age following the Greulich and Pyle methodology (20).

Data analysis

The univariate descriptive measurements of the two groups' characteristic variables were calculated along with 95 % confidence intervals and p values of two sample t-tests for differences between two group means. The Gaussian distribution was assumed in the inference procedures, i.e. confidence intervals and two sample t-tests. The laboratory data and nutritional intake data were analysed using the same method as the characteristic variables assuming Gaussian distribution. A paired t-test was used to compare the mean difference between the chronological and bone age of Mexico City children and the controls assuming Gaussian distribution. The Z-score of the BMD DXA was calculated based on the control population with life exposures to air pollutants below the standards. For calculating Z scores, the BMD value was X, while MU and SIGMA were the mean and standard deviation of that variable of the control population, respectively. The Z-score of X was defined as (X-MU)/SIGMA. The Z-scores were analysed using inference procedures such as laboratory data and nutritional intake data. Simple linear regression was conducted between total peripheral neutrophils and DXA variables. The r-squares of linear regression and p values for testing linear relationship were calculated. Statistical analyses were performed using R version 2.13 (http://www.r-project.org/). Univariate descriptive measurements were summarized as mean values ±standard deviations. Significance was assumed if either 95 % confidence intervals did not cover zero or at $p<0.05$. The strength of the simple linear relationship was summarized as r-squares.

RESULTS

Air pollution levels

Mexico City children involved in this study had been exposed to significant concentrations of O_3 and particulate matter (PM) for their entire life (15). The climatic conditions in MC are relatively stable and thus pollutant concentrations are consistent year after year. In the years from 2007 through 2009, which includes the study period, $PM_{2.5}$ annual average concentrations in SW MC were (16.84±8.04) µg m^{-3}; (17.96±8.34) µg m^{-3}; and (18.39±8.69) µg m^{-3}, respectively (the US EPA annual standard calls for an $PM_{2.5}$ annual average limit of 15 µg m^{-3}). In addition,

the fourth highest daily maximum 8-hour average ozone concentrations for the same years in that same part of MC were 240 µg m^{-3} (0.121 ppm), 250 µg m^{-3} (0.126 ppm) and 244 µg m^{-3} (0.123 ppm), respectively (the ozone US EPA air quality standard stands for an annual fourth-highest daily maximum 8-hour concentration of 149 µg m^{-3} (0.075 ppm). In the control location, pollutant concentrations had been below the current US EPA standards since 2003 (21). UV-B levels at ground level had been lower in MC than in the surrounding rural locations because of the attenuation effect of the polluted urban air in Mexico City (22). Typically, the UV-B reduction increases from Northeast towards Southwest over MC following the same spatial pattern of the oxidant levels. Acosta and Evans (22) reported that by 1996, the reduction in UV-B in the urban area was greater than 40 % compared with surrounding rural areas in highly polluted days, while the yearly average reduction was around 20 %. Even though this effect has been diminished in the last decade due to the decrease in air pollution levels, the UV light reduction persists due to the presence of highly absorbing aerosols (17).

Demographic data and physical exams

Table 1 summarizes the characteristics of the matched samples. There were 15 children from Polotitlán (8 female, 7 male) and 20 children from Mexico City (8 female, 12 male). Controls spent more time outdoors than MC kids [CTL (4.3±0.5) h, MC (3.5±0.2) h, $p<0.001$]. The predominant skin type was type IV with 14 % of children with type III skin.

Laboratory findings

Mexico City children exhibited significantly higher IL6 concentrations ($p=0.001$), while they had significant reductions in WBC ($p=0.0002$) and total neutrophils ($p=0.0002$), and increases in monocytes ($p=0.005$) (Table 2). There were no significant differences in the concentrations of the custom made human Multiplexing Laser Bead Technology inflammatory panel, except IL-6 (Supplemental Table 1). Total serum alkaline phosphatase was within normal limits in both cohorts. GGT and Ca concentrations were significantly higher in Controls ($p<0.0001$ and $p<0.0002$, respectively) but remained within our normal concentration range for age and gender.

Table 1 *Characteristics of the groups of children studied: Mexico City versus controls*

	Groups			
	Controls **N=15**	**Mexico City** **N=20**	**Confidence Intervals** **Lower / upper limits**	***p* value**
Birth weight / g	2952±655	3144±411	-206.8039/592.5039	0.32
Weight / kg	20.4±3.94	21.2±3.5	-1.9129/3.3795	0.57
Height / m	1.15±0.06	1.14±0.04	-0.0442/0.0375	0.86
BMI / kg m^{-2}	15.14±2.23	15.99±1.84	-0.6066/2.3009	0.24
Outdoor / h	4.3±0.5	3.5±0.2		<0.001
Gender	8F/7M	8F/12 M		
Skin type *	IV13/III 2	IV 17/III 3		

*Note: The numerical values shown in the table represent means ± 1 standard deviation as well as 95 % confidence intervals and p values of two sample t-test for difference between two group means. *The skin classification was based on the Fitzpatrick Classification Scale*

Supplemental Table 1 *Profile of inflammatory mediators and differences between controls and Mexico City children*

	LCI95*	**UCI95****	***p* values**
Eotaxin	-23.8085	22.0638	0.9350
FGF-2	-9.4529	27.7549	0.3200
Flt3Ligand8	-2.9167	11.8896	0.2215
Fractalkine	-81.9580	72.7337	0.9013
G-CSF	-5.8497	0.3504	0.0773
M-CSF	122.3757	35.4183	0.2625
GRO	102.0811	131.2136	0.7980
IFN alpha	-17.2324	16.1459	0.9457
IFN gamma	-64.6281	22.3666	0.3010
IL-1alpha	-2.9672	2.2823	0.7801
IL-1beta	-3.4480	11.8436	0.2688
IL-1ra	-4.7605	4.1485	0.8843
IL-2	-3.0833	3.6024	0.8742
IL-4	-4.0242	0.9682	0.2056
IL-8	-1.9160	1.9586	0.9819
IL-9	-0.3029	0.1940	0.6498
IL10	-3.9318	1.3598	0.3017
IL-12	-54.3925	26.4674	0.4658
IL-12 (p70)	-15.1739	5.2806	0.3061
IL13	-8.4969	2.1742	0.2135
IL15	-1.0563	1.6898	0.6392
IL-17	-1.5796	0.7987	0.4978
IP-10	-60.0868	92.6195	0.6615
MCP 1	-28.1585	103.9692	0.2471
MCP	-11.6111	21.6422	0.5380
MIP-1alpha	112.6877	41.7140	0.3314
MIP-1beta	-47.6089	85.4206	0.5599
PDGF-AB/BB70	-98.9755	4306.0748	0.0604
RANTES	107.6555	95.9740	0.9068
CD40	371.0274	953.0365	0.3716
sIL-2Ralpha	-3.6297	8.1739	0.4344
TGFalpha	-5.8105	2.9514	0.4942
TNFalpha	-1.4192	1.8143	0.7978
TNFbeta	-7.0867	2.5105	0.3104
VEGF	-13.0654	8.5096	0.6531

* Lower limit of 95 % Confidence Interval
** Upper limit of 95 % Confidence Interval

Table 2 *Peripheral blood variables and comparisons in control and Mexico City children.*

	Groups			
	Controls N=15	**Mexico City** N=20	**95 % Confidence Interval** lower/upper limits	*p* value
Haemoglobin / g dL^{-1}	13.0±1.2	13.8±0.6	-0.0073/ 1.5273	0.05
Haematocrit / %	38.5±3.5	40.4±1.8	-0.2246/3.9480	0.07
White blood cells / x10^3 L^{-1}	7460±1464	5635±1001	-3081.8506/-195.1894	0.02
Neutrophils / %	52.1±7.3	46.5±8.4	-11.0289/-0.1244	0.04
Lymphocytes / %	38.7±7.5	43.9±9	-0.5440/10.9007	0.07
Monocytes / %	5.6±1.8	7.4±1.7	0.5693/3.0774	0.0059
Eosinophils / %	2.8±1.9	1.6±1.0	-2.4372/-0.0895	0.0362
Neutrophils total	3.8±0.9	2.6±0.67	-1.8623/-0.6544	0.0003
Lymphocytes total	2.8±0.7	2.4±0.6	-0.8728/0.0828	0.010
Platelets	309467±47715	331800±57239	-13827/58494	0.21
Glucose / mg dL^{-1}	79.6±11.3	81.0±7.3	-6.2767/9.0254	0.71
Calcium / mg dL^{-1}	9.9±0.25	9.5±0.2	-0.5729/-0.2048	0.0002
Phosphorus / mg dL^{-1}	5.14±0.59	5.3±0.39	-0.1816/0.620	0.26
Cholesterol / mg dL^{-1}	158.9±17.7	167.8±37.0	-13.7331/31.0869	0.42
Triglycerides / mg dL^{-1}	109.8±81.5	97.3±42.6	-65.4600/40.4344	0.62
AST / UI L^{-1}	32.2±3.2	31.2±8.0	-57517/3.6902	0.65
ALT / UI L^{-1}	15.1±3.2	19.4±12.8	-2.9757/11.6013	0.22
GGT / UI L^{-1}	27.8±2.1	16.0±2.9	-13.7646/-9.7944	<0.0001
ALP / UI L^{-1}	235.5±31.8	239.4±47.4	-27.2325/35.0889	0.79
IL-6	2.27±1.5	4.8±2.3	1.0413/4.0187	0.001

Note: The numerical values shown in the table represent means ± 1 standard deviation as well as 95 % confidence intervals and p values of two sample t-test for difference between two group means. (AST= aspartate aminotransferase, ALT= alanine aminotransferase, GGT= gamma glutamyl transferase, ALP= total alkaline phosphatase).

TLR4 genotyping

All included children lack the Asp299Gly Toll-like receptor-4 (TLR-4) polymorphism, which is associated with a blunted response to lipopolysacharide (23).

Nutritional intake findings

The nutritional intake based on a seven-day recall showed no differences in control and Mexico City children (Table 3), except for the intake of ascorbic acid that was significantly higher in Mexico City children (*p*= 0.005). Both cohorts exhibited a Vitamin D intake below the daily requirement of 600 UI, the deficiency being more severe in MC children [(429±70) IU and (389±141) IU CTL and MC respectively]. For both cohorts, the Ca intake was near the lower acceptable threshold of the requirement for age (800 to 1200) mg day^{-1}.

Bone age results

There was a significant difference between chronological and bone age in Mexico City children (*p*=0.03) based on the evaluation of the left wrist (20), a difference that was not seen in the controls (*p*=0.15).

DXA results

There were no statistical differences between groups in the BMD Z scores in the different anatomical regions (Table 4). Given the importance of inflammation and air pollution, we performed a linear regression analysis between total peripheral neutrophils and DXA and other CBC variables including WBC, percentages and total numbers of monocytes, lymphocytes, etc. The analysis showed a positive association between the percentage of body fat and neutrophils (*r*^{2}=0.12, *p*=0.03), while the number of monocytes correlated with IL6 (*r*^{2}=0.22, *p*=0.0116).

DISCUSSION

Mexico City six-year-olds exhibit significantly higher concentrations of IL-6, have a Vitamin D insufficiency (24, 25), and spend less time outdoors.

Table 3 *Nutritional intake based on a seven day recall and comparisons in Control and Mexico City children age (6.2±0.69) years*

	Groups		Confidence Intervals lower/upper limits	*p* value	RDA*
	Controls N=15	Mexico City N=20			
Energy / kcal day^{-1}	1438±233	1463±234	-137.8892/188.6137	0.75	1600 to 1700
Protein / g day^{-1}	58.5±9.2	62.5±12.8	-3.7024/11.5485	0.30	1.35 g kg^{-1} b.m.
Carbohydrates / g	177±39.8	174.6±32	-29.0989/22.7039	0.80	(50 to 60) % of energy
Fat / g	54.8±10.4	58.1±11.9	-4.4168/10.9994	0.39	(25 to 30) % of energy
Fibre (g)	6.3±2.2	6.1±2.1	-1.6960/1.4359	0.86	(8 to 10) g per 1000 kcal
Cholesterol / mg	277±68	325±100	-9.6840/106.9574	0.09	100 mg per 1000 kcal
Calcium / mg	824.7±67.4	785.4±193	-135.2528/56.5389	0.40	800 to 1200
Phosphorus / mg	1006.5±222	953.2±192	-200.5850/93.8912	0.46	800
Iron / mg	10.6±4.0	11±3.8	-2.4078/3.1418	0.78	15
Magnesium / mg	136.8±19	146±37	-10.1170/29.9978	0.31	120
Potassium / mg	1464±259	1647±429	-55.4186/421.5431	0.12	1400 to 1600
Retinol / μg	647±230	792±281	-30.4543/321.6284	0.10	500 to 700
Ascorbic acid / mg	48.9±26	89.8±52	13.2615/68.4444	0.005	45
Vitamin D / IU	429±70	389±141	-114.6085/34.6478	0.28	600

*Note: The numerical values shown in the table represent means ± 1 standard deviation as well as 95 % confidence intervals and p values of two sample t-test for difference between two group means. *Recommended dietary allowance (RDA) for age and gender.*

Even though changes in the DXA Z scores are not evident in this small cohort, the high concentrations of IL-6 and the vitamin D insufficiency are cause for serious concern. Interleukin 6 plays a key role in bone resorption and osteoporosis (26-32). Bones are targets of inflammation, with inflammatory cytokines promoting bone resorption which results in systemic bone loss (30). A clear example of an inflammatory entity and a bone detrimental effect is inflammatory bowel disease (IBD) (33). Patients with IBD demonstrate elevated levels of IL-6, TNF α and IL1β, all of which are associated with osteoclastic activity through the activation of p38 mitogen-activated protein kinase (MAPK) pathways (33, 34). Our previous study on older Mexico City children had shown elevated concentrations of TNF α and IL1β (5), thus a osteoclastic synergistic action is likely to be taking place as the children grow up in their polluted environment. TNF α and RANKL interact to potentiate two osteoclastogenic pathways: NFκB and the stress activated protein kinase/c-Jun NH2-terminal kinase activity (36).

TLR4 plays a critical role in the activation of IL-6 producing leukocytes (32) and LPS promotes osteoclast activity and bone resorption (37). Both associations are also very relevant in Mexico City where children are exposed to significant concentrations of lipopolysaccharides associated with particulate matter (6). Since 85 % of the Mexican population lacks the Asp299Gly Toll-like receptor-4 (TLR-4) polymorphism associated with a blunted response to lipopolysacharide (Calderón-Garcidueñas personal data), most Mexican children will be responsive to LPS and the activation of TLR4.

The characterization of inflammatory osteoclastic pathways in exposed children, along with the genotyping of TLR4 key polymorphisms, is mandatory to define whether critical mediators of bone loss are up-regulated and how bone responses evolve as the child grows up.

Exposure to sunlight is key for the skin conversion of 7-dehydrocholesterol to pre-vitamin D$_3$ and vitamin D$_3$; however, in the setting of air pollution, these children get less UV light, spend less time outdoors, and their skin phototype is mostly type IV. All of these factors act synergistically to decrease Vitamin D availability. These issues are important for our paediatric populations because air pollution reduces

Table 4 *Bone mineral density (BMD / g cm^{-2}) Z score, 95 % confidence intervals, mean and SD, and* p *values between Controls and Mexico City children*

BMD Region	p values	LCI95*	UCI95**	Mean	SD
BMD Total	0.2271	-0.0119	0.0478	0.3918	0.8215
BMD head	0.0537	-0.0013	0.1484	0.6359	0.8056
BMD pelvis	0.6361	-0.0394	0.0244	-0.1914	1.3711
BMD column	0.0885	-0.0031	0.0426	0.6648	1.2386
BMD L1L4	0.4760	-0.0306	0.0640	0.2692	1.1635
BMD L1	0.1316	-0.0133	0.0970	0.5795	1.1701
BMD L2	0.5721	-0.0319	0.0567	0.2101	1.1726
BMD L3	0.2596	-0.0217	0.0779	0.4236	1.1803
BMD L4	0.3205	-0.0223	0.0660	0.4228	1.4769
BMD left femoral neck	0.9056	-0.0559	0.0628	0.0367	0.7372
BMD right femoral neck	0.8636	-0.0736	0.0621	-0.0553	0.8348
BMD mean femoral neck	0.9686	-0.0641	0.0617	-0.0124	0.7907
BMD left sup femoral neck	0.6477	-0.0492	0.0777	0.1452	0.8001
BMD right sup femoral neck	0.6768	-0.0903	0.0595	-0.1371	0.8858
BMD mean femoral neck	0.9857	-0.0687	0.0675	-0.0058	0.8463
BMD Ward left	0.6905	-0.0893	0.0600	-0.1256	0.7822
BMD Ward right	0.3624	-0.1316	0.0498	-0.2877	0.7687
BMD Ward mean	0.4932	-0.1097	0.0543	-0.2157	0.7690
BMD right diaphysis	0.8602	-0.0799	0.0946	0.0683	0.6181
BMD right femur total	0.9347	-0.0766	0.0829	0.0312	0.5885
BMD left diaphysis	0.8536	-0.0764	0.0913	0.0676	0.6119
BMD left femur total	0.5751	-0.0492	0.0860	0.2005	0.5695
BMD T5	0.2428	-0.0870	0.2860	0.7940	1.1416
BMD T6	0.6886	-0.2116	0.2937	0.2396	0.8105
BMD T7	0.2781	-0.1839	0.0581	-0.9788	2.2122

* Lower limit of 95 % confidence interval
** Upperlimit of 95 % confidence interval

UV light by 20 % on average (22) and the children's diet becomes a critical source of vitamin D due to the inadequate exposure to sunshine (38). Our cohorts have an adequate caloric, protein, fat and carbohydrate intake but restricted access to fortified Vitamin D foods and a low calcium intake. In a recent study on Mexican children, Elizondo-Montemayor et al. (39) found that 62.1 % of urban children had an insufficiency of 25-OHD (21 to 29) ng mL^{-1} and 20.2 % had a deficiency (<20 ng mL^{-1}). The problem of Vitamin D deficiency among Mexican children is a serious health issue shared with one billion people worldwide (25, 39).

Given that the majority of bone mass is attained during adolescence and young adulthood, with almost 90 % of skeletal mass accumulated by age 18 (40), factors that influence bone mass accrual during growth, including mechanical factors and calcium and vitamin D intake, become critical in populations like such as that of Mexico. Thus, we face serious social issues involving the negative impact on our children's bone health: 1. during the lifetime of our cohorts, there had been a significant rise in crime and violence in neighbourhoods (41) resulting in a marked decrease in the use of public spaces that used to host much of the children's outdoor activities after school; 2. There are 52 million people in the country with incomes below the poverty line, including 11.7 million in extreme poverty, 41 % of who are children (42). Twenty one million children are in households below the poverty level and 77 % of these children live in urban areas. Poverty, food insecurity, and the deteriorating security conditions are affecting the

children's quality of life and potentially even their bone health.

Bone health is critical in childhood and adolescent years (43). A compromised bone with decreased strength and altered microarchitecture will have detrimental effects during childhood and may leave permanent bone damage (43, 44). Low bone density in children is a serious issue; one that remains difficult to evaluate particularly in terms of long-term consequences (44-48). The association of low BMD and a history of at least one fragility fracture are required in order to diagnose osteoporosis in children (44, 47). The evidence of an association between lower than average BMD and a higher fracture rate, along with sustaining repeated fractures, especially at sites specific for bone weakness (i.e., the ultradistal radius), are pieces of information we need to include in future studies on older paediatric populations exposed to pollution.

Our studies in Mexico City have shown that lifelong exposures to a polluted environment, including above standard concentrations of $PM_{2.5}$, are associated with significant systemic inflammation and immunodysregulation (4-6). The marked reduction of peripheral blood neutrophils observed in highly exposed Mexico City children in this work and in previous studies, along with high concentrations of endothelin-1, have been taken as strong evidence for endothelial activation and inflammation (4,6) Moreover, the increase in monocytes has been described upon exposure to particulate matter (49). An interesting association in this work is the positive relationship between body fat and the numbers of peripheral neutrophils, as well as between monocytes and IL-6 concentrations in keeping with the literature pointing to low-grade systemic inflammation related to higher levels of body fat in children (50, 51).

The impact of the increased production of inflammatory mediators with osteoclastic activity has to be taken into account when selecting DXA reference urban populations from polluted cities (52). Especially relevant are cities with concentrations of particulate matter (PM) above the current US National Ambient Air Quality Standards, and since people travel to and from larger cities, PM exposures should be taken into consideration through their well-documented systemic effects (9).

This pilot study is limited to the small number of children included; however, since the cohorts represent clinically healthy matched children with unremarkable clinical histories and representative of low and high polluted urban areas, the results will be useful for planning future longitudinal studies.

In summary, this pilot study on Mexico City six-year-olds is a warning to paediatricians to carefully assess bone health in children residents of polluted megacities and small communities with high PM pollution, taking into consideration the presence of systemic inflammation and Vitamin D and calcium dietary deficiencies (53, 54). There is a lack of appreciation among paediatricians that vitamin D status and physical activity are critical for skeletal health in young children and that the physician's role is to provide recommendations to maximize the health of each individual child (25, 55). More paediatric research is needed to characterize the changes in bone metabolism in the setting of urban air pollution; measurements of circulating levels of 25-hydroxyvitamin D [25(OH)D], serum calcidiol (25 OH vitamin D(3), and the parathyroid hormone ought to be included.

There is urgent need to assess protective measures and nutritional supplementation in disadvantaged highly exposed Mexico City children.

The impact of air pollution, nutritional deficiencies, urban insecurity and poverty may have long-term bone detrimental outcomes in exposed urban paediatric populations, potentially increasing their risk of low bone mass and osteoporosis.

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Sažetak

IZLOŽENOST GRADSKOM ONEČIŠĆENJU ZRAKA I ZDRAVLJE KOSTIJU U KLINIČKI ZDRAVOJ ŠESTOGODIŠNJOJ DJECI

Onečišćenje zraka uzrokuje sistemsku upalu, kao i respiratorne, miokardijalne i moždane upale kod djece. Čimbenici iz okoliša utječu na vršnu koštanu masu. Ispitali smo hipotezu da šestogodišnjaci s cjeloživotnom izloženosti gradskom onečišćenju zraka imaju drugačije upalne pokazatelje i mineralnu gustoću kostiju (BMD) od djece iz gradova s niskom razinom onečišćenja kada usporedimo tjelesni indeks mase, povijest dojenja, fototip kože, dob, spol i društveno-ekonomski položaj. Ova pilot-studija uključuje dvadesetero djece iz glavnoga grada Méxica (6,17 ± 0,63) godina i petnaestero kontrolne djece (6,27 ± 0,76) godina. Obavili smo cjelovite pedijatrijske preglede, prikupili povijesti vanjske izloženosti, sedmodnevne analize prehrane, serumske razine upalnih pokazatelja i dvoenergetsku apsorpciometriju X-zraka (DXA). Djeca iz Méxica imala su značajno više koncentracije IL-6 (*p*=0,001), izrazito smanjen ukupni broj krvnih neutrofila (*p*=0,0002) i povećan broj monocita (*p*=0,005). Djeca iz Méxica također su unosila nedovoljnu količinu vitamina D i provodila manje vremena na otvorenome od kontrolnih ispitanika (*p*<0,001), i to u okružju obilježenom smanjenom UV svjetlošću te koncentracijama ozona i čestične materije iznad standardnih vrijednosti. Nije bilo značajnih razlika između kohorta u Z-vrijednostima DXA. Učinci sistemske upale, insuficijencije vitamina D, onečišćenja zraka, gradskog nasilja i siromaštva mogu imati dugoročne štetne posljedice na kosti u izloženim pedijatrijskim populacijama kako one odrastaju, povećavajući tako njihov rizik od smanjenja koštane mase i osteoporoze. Pri odabiru referentne populacije za DXA bi se ubuduće trebala uzimati u obzir i izloženost onečišćenom zraku.

KLJUČNE RIJEČI: *čestična materija, DXA, insuficijencija vitamina D, sistemska upala, UV svjetlo*

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