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Lifestyle and environmental factors associated with inflammation, oxidative stress and insulin resistance in children

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Abstract

Background: Reaching a better understanding of the modifiable factors associated with inflammatory and oxidative biomarkers in children would be relevant to the design of further investigation and prevention strategies.
Objective: To determine the association of air pollution as well as dietary and physical activity habits with markers of inflammation, oxidative stress and insulin resistance for the first time in a population-based sample of children.
Methods: We conducted a population-based study of 374 children, aged 10–18 years, and assessed the exposure of participants to air pollutants as well as their dietary and physical activity habits. In addition to anthropometric and blood pressure measurements, we determined the fasting serum levels of lipid profile, insulin and markers of inflammation and oxidation.
Results: We found independent associations between improper air quality and plasma markers of inflammation, oxidative stress and insulin resistance. The Pollutant Standard Index (PSI) and the level of fine particulate matter were significantly associated to all biomarkers studied. The associations between different markers of air pollutants and markers of inflammation, oxidative stress and insulin resistance remained significant after adjustment for age, gender, body mass index, waist circumference, healthy eating index and physical activity level. The association of healthy eating score with CRP and insulin resistance was mediated through anthropometric indices, and physical activity had independent association with insulin resistance.
Conclusion: The independent influence of inflammatory/oxidative mechanisms of air pollution effects on surrogate markers of atherosclerosis from early life should be highlighted.

Keywords: Air pollution; Lifestyle; Children; Inflammation; Oxidation

1. Introduction

Primordial and primary prevention of atherosclerosis might be effective for prevention of the development of atherosclerotic diseases [1]. Inflammation is thought to play a central role in the pathogenesis of atherosclerosis and consequently coronary heart diseases (CHD) [2]. As documented by the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study, immunological-inflammatory cells are present in the earliest stages of atherogenesis [3].

Markers of inflammation, as C-reactive protein (CRP), are associated with an increased risk of incident CHD. The causes of increased CRP, however, are not completely understood. It is suggested that oxidative stress may be a determinant of CRP levels and is suggested to promote pro-atherosclerotic inflammatory processes in adults [4] and children [5]. Studying the early relationships between markers of inflammation and atherosclerosis would help to better understand the pathogenesis.

Although the biologic mechanisms responsible for the association of inflammation and oxidative stress with CHD

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remain to be fully understood, reaching a better understanding of the modifiable factors associated with increasing levels of these biomarkers in children would be relevant to the design of further investigation and prevention strategies.

A seamless progression of the effects of the modifiable risk factors on atherosclerosis is documented from childhood [6]. The few studies that have assessed the contribution of lifestyle factors to markers of inflammation among adult population revealed conflicting results [7–9]. Similar studies among youth have been limited to obese children [10,11], and no previous population-based study has been conducted in this age group.

The association between air pollution and cardiovascular and other chronic diseases may be mediated through systemic inflammatory responses [12,13]. Air pollution may result in the formation of reactive oxygen species that contribute to a decline in cellular function. Generation of these substances is linked to a variety of environmental factors. The effect of air pollution with inflammation/oxidative stress has been demonstrated in *in vitro* [14] and *in vivo* [15] studies, as well as in patients with cardiovascular disease [16], and in young adults [17], but it has not been assessed in children who might have the early stages of atherosclerosis [3,6].

Long-term longitudinal studies have documented that metabolic syndrome/insulin resistance in childhood is associated with increased risk of CHD in adulthood, reactive oxygen species are considered to have a pivotal role in mediating inflammation in the metabolic syndrome [18]. Metabolic syndrome, although more prevalent in overweight individuals, but is also present in normal-weight children [19]. Determining modifiable associated factors with insulin resistance may be important for planning preventive measures.

The aim of this study was to determine the association of dietary and physical activity habits as well as air pollution with markers of inflammation, oxidative stress and insulin resistance in a population-based sample of children in order to have a better understanding of the potentially modifiable risk factors and environmental exposures associated with inflammatory processes linked to atherosclerosis in its early stages.

2. Methods

2.1. Study population

Study subjects were selected from among participants of a community-based cross-sectional study of risk factors for CHD among children aged 10–18 years [5] living in Isfahan, the second largest city in Iran. Individuals were eligible if they were living in those urban areas of Isfahan city with air pollution monitoring stations, have stayed in the same area for at least 1 week prior to the study, were non-smokers and lived with nonsmokers. Children with chronic disease, long-term medication use or a history of acute infectious diseases in the past 2 weeks were excluded. The Ethics Committee of the Isfahan Cardiovascular Research Center (NIH member) approved the study. Written informed consent was obtained from parents and oral assent from eligible children. The study was conducted from November 2004–2005. From among the 512 participants of the main study, 374 children were eligible.

2.2. Clinical examination and laboratory methods

Trained nurses completed validated questionnaires about demographic characteristics as well as dietary and physical activity habits of participants [20]. The address and the sampling date were recorded for each participant to match with air pollution data.

Nutritional assessment was performed by means of three 24-h food records (once per week; two school days and one weekend). However, quality control measures, including protocols for interviewing and the training of interviewers, as well as the verification of home recipes, have also been undertaken. The Healthy Eating Index (HEI) was computed to measure participants' diet quality. The index consisted of 10 components based on the analysis of the three 24-h dietary records. Components 1–5 measured the degree to which a participant's diet conformed to the Food Guide Pyramid serving recommendations for the 5 major food groups and are based on the average number of servings from the 3 days of intake data. Similarly, components 6–9, which measured total fat, saturated fat, and cholesterol and sodium intakes, were based on 3-day averages; component 10 measured variety in participants' diets by examining number of different foods consumed over the 3 days of dietary intake. A maximum score of 10 was obtained if 16 or more different food items were consumed over the 3 days. The other components of the index were scored similarly with maximum scores of 10 and minimum scores of 0, where high scores were closer to recommended ranges or amounts; therefore, a combined maximum score was 100 [21].

The participants' physical activity pattern was assessed by a scaled questionnaire organized in 9 different metabolic equivalent (MET) levels ranging from sleep/rest (0.9 METs) to high-intensity physical activities (>6 METs); this instrument was assessed by its concurrent validity against accelerometry and PA dairy, and found that it was significantly associated with the International Physical Activity Questionnaire (IPAQ). For each activity level, the MET-value was multiplied by the time spent on that particular level. MET-time from each level was added to total 24-h MET-time, representing physical activity level on an average weekday [22]. We have previously modified and validated it among Iranian youths [20]. We stratified the physical activity level according to quartiles of the population studied.

The same team of pediatricians, general physicians and nurses examined all study participants. Anthropometric measurements including weight, height and waist circumferences were measured by calibrated instruments following standard protocol. Body mass index (BMI) was categorized on the basis of CDC growth charts: as underweight (BMI < 5th per-

centile, normal weight (BMI 5th–84th percentile), at risk of overweight (BMI 85th–94th percentile) and overweight (>95th percentile). Subcutaneous fat of the biceps and triceps muscles were measured with a skinfold caliper (Mojtahedi, Iran), and the percent body fat was determined by bioelectrical impedence using a Body Fat Monitor (Omron HBF-300, Japan).

Blood pressure (BP) was measured using mercury sphygmomanometers under standard protocol. The readings at the first and the fifth Korotkoff phase were taken as systolic and diastolic BP (SBP and DBP), respectively. The average of the two BP measurements was recorded and included in the analysis [23].

Fasting venous blood sample was obtained from all participants. Compliance with fasting and staying at least for the last 1 week in the same area was determined by interview on the morning of blood collection. Fasting blood glucose (FBG), lipid profile, insulin, CRP and stress oxidants, i.e. malondialdehyde (MDA), and conjugated diene (CDE) were measured according to standard methods that we have previously described in details [5]. The concentration of oxidized-LDL (ox-LDL) in plasma was measured with a sandwich enzyme-linked immunosorbent assay (ELISA) procedure by using the murine monoclonal antibody mAb-4E6 as capture antibody (bound to microtitration wells) and a peroxidase conjugated antiapolipoprotein B antibody recognizing ox-LDL bound to the solid phase (Mercodia AB, Uppsala, Sweden). Plasma insulin was measured by radioimmunoassay (RIA) (LINCO Research Inc.) which is 100% specific for human insulin with less than 0.2% cross-reactivity with human proinsulin and no cross-reactivity with c-peptide or insulin like growth factor. Insulin resistance (IR) was calculated on the basis of homeostasis model assessment of IR [HOMA-R = (fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5]. Sera were analyzed in the central laboratory at Isfahan Cardiovascular Research Center. This laboratory meets the standards of the National Reference Laboratory (WHO-Collaborating Center), and is also under the quality control of the Centers for Disease Prevention and Control, USA, and the Department of Epidemiology, St. Rafael University, Leuven, Belgium.

Because no universally accepted definition of the metabolic syndrome exists for children, we used a definition similar to that used by Cook et al. [24]. It should be noted that they used the cutoff of FBS > 110 mg/dL, but we used the last recommendation of the American Diabetes Association, i.e. the cutoff value of 100 mg/L [25].

2.3. Air pollution data

Data of the three existing fixed-site air-monitoring stations in Isfahan city were used to represent the exposure of participants to air pollutants. Daily concentrations of particulate air pollutants of particles with aerodynamic diameters less than 10 $\mu g/m^3$ (PM_{10}), ozone (O_3), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and carbon monoxide (CO) were obtained.

Particles in the air are a mixture of solids and liquid droplets that vary in size and are often referred to as "particulate matter." Those particles less than 10 mm in diameter (PM_{10}) pose the greatest health concern because they can pass through the nose and throat and get deep into the lungs. According to the US Environmental Protection Agency (US EPA), the standard annual mean is 50 $\mu g/m^3$ for PM_{10}, 0.053 ppm For NO_2, and 0.03 ppm for SO_2. The standard 8-h average is 9 ppm for CO and 0.08 ppm for O_3. For categorizing the air quality measurement, we used the Pollutant Standard Index (PSI) for five major pollutants (CO, O_3, NO_2, SO_2 and PM_{10}). The PSI converts air pollution concentrations to a simple number between zero and 500 and assigns a descriptive term such as "good" or "moderate" to that value. PSI values of 0–50 and 51–100 indicate good and moderate air quality, respectively. A PSI value of 100 indicates that at least one pollutant reached its ambient air quality standard on that day. PSI values of 101–150 show that the air quality is unhealthy for sensitive groups; and values of 151–200 indicate unhealthy air. A PSI of 200 corresponds to a first stage alert during which time elderly persons with existing heart or lung disease are advised to stay indoors and reduce their physical activity; and PSI values of 201–300 indicate very unhealthy air. A second stage alert is called when the PSI reaches 300, at which point the general public is advised to avoid outdoor activity, i.e. PSI values of 301–500 indicate hazardous air quality. Pollution data on the previous 7 days when blood sampling was done were collected and the average was matched for each individual subject. All environmental data were matched with the sampling time of blood for each participant to estimate pollution effects on blood markers.

2.4. Statistical analysis

Data were analyzed by the SPSS software package version 14.0 (SPSS, Inc. Chicago, IL). We used log-transformed concentrations of air pollution markers to achieve normal distributions. Multiple linear regression was used to assess the relation of HEI score, physical activity and PSI with biomarkers (CRP, ox-LDL, MDA, CDE and HOMA-IR). Four models were used to assess the relationships, the first model was adjusted for age and gender but not for anthropometric measures because body composition might act as intermediate factor in the causal pathways; the second model was adjusted for age, gender and BMI; the third model was adjusted for age, gender and WC; and in an additional analysis, we adjusted for age, gender, BMI and WC. We adjusted for the separate and combined effect of BMI and WC because we had previously found relationships between CRP and WC but not with BMI [5]. The concentrations of biomarkers and air pollutants were categorized to quartiles, and the upper quartile was considered as elevated value. We examined the association of the dichotomized concentrations of biomarkers (upper quartile vs. lower quartiles) across the quartiles of HEI score, physical activity level and PSI by using logistic regression analysis.

Medians for quintiles were used to test linear trend in logistic regression analyses. The associations between different markers of air pollutants and biomarkers were assessed by multiple linear regression after adjustment for age, gender, body mass index, waist circumference, healthy eating index and physical activity level.

3. Results

In this study, 374 children and adolescents including 191 boys and 183 girls, aged 10–18 years, with a mean age of 13.7 ± 2.5 years were studied. Of children studied, 12% were underweight, 9% were at risk of overweight and 7% were overweight. The metabolic syndrome was detected in 14.1% of participants.

Table 1 presents the mean (S.D.) values of the participants' characteristics and air pollution data. Anthropometric measures, serum CRP and oxidative stress markers were not significantly different in terms of gender, and the data is presented for the whole population studied. The mean HEI score was not significantly different between genders, but the mean physical activity level was significantly higher in boys than in girls (48.1 ± 4.5 MET-hour/day vs. 39.2 ± 3.5 MET-hour/day).

Table 2 presents the mean and quartiles of air pollutants in different parts of the city, and shows that during the study period, the range of air pollutants varied largely. The mean PSI value corresponded to a moderate air quality; however the mean PSI value of two parts of the city was above 100 and indicated unhealthy air quality for sensitive groups. During the study period, the total PSI value has been above 100 in 171 days. Overall, the mean PM_{10} value was more than 2-fold higher than standard ($122\ \mu g/m^3$ vs. $50\ \mu g/m^3$, respectively), the mean SO_2 level corresponded with the upper limits of standard values, and the mean NO_2 level was in standard limits. Given that the existing air quality standards for CO and O_3 correspond to 1- and 8-h averages, we could not compare our data with these standard values.

Table 1
Mean (S.D.) values of the variables studied

Age (years)	13.7(2.5)
Weight (kg)	49.9(12.4)
Height (cm)	163.9(7.8)
Body mass index (kg/m^2)	19.5(4.1)
Waist circumference (cm)	71.6(6.8)
Percent body fat (%)	22.4(3.7)
Fat mass (kg)	11.4(2.5)
Subcutaneous fat (mm)	10.7(2.1)
Systolic blood pressure (mmHg)	101.4(12.1)
Diastolic blood pressure (mmHg)	64.2(9.7)
Fasting blood sugar (mg/dL)	84.5(7.2)
Total cholesterol (mg/dL)	175.2(24.5)
LDL-C (mg/dL)	107.1 ± 24.2
HDL-C (mg/dL)	42.1(8.4)
Triglycerides (mg/dL)	115.7(22.4)
C-reactive protein (mg/L)	1.1(0.2)
Oxidized-LDL (U/L)	57.2(17.4)
Malondialdehyde (μmol/L)	0.7(0.1)
Conjugated Diene (μmol/L)	2.5(0.2)
Insulin (μU/mL)	19.2(2.7)
HOMA-IR	1.4(0.07)
Healthy eating index score (0–100)	72.4(12.5)
Physical activity (MET-hour/day)	45.2(11.7)
PM_{10} (μg/m^3)	122.08(43.6)
CO (ppm)	8.6(4.9)
SO_2 (ppb)	35.8(24.3)
NO_2 (ppb)	34.4(11.8)
O_3 (ppb)	38.4(18.7)
PSI	93.5(22.1)

HOMA-IR = (fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5. PSI: Pollutant Standard Index.

Table 2
Mean (S.D.) and quartiles of air pollutants in different monitoring stations during the study period

		Quartile			
	Mean (S.D.)	0th	25th	75th	100th
Station 1: Laleh square (North of the city)					
PM_{10}	157.25 (19.44)	135.00	140.50	178.00	183.00
CO	6.82 (1.57)	3.20	5.60	7.86	10.90
SO_2	32.71 (18.19)	1.70	17.80	41.20	271.00
NO_2	31.57 (17.08)	12.10	21.30	37.15	412.00
O_3	40.96 (11.93)	14.90	32.40	51.40	61.50
PSI	73.66 (15.58)	36.00	63.00	81.00	135.00
Station 2: Bozorgmehr square (Center of the city)					
PM_{10}	153.51 (16.78)	115.00	142.00	165.00	183.00
CO	9.85 (6.49)	4.20	8.50	10.70	96.00
SO_2	34.03 (19.02)	3.10	22.20	42.70	98.10
NO_2	34.57 (12.91)	14.60	26.90	39.25	95.10
O_3	35.41 (12.29)	6.70	6.70	47.20	67.20
PSI	106.33 (20.91)	48.00	94.00	121.00	161.00
Station 3: Azadi Square (South of the city)					
PM_{10}	111.76 (44.71)	11.00	81.00	145.00	191.00
CO	9.45 (5.02)	2.40	8.27	10.50	105.00
SO_2	48.09 (26.69)	3.40	20.60	66.40	93.50
NO_2	37.62 (13.37)	17.90	29.40	41.80	88.40
O_3	51.02 (5.25)	43.20	46.20	55.60	58.30
PSI	104.08 (23.17)	27.00	89.00	121.00	147.00
Total					
PM_{10}	122.08 (33.63)	11.00	86.50	153.0	191.00
CO	8.67 (3.95)	2.40	6.90	10.30	105.00
SO_2	35.81 (14.37)	1.70	19.20	47.20	271.00
NO_2	34.48 (12.73)	12.10	26.02	39.40	412.00
O_3	38.48 (11.96)	6.70	27.80	153.0	67.20
PSI	93.59 (22.11)	27.00	73.00	115.00	161.00

The mean and quartile values in each station were significantly different ($p < 0.05$) with the corresponding value in other stations. PM_{10} (μg/m$^{3)}$, CO (ppm), SO_2 (ppb), NO_2 (ppb) O_3 (ppb), PSI: Pollutant Standard Index.

The associations of lifestyle factors (diet and physical activity) and the marker of air pollution (PSI) with biomarkers of inflammation, oxidation and insulin resistance are presented in Table 3. The age and sex-adjusted association of CRP, MDA and HOMA-IR was significant with the HEI score, however after adjustment for anthropometric this association decreased to non-significant levels. While HOMA-IR had significant association with physical activity both before and after adjustment for anthropometric indices,

Table 3
Regression coefficients for the relation of Pollutant Standard Index (PSI), healthy eating index (HEI) score and physical activity (PA) with concentrations of biomarkers

	PSI				HEI				PA			
	Model1	Model2	Model3	Model4	Model1	Model2	Model3	Model4	Model1	Model2	Model3	Model4
CRP	0.38*	0.35*	0.35*	0.34*	−0.31*	−0.27	−0.23	−0.21	−0.19	−0.16	−0.17	−0.17
Ox-LDL	0.31*	0.29*	0.28*	0.28*	−0.29*	−0.25	−0.21	−0.22	−0.21	−0.18	−0.16	−0.16
MDA	0.35*	0.32*	0.34*	0.32*	−0.21	−0.17	−0.18	−0.16	−0.24	−0.17	−0.18	−0.17
CDE	0.29*	0.29*	0.27*	0.27*	−0.19	−0.21	−0.18	−0.19	−0.18	−0.18	−0.17	−0.15
HOMA-IR	0.39*	0.36*	0.32*	0.31*	−0.34*	−0.27	−0.25	−0.21	−0.31*	−0.27*	−0.25*	−0.24*

Model1 was adjusted for age and gender; Model2 was adjusted for age, gender and BMI; Model3 was adjusted for age, gender and WC; Model4 was adjusted for age, gender, BMI and WC. HEI: healthy eating index, PA: physical activity, PSI: Pollutant Standard Index, CRP, C-reactive protein (mg/L), Ox-LDL: oxidized-LDL (U/L), MDA: malondialdehyde (μmol/L), CDE: conjugated diene (μmol/L), HOMA-IR: HOMA-IR = (fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5.

* $p < 0.05$.

other biomarkers had no significant relation with physical activity. Moreover, all biomarkers were significantly associated with PSI before and after adjustment for anthropometric indices.

The odds ratio of elevated CRP and HOMA-IR decreased progressively as the quartiles of the HEI score increased, however this association reached to significant level only in the highest quartile of the HEI score (Table 4).

In order to examine the independent associations between different markers of air pollutants and biomarkers of inflammation, oxidative stress and insulin resistance, the analysis was conducted after adjustment for age, gender, BMI, WC, HEI score and physical activity level. As presented in Table 5, different significant relationships were documented, and PSI had significant independent association to all biomarkers studied.

Table 5
Adjusted[a] associations between different markers of air pollutants and markers of inflammation, oxidative stress and insulin resistance

	B(S.E.)				
	CRP	Ox-LDL	MDA	CDE	HOMA-IR
PM_{10}	1.5(0.2)¶	1.4(0.1)¶	1.3(0.1)*	1.1(0.1)*	1.1(0.3)*
CO	1.3(0.4)¶	1.3(0.2)¶	1.2(0.1)*	0.9(0.1)	1.1(0.4)*
SO_2	0.8(0.1)	0.6(0.1)	1.1(0.2)*	0.7(0.2)	0.7(0.1)
NO_2	1.1(0.1)*	1.2(0.2)*	1.1(0.4)*	0.9(0.2)	0.8(0.1)
O_3	1.1(0.2)*	0.8(0.3)	1.1(0.1)*	0.7(0.4)	0.6(0.1)
PSI	1.6(0.2)¶	1.4(0.2)¶	1.2(0.2)*	1.1(0.3)*	1.2(0.4)*

B: regression coefficient, S.E.: standard error. Ox-LDL: oxidized-LDL, MDA: malondialdehyde, CDE: conjugated diene, HOMA-IR: HOMA-IR = (fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5.

[a] Adjusted for age, gender, body mass index, waist circumference, healthy eating index and physical activity level.

* $p < 0.05$.

¶ $p < 0.0001$.

Table 4
Association of the quartiles of Pollutant Standard Index (PSI), healthy eating index (HEI) score and physical activity (PA) with upper quartiles of markers of inflammation, oxidative stress and insulin resistance

	Quartile1[a]	Quartile2	Quartile3	Quartile4	*P*-value for linear trend
PSI					
CRP	1:00	1.02 (0.7–1.5)	1.1 (0.5–1.4)	1.4 (1.07–1.2)	0.04
Ox-LDL	1:00	1.03 (0.8–1.4)	1.2 (1.1–1.5)	1.5 (1.1–1.7)	0.03
MDA	1:00	1.01 (0.4–1.7)	1.1 (1.02–1.5)	1.3 (1.1–1.5)	0.04
CDE	1:00	1.04 (0.6–1.8)	1.1 (0.7–1.7)	1.1 (0.9–1.6)	0.06
HOMA-IR	1:00	1.02 (0.8–1.7)	1.2 (0.9–1.3)	1.3 (1.1–1.5)	0.04
HEI					
CRP	1:00	0.9 (0.7–1.8)	0.8 (0.6–1.5)	0.7 (0.5–0.9)	0.04
Ox-LDL	1:00	0.9 (0.5–2.1)	0.8 (0.5–1.4)	0.8 (0.6–1.3)	0.1
MDA	1:00	1.02 (0.8–2.6)	0.9 (0.7–1.8)	0.8 (0.6–1.4)	0.08
CDE	1:00	0.9 (0.4–1.8)	0.9 (0.5–1.7)	0.8 (0.6–1.3)	0.07
HOMA-IR	1:00	0.8 (0.2–1.7)	0.7 (0.4–1.3)	0.6 (0.4–0.8)	0.04
PA					
CRP	1:00	0.9 (0.6–2.8)	0.8 (0.4–1.7)	0.7 (0.5–1.4)	0.06
Ox-LDL	1:00	1.01 (0.7–1.9)	0.9 (0.5–1.6)	0.9 (0.6–1.5)	0.1
MDA	1:00	0.9 (0.3–1.8)	0.9 (0.5–1.9)	0.9 (0.6–1.5)	0.4
CDE	1:00	0.9 (0.2–1.8)	0.9 (0.5–1.9)	0.9 (0.6–1.8)	0.5
HOMA-IR	1:00	0.9 (0.1–1.7)	0.9 (0.3–1.8)	0.8 (0.4–1.5)	0.2

CRP: C-reactive protein, Ox-LDL: oxidized-LDL, MDA: malondialdehyde, CDE: conjugated diene, HOMA-IR: HOMA-IR = (fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5.

[a] Values represent odds ratio (95% CI) adjusted for age, sex, body mass index, waist circumference, skinfold thickness and body fat mass.

4. Discussion

In this study, that is the first of its kind in the pediatric age group, the association of lifestyle and environmental factors with biomarkers of inflammation, oxidation and insulin resistance were assessed. Of special interest in the context of this study was our finding on independent associations between improper air quality and plasma markers of inflammation, oxidative stress and insulin resistance. Furthermore, we found that the association of HEI score with CRP and HOMA-IR was mediated through anthropometric indices, and physical activity had independent association with HOMA-IR.

The World Health Organization has identified ambient air pollution as a high public health priority, based on estimates of air pollution related death and disability-adjusted life years; it is estimated that 4.6 million people die each year from causes directly attributable to air pollution [26]. The exact mechanisms linking air pollution to the risk of cardiovascular diseases remain to be determined. According to the statement of the American Heart Association, in addition to the deleterious direct effects on the lung, and subsequently the cardiovascular system, air pollutants have indirect effects mediated through pulmonary inflammation and oxidative stress that develop into a systemic inflammatory response [12].

Different studies found that air pollution was associated with activation of inflammatory/oxidative stress pathways in healthy individuals as well as CHD patients. Studies among elderly [27] and CHD patients [16] demonstrated significant association between air pollutants, notably particulate matter, on serum CRP and suggested that inflammation may have a role in pathogenesis relating to the deleterious effects of air pollution on human health. A large study on patients who underwent coronary angiography found that the effects of air pollutants on the risk of ischemic heart disease were larger for those with abnormal findings in angiography than in other patients [28]. Some researches found modest positive associations between air pollutants and indicators of systemic inflammation, with larger associations suggested for individuals with diabetes, obesity, hypertension, and elevated mean inflammatory markers [29]. In addition, many previous studies among healthy adults provided evidence of positive associations between air pollutants and indicators of systemic inflammation, and suggested that systemic inflammation is a pathway through which airborne PM leads to short-term increases in cardiac risk [30,31].

A growing body of evidence supports a pivotal role for environmental factors during the development of a child [32], as well as the origins of atherosclerosis from early life, whereas most studies and policies focused on the association of air pollutants with respiratory and allergic disorders [33,34]. No previous study has assessed the relationship between environmental factors and biomarkers of inflammation/oxidation and insulin resistance among children. A recent research in Taiwan documented an association of urban air pollution with inflammation, oxidative stress, blood coagulation and autonomic dysfunction simultaneously in healthy young adults, aged 18–25 years [17]. We found association between improper air quality and plasma concentrations of markers of inflammation and oxidative stress among 10–18-year-old children, of special interest that this association was independent of covariates as anthropometric measures and lifestyle factors. Our findings are in agreement with the AHA statement on inflammatory/oxidative mechanisms of air pollution effects on progression of atherosclerosis [12]; however the influence of such factors from early life should be highlighted.

Noteworthy to mention that similar to a previous study [35], we found harmful levels of air pollutants notably high levels of PM_{10} in Isfahan city. This city is situated in the central part of Iran, and is located between the Zagros Mountain and central plain of Iran. It is the second large city of the country with a population of near 2 million, and is facing a significant atmospheric pollution arising from the rapid urbanization and rapid industrial development, as well as heavy traffic of motor vehicles, and is the second air polluted city of the country. Given that the main sources of PM production are motor vehicle emissions, tire fragmentation and resuspension of road dust, as well as power generation and other industrial combustion [12], the main sources of air pollution of the city can be the increasing daily number of motor vehicles in Isfahan city (approximately 450 new cars/day) and the large number of factories surrounding this city. Although during the study period, the mean PSI value corresponded to moderate air quality, and the mean value of some pollutants was within normal limits, but the air quality was associated with markers of inflammation and oxidation among the study participants, this might be because in near half of the study period, the air quality was unhealthy for sensitive groups including children. Furthermore, as the mean PM_{10} value was more than 2-fold higher than standards and was significantly associated with all biomarkers studied, it can be suggested that among children, these fine particles might have important role in the process of oxidation and inflammation in addition to their other documented health hazards.

The prevalence of CHD and related risk factors is high among Iranian population, and Iran is considered to be at the end of the second stage of health and demographic transition, and leading causes of death are shifting to chronic diseases [36]. On the other hand, large cities of the country are suffering from air pollution, it is suggested that part of the burden of chronic diseases and related risk factors might be mediated through deleterious effects of air pollution; hence additional approaches to reduce the burden of disease related to air pollution should be highlighted.

Some previous studies have shown that nutrition can affect inflammation by an excessive production of proinflammatory cytokines associated with a reduced production of anti-inflammatory cytokines [37]. However, the results of different studies are controversial, most studies confirmed such association [37–40], but few studies did not [8]. Previous studies

about the association of dietary habits on the concentration of CRP in the pediatric age group are limited to obese children [11,41]. In a previous population-based study, we found that consumption of solid hydrogenated fat and white-flour bread increased the risk of having the metabolic syndrome, whereas the frequency of consumption of fruits and vegetable, as well as dairy products decreased this risk [42]; in the current study, the odds ratio of high CRP and HOMA-IR decreased progressively as the quartiles of the HEI score increased, however this association reached to significant level only in the highest quartile of the HEI score. Our findings suggest that one mechanism underlying the relation between diet and CVD may involve influencing the process of systemic inflammation. Hence, the whole diet approach seems particularly promising to reduce the inflammation associated with the metabolic syndrome in order to help prevention of chronic diseases from childhood. However, considering that children with magnesium consumption of less than 75% recommended daily allowance (RDA) are 58% more likely to have elevated CRP [43], and considering the possible role of micronutrients in insulin resistance [44,45], the intake of micronutrients should also be considered in such diet approach.

Increase in oxidative stress has a central role in the molecular mechanism of atherogenesis [46]. The antioxidant substances in diet are considered to protect different organs against the harmful effects of oxidative stress and free radicals [47]. Different animal studies [48], as well as clinical trials on normal population [49] and obese individuals [50] showed that a balanced diet rich in antioxidants might have beneficial effects on lipid oxidation. We could not find any association between dietary habits and markers of oxidation; this might be because of the young population in our study; longitudinal studies of youths until young adulthood are necessary to find the effects of diets in childhood and oxidation in later life.

The results about the association of physical activity and markers of inflammation are conflicting. While some studies among adults confirmed such associations [51,52], another study did not confirm it [8]. The few studies conducted among children demonstrated an inverse independent association between physical activity and the metabolic syndrome [53,54]. However, as a longitudinal study among adults indicated that BMI, but not previous-year or current physical activity, predicted CRP concentrations [55], our findings need to be confirmed in a cohort study among children.

In addition to improving the major risk factors associated with the metabolic syndrome, it is suggested that some of the beneficial effects of physical activity is mediated through decreasing subclinical inflammation involving cytokines derived from adipose tissue [56]. Our finding about the independent inverse association between physical activity and insulin resistance and CRP might confirm this suggestion. Physical activity might lowers CRP levels in a dose–response manner [57], and as an association of highest levels of physical activity with metabolic syndrome is documented in a previous study [53] and with insulin resistance in the current study, children and adolescents should be encouraged to vigorous daily physical activity.

Our study has several limitations. First, we cannot infer causality because of the cross-sectional nature of the associations, second the presumptions on the recall bias for the process of recalling and recording the dietary and physical activity habits, which requires attention and involves perception. Third, dietary assessment involves perception, and underreporting is likely due to the participant's awareness of the importance of diet and their ability to readily identify healthful and not so healthful foods; therefore, it is probable that some databases are unable to reflect the precise food intake. Fourth, we used general environmental data to represent participants' exposures rather than personal monitoring data. Fifth, the serum concentrations of biomarkers studied are indicators of overall systemic response rather than specific local responses in the respiratory system, such as pulmonary inflammation from cells and fluid in bronchoalveolar lavage. Sixth, because of the lack of a food guide pyramid adapted for Iranian population, we used that of the United States Department of Agriculture (USDA). Seventh, although pubertal stage have potential role on variables studied, we did not document it.

The main strengths of our study are the novelty in the pediatric age group, its population-based design and assessment of known potential confounding factors and controlling them in our analysis.

5. Conclusion

The independent influence of inflammatory/oxidative mechanisms of air pollution effects on surrogate markers of atherosclerosis from early life should be highlighted. Further longitudinal investigations are needed to confirm these associations.

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