Myeloperoxidase Is an Early Biomarker of Inflammation and Cardiovascular Risk in Prepubertal Obese Children

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OBJECTIVE—Obesity is associated with a state of chronic low-grade inflammation. Myeloperoxidase (MPO) plays an important role in the initiation and progression of acute and chronic inflammatory diseases, such as cardiovascular disease (CVD). The objectives of the current study were to evaluate plasma MPO levels in prepubertal obese children and to determine whether MPO could be an early biomarker of inflammation and CVD risk.

RESEARCH DESIGN AND METHODS—In a prospective multicenter case-control study paired by age and sex of 446 Caucasian prepubertal children ages 6–12 years, 223 normal-weight and 223 obese children were recruited. Blood pressure, waist circumference, weight, and height were measured. In addition to MPO, glucose, insulin, metabolic lipid parameters, oxidized low-density lipoproteins, adiponectin, leptin, resistin, C-reactive protein (CRP), interleukin 6, tumor necrosis factor α, matrix metalloproteinase-9 (MMP-9), and plasminogen activator inhibitor 1 were determined.

RESULTS—We found that MPO was elevated in prepubertal obese children and that this enzyme was associated with such proinflammatory and cardiovascular risk biomarkers as CRP, MMP-9, and resistin. Insulin resistance calculated by the homeostatic assessment model was the best predictor of MPO.

CONCLUSIONS—MPO is an early biomarker of inflammation associated with CVD risk in obese children at the prepubertal age.

O besity is characterized by a chronic low-grade inflammation (1). Obese children have been shown to develop many proinflammatory and proatherogenic changes associated with vascular diseases in adults, including high plasma levels of C-reactive protein (CRP) (2), interleukin 6, and tumor necrosis factor-α (TNF-α) (3). In addition, it has been demonstrated that some factors involved in vascular homeostasis, such as plasminogen activator inhibitor 1 (PAI-1) (4), and in angiogenesis, such as matrix metalloproteinase 9 (MMP-9) (5), are elevated in the plasma of obese children, whereas others, such as adiponectin, are decreased.

Myeloperoxidase (MPO) is an enzyme most abundantly expressed in neutrophils and, to a lesser extent, in monocytes (6). This enzyme has long been viewed as functioning primarily as a bactericidal agent (7),

generating reactive oxygen species that contribute to the destruction and killing of the engulfed pathogens (6). It has been demonstrated that MPO is involved in cellular homeostasis and plays an important role in the initiation and progression of acute and chronic inflammatory diseases, fundamentally cardiovascular diseases (CVD). Recent studies have reported elevated MPO levels in obese adults (8,9). Furthermore, some studies in children have shown that MPO is elevated in subjects with diabetes mellitus type 1 (10) and with hypercholesterolemia (11), although as far as we know, no data have been reported in obese children at the prepubertal period. The objectives of the current study were therefore to evaluate plasma MPO levels in prepubertal obese children and to determine whether MPO could be an early biomarker of inflammation and CVD risk.

RESEARCH DESIGN AND METHODS

Study design
A prospective, case-control multicenter study was performed in prepubertal children paired by sex and age. We recruited 223 obese children and 223 normal-weight children (119 male and 104 female in each group), ages 6–12 years, all Caucasian, from primary care centers and schools in three Spanish cities (Cordoba, Santiago de Compostela, and Zaragoza). Childhood obesity was defined according to the International Obesity Taskforce (IOTF) reference for children (12). Inclusion criteria were: prepubertal stage (Tanner I) and absence of congenital metabolic diseases. Exclusion criteria were: pubertal stage, the presence of congenital metabolic diseases (e.g., diabetes or hyperlipidemia) or undernutrition, and the use of medication that alters blood pressure (BP), glucose, or lipid metabolism. After initial assessments at the school or primary care center, children fulfilling the inclusion criteria were invited for a clinical examination in the appropriated, participating hospital (Reina Sofia University Hospital, Córdoba, Unit of Clinical Analyses, Valle de los Pedroches Hospital, Cordoba, Pediatric Department,

Lozano Blesa University Hospital, Unit of Investigation in Nutrition, Growth and Human Development of Galicia, Pediatric Department, Clinic University Hospital of Santiago). The parents or guardians were informed about the purpose and procedures of the study before written consent was obtained, and all children gave their assent. The study was approved by the Ethics Committees of all participating institutions and complied with the Declaration of Helsinki (Edinburgh 2000 revised) and following the recommendations of the Good Clinical Practice of the CEE (Document 111/3976/88 July 1990) and the legal in force Spanish regulation, which regulates the Clinical Investigation in human beings (RD 223/04 about Clinical Assays).

Clinical examination
Trained pediatricians performed the clinical examinations according to standardized methods. Pubertal stage was determined in each patient according to the Tanner criteria and validated by plasma sex hormone concentrations.

Anthropometric measurements
Anthropometric measurements were taken by a single examiner (M.G.-C., R.L., G.B., M.D.M.-J., M.V., and R.C.) with the children barefoot and in their underwear. Body weight (kg), height (cm), and waist circumference (WC) were measured using standardized procedures, and BMI was calculated as weight (kg) divided by the square of the height (m^2). Obesity was defined according to BMI, with the age- and sex-specific cutoff points proposed by the International Obesity Taskforce (linked to adult cutoffs of 25 and 30 kg/m^2) (12). The z score for BMI was based on Spanish reference standards published by Sobradillo et al. (13).

BP
Systolic and diastolic BP were measured three times by the same examiner (M.G.-C., R.L., G.B., M.D.M.-J., M.V., and R.C.), with a mercury sphygmomanometer and according to international recommendations (14).

Hematological and biochemical analysis
Blood samples were drawn from the antecubital vein between 8:00 and 9:30 A.M. after an overnight fast. The routine blood tests were analyzed at the general laboratory of each hospital. The plasma aliquots for CVD risk and inflammatory biomarkers

were centrifuged immediately and frozen at −80°C until analyzed.

White blood cells were counted with a hematology autoanalyzer. Glucose was analyzed according to the glucose oxidase method in automatic analyzers (coefficient of variance [CV], 1%) (Roche-Hitachi Modular P and D Autoanalyzer; Roche Laboratory Systems, Mannheim, Germany), and plasma insulin was analyzed by radioimmunoassay (CV, 2.6%) with automatic microparticle analyzers (Axsym; Abbott Laboratories, Chicago, IL). Insulin resistance (IR) was calculated by the homeostatic assessment model (HOMA-IR), as defined by the equation HOMA-IR = fasting glucose (mmol) × fasting insulin (μU/mL)/22.5 (15). Plasma triacylglycerols (TAG) (CV, 1.5%), total cholesterol (CV, 0.9%), HDL cholesterol (HDL-c) (CV, 0.8%), LDL cholesterol (LDL-c) (CV, 1.5%), apolipoprotein (apo) AI (CV, 1.7%), and apoB (CV, 2.6%) were measured with an automatic analyzer (Roche-Hitachi Modular P and D Autoanalyzer).

Cardiovascular risk and inflammatory biomarkers
LINCOplex kits of human monoclonal antibodies (Linco Research, St Charles, MO) were used on a Luminex 200 System (Luminex Corporation, Austin, TX). To determine adiponectin (CV, 7.9%), resistin (CV, 6.0%), and active PAI-1 (CV, 6.6%) (Cat. HADK1–61K-A); IL-6 (CV, 7.8%), TNF-α (CV, 7.8%), and leptin (CV, 7.9%) (Cat. HADK2–61K-B); and MPO (CV, 11.2%), MMP-9 (CV, 6.8%), and total PAI-1 (CV, 6.6%) (Cat. HCVD1–67 AK). CRP (CV, 4%) was determined with a particle-enhanced turbidimetric immunoassay (Dade Behring Inc., Deerfield, IL), and oxidized LDL-c (CV, 7.6%) was detected with an ELISA kit (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria).

Statistical analysis
Data were expressed as means ± SE. Normality of distribution of data was assessed by the Kolmogorov-Smirnov test. Homogeneity of variances was estimated with the Levene test. Mean comparisons between obese and normal-weight children for continuous variables with normal distribution were compared by a Student t test for unpaired samples and those with an asymmetric distribution by the Mann-Whitney U test. The Pearson test was applied to assess correlations of MPO and HOMA-IR with anthropometric, metabolic, inflammatory, and CVD risk variables. Backward-step multiple linear regression analysis was performed to evaluate the independent risk factors and the best predictor for MPO. $P < 0.05$ was considered significant. Statistical Package for Social Science software (IBM SPSS Statistics 20; IBM Corporation, Somers, NY) was used for all the statistical analyses.

RESULTS

General, anthropometric, hematological, and metabolic characteristics
Table 1 shows the general and anthropometric characteristics of the children, confirming the similar age and sex distributions between normal-weight and obese groups. The mean weight, height, BMI, BMI z score, and WC were all significantly higher in the obese children. In comparison with the normal-weight group, all obese children had higher neutrophil and monocyte counts, systolic and diastolic BP, plasma TAG, apoB, insulin, and HOMA-IR and lower plasma total cholesterol, HDL-c, and apoAI levels. No differences were found in fasting plasma glucose or LDL-c concentrations (Table 2). Plasma resistin and leptin were

Table 1—General characteristics of the studied prepubertal children

	Normal weight	Obese	*P* value
N	223	223	
Sex (male/female)	119/104	119/104	
Age (years)	8.96 ± 0.1	8.88 ± 0.1	0.574
Weight (kg)	28.9 ± 0.4	52.9 ± 0.8	**<0.001**
Height (m)	1.33 ± 0.01	1.38 ± 0.01	**<0.001**
BMI (kg/m^2)	16.57 ± 0.10	27.48 ± 0.24	**<0.001**
BMI z-score	−0.20 ± 0.04	3.61 ± 0.09	**<0.001**
WC (cm)	58.9 ± 0.5	82.8 ± 0.9	**<0.001**

Data are mean ± SE. Boldface *P* values are statistically significant.

Table 2—Clinical and metabolic characteristics of the studied prepubertal children

	Normal weight	Obese	P value
N	223	223	
Systolic BP (mmHg)	96 ± 1	109 ± 1	**<0.001**
Diastolic BP (mmHg)	59 ± 1	68 ± 1	**<0.001**
Blood neutrophils (units/μL)	3308 ± 101	3 876 ± 102	**<0.001**
Blood monocytes (units/μL)	439 ± 22	780 ± 53	**<0.001**
Glucose (mmol/L)	4.7 ± 0	4.7 ± 0	0.291
Insulin (pmol/L)	35.98 ± 1.25	77.85 ± 3.82	**<0.001**
HOMA-IR	1.09 ± 0.04	2.38 ± 0.13	**<0.001**
Adiponectin (mg/L)	26.11 ± 0.76	21.31 ± 0.75	**<0.001**
Resistin (μg/L)	9.27 ± 0.28	12.04 ± 0.45	**<0.001**
Leptin (μg/L)	4.05 ± 0.26	23.33 ± 0.96	**<0.001**
TAG (mmol/L)	0.61 ± 0.01	0.87 ± 0.03	**<0.001**
ApoAI (g/L)	1.54 ± 0.02	1.32 ± 0.02	**<0.001**
ApoB (g/L)	0.67 ± 0.01	0.72 ± 0.01	**0.007**
Cholesterol (mmol/L)	4.45 ± 0.05	4.24 ± 0.05	**0.004**
HDL-c (mmol/L)	1.71 ± 0.03	1.34 ± 0.03	**<0.001**
LDL-c (mmol/L)	2.43 ± 0.05	2.48 ± 0.05	0.408

Data are mean ± SE. Boldface P values are statistically significant.

significantly higher in the obese group than in the normal-weight group, whereas levels of adiponectin were lower.

Inflammatory and cardiovascular risk biomarkers

In comparison with the normal-weight group, MPO and plasma levels of CRP, IL-6, and TNF-α were higher in the obese group. Likewise, plasma levels of cardiovascular risk biomarkers (total and active PAI-1) were significantly higher in the obese group than in the normal-weight group, whereas no differences were found in oxidized LDL-c and MMP-9 (Table 3).

Relationships of MPO with anthropometric, hematological, inflammatory, CVD risk, and IR markers

Table 4 shows the statistically significant correlations of MPO with anthropometric, hematological, inflammatory, CVD risk, and IR markers. After elimination of the multicollinearity, a multiple linear regression analysis of MPO with associated risk factors was carried out. This analysis showed that HOMA-IR (β = 0.273; $P < 0.001$), MMP-9 (β = 0.156; $P = 0.009$), resistin (β = 0.125; $P = 0.032$), and CRP (β = 0.233; $P = 0.013$) were independently associated with MPO. In addition, we performed a backward-step multiple linear regression analysis including sex, age, WC, BMI z score, and HOMA-IR to evaluate whether the inflammation was dependent on obesity, IR, or both. We found that the best predictor for MPO was HOMA-IR (β = 0.254; $P < 0.001$).

CONCLUSIONS—The main findings of this study were that plasma MPO was elevated in prepubertal obese children and that this enzyme was associated with proinflammatory and cardiovascular risk factors, particularly CRP and MMP-9, and also with IR, specifically HOMA-IR and resistin. In fact, HOMA-IR was the best predictor of plasma MPO.

Obesity is related to specific anthropometric, physiological, and biochemical abnormalities, which predispose toward the development of IR and CVD (16). In this regard, several studies have demonstrated relationships among obesity, inflammation, IR, and CVD in adults and children (17), and associated changes in vascular functionality and structure have been reported to be indicators of early development of atherosclerosis (18). Furthermore, prospective studies have shown that childhood obesity and the presence of risk factors negatively affect vascular health in adulthood (19). MPO has proved to be an active mediator of endothelial dysfunction in cell culture, animal models (20), and adult humans (21). A strong relationship has been found between serum MPO levels and endothelial dysfunction in an overweight adult population, even after adjustment for prevalent CVD, CVD risk factors, and CRP levels (21). In addition, elevated serum MPO levels have been associated with increased CVD risk in apparently healthy adults (22). In addition, an elevated level of MPO has been observed in obese adults, and this biomarker serves as an independent marker in risk assessment for future coronary arteriosclerosis (8). Likewise, MPO has been reported to be elevated in obese hypercholesterolemic children (11) and in obese adolescents (23), increasing the risk of endothelial dysfunction in adulthood. No study to our knowledge, however, has considered MPO plasma levels in prepubertal obese children as we did here.

In our study, plasma levels of such proinflammatory factors as CRP, TNF-α, and IL-6 were all increased. Thus our results add further support to the concept that childhood obesity could lead to CVD later in life (19), because elevated CRP, TNF-α, IL-6, and PAI-1 levels have all been proposed as CVD risk factors.

Mechanisms of action of MPO in CVD involve the modulation of the immune responses and inflammation, inhibition of nitric oxide production, and impairment of lipoprotein function (24,25). In the current study, neutrophil and monocyte counts were higher in obese children, and they were associated with increased MPO levels. It has also been shown that modification of LDL with MPO

Table 3—Biomarkers of inflammation and cardiovascular risk in prepubertal children

Biomarker	Normal weight	Obese	P value
N	223	223	
MPO (μg/L)	13.63 ± 1.27	21.97 ± 1.83	**<0.001**
MMP-9 (μg/L)	84.9 ± 3.3	92.1 ± 4.6	0.204
Active PAI-1(μg/L)	5.00 ± 0.27	13.54 ± 0.73	**<0.001**
Total PAI-1 (μg/L)	18.57 ± 0.84	28.66 ± 1.32	**<0.001**
Oxidized LDL (ng/mL)	1.99 ± 0.14	1.68 ± 0.13	0.109
Interleukin 6 (ng/L)	4.37 ± 0.55	7.45 ± 0,89	**0.003**
TNF-α (ng/L)	3.14 ± 0.11	3.91 ± 0.14	**<0.001**
CRP (mg/L)	0.50 ± 0.08	2.45 ± 0.19	**<0.001**

Data are mean ± SE. Boldface P values are statistically significant.

Table 4—*Correlation of MPO with anthropometric, inflammatory, endothelial dysfunction, cardiovascular risk, and insulin resistance biomarkers*

Biomarker	r	P value
BMI	0.155	**0.001**
WC	0.108	**0.027**
Systolic BP	0.143	**0.003**
Diastolic BP	0.151	**0.002**
Neutrophils	0.159	**0.001**
Monocytes	0.198	**5.22×10^{-5}**
TAG	0.106	**0.027**
ApoAI	−0.113	**0.021**
ApoB	0.103	**0.037**
Insulin	0.242	**4.53×10^{-7}**
HOMA-IR	0.256	**8.88×10^{-8}**
Resistin	0.315	**1.32×10^{-11}**
Adiponectin	−0.093	0.054
Leptin	0.115	**0.016**
CRP	0.198	**4.07×10^{-5}**
MMP-9	0.224	**2.07×10^{-6}**
Total PAI-1	0.213	**6.87×10^{-6}**

Boldface P values are statistically significant.

generates a high-uptake form of LDL-c that can induce foam cell formation (24) and affects HDL cholesterol function by an oxidative modification of apoAI, attenuating its capacity to promote cholesterol efflux (25) and catalyzing oxidation of HDL-c, generating a particle that not only loses its anti-inflammatory activity but also gains a proinflammatory function. In addition, it has been reported that in hypercholesterolemic children MPO is positively correlated with oxidized LDL-c (26). In the current study, however, levels of oxidized LDL-c were similar between obese and normal-weight children, suggesting that MPO alteration precedes lipoprotein modifications and likely later foam cell formation. A very interesting finding of this study is the relationship between MPO and IR. Our results suggest that MPO is dependent on IR but not on obesity. Both MPO and IR are associated with markers of endothelial dysfunction, which indicates that very young obese children could have abnormal vascular changes early in life.

In summary, MPO is an early biomarker of inflammation associated with CVD risk in obese children at the prepubertal age.

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