CLINICAL STUDY

Endothelial dysfunction is related to insulin resistance and inflammatory biomarker levels in obese prepubertal children

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Abstract

Background: The metabolic syndrome (MS) is associated with insulin resistance (IR), a systemic low-grade inflammatory state and endothelial dysfunction. These disorders may arise at a very early age in obese children. This study aimed to investigate the relationship between endothelial dysfunction and both IR and inflammation in prepubertal obese children.

Methods and results: Von Willebrand factor (vWF) and soluble intercellular adhesion molecule-1 (sICAM-1) levels were measured in 46 obese prepubertal children aged 6–9, and in 46 non-obese, age- and sex-matched controls; the possible association of these levels with MS, various inflammatory biomarkers and plasminogen activator inhibitor-1 (PAI-1) was analyzed. Obese children displayed significantly elevated values for sICAM-1 ($P=0.008$), vWF ($P=0.034$), insulin ($P=0.006$), homeostasis model assessment for IR (HOMA-IR; $P=0.003$), C-reactive protein (CRP) ($P<0.001$), PAI-1 ($P=0.002$) and leptin ($P<0.001$). Nonsignificant differences were found in interleukin 6 (IL-6) levels. In the obese group, sICAM-1 showed a positive correlation with insulin ($P=0.013$), HOMA-IR ($P=0.015$), CRP ($P=0.020$), IL-6 ($P=0.023$) and PAI-1 ($P=0.015$). Corrected for age and sex, insulin, HOMA-IR, IL-6 and CPR were found to be independent predictive factors for sICAM-1.

Conclusions: Prepubertal obese children displayed alterations indicative of endothelial dysfunction as well as disorders typical of MS. An association was established between endothelial dysfunction, IR, inflammation and inappropriate fibrinolysis in the children studied.

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Introduction

Obesity is a chronic pathology with high morbidity–mortality rates which is frequently associated with various metabolic disorders grouped under the heading of the metabolic syndrome (MS) (1–3); its prevalence has increased considerably over the last few years. The MS is associated with high risk for diabetes and atherosclerotic cardiovascular disease (CVD) (4, 5).

MS is associated with insulin resistance (IR) and a systemic low-grade inflammatory state (6). Subclinical inflammation could be a unifying factor since it is a precursor of CVD, associated with IR and precedes development of type 2 diabetes (7, 8).

In adults, obesity and IR are associated with higher levels of circulating endothelial dysfunction biomarkers such as soluble intercellular adhesion molecule-1 (sICAM-1) and von Willebrand factor (vWF) (9–11).

The sICAM plays an important role in the initiation of the inflammatory process (12, 13) and is a biochemical marker associated with atherosclerotic progression and with other inflammatory disease processes (14).

Elevated levels of this molecule are indicative of endothelial dysfunction and imply enhanced leukocyte adhesion to the endothelium (15), a physiopathologically decisive stage in atherogenesis (16).

Vascular endothelial dysfunction is considered the earliest stage in the atherogenic process (17, 18). Elevated sICAM levels are reported in obese subjects (19, 20), prompting a pathological upregulation of endothelial adhesion and permeability, favoring atherogenesis; they have also been associated with an increased risk of ischemic heart disease and peripheral artery disease (21–26).

Findings about obese children include not only the alterations characteristic of MS (27, 28) but also systemic low-grade inflammation (29) and disorders indicative of endothelial dysfunction (30). However, little research has addressed the relationship between these variables, particularly prior to puberty.

The early onset of the MS gives rise to prolonged exposure to the adverse effects of the cardiovascular risk factors associated with the syndrome. This may lead to an increased risk of developing type 2 diabetes and atherosclerotic CVD.

Hyperinsulinemia and IR have been involved in vascular reactivity, and a growing body of evidence suggests the role of low grade inflammation as a link between obesity, IR and endothelial dysfunction (31).

The aim of this study was to investigate the relationship between endothelial dysfunction and both IR and inflammation in prepubertal obese children.

Material and methods

Subjects

A case-control study was carried out in obese children of both sexes. One group comprised 46 obese children (body mass index (BMI) over percentile 95 in growth curves for the study population (32)), and the other (control group) comprised an equal number of non-obese children (percentile <85) paired by age and sex (aged 6–9 years). The study included only prepubertal children (Tanner stage 1; 33).

Children with primary hyperlipidemia, hypertension, diabetes or glucose intolerance were excluded from both the test group and the control group, as were children with secondary obesity. Any child receiving pharmacological treatment was also excluded. All parents gave their written consent, and the study was authorized by the hospital ethical research committee.

Blood sampling and analysis

Blood samples were collected after 12 h fasting from a vein in the antecubital fossa, without venous occlusion. All collections were made between 0800 and 0900 h. The samples were separated into aliquots and frozen immediately at -45°C until analysis.

The following were measured in all children: sICAM protein, vWF, C-reactive protein (CRP), interleukin 6 (IL-6) and leptin levels, together with a range of MS-related variables (insulin, lipids, blood pressure, hydrocarbonate metabolism, hemostasis and uric acid).

Glucose, uric acid, cholesterol and triglyceride (TG) concentrations were measured using a random access analyzer (Axon, Bayer Diagnostics) with reagents from Bayer Diagnostics. The homeostasis model assessment for IR (HOMA-IR) was used to detect the degree of IR. Resistance was assessed from fasting glucose and insulin concentrations using the formula: resistance (HOMA-IR) = (insulin (mU/l) $\times$ glucose (mmol/l))/22.5.

High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of chylomicrons, very low-density lipoproteins and low-density lipoproteins with phosphotungstic acid and magnesium ions.

Insulin was quantified using an Access2-Immunoassay System (Beckman Coulter, Brea, CA, USA). Apolipoprotein A-I (Apo A-I) and CRP were measured by nephelometry (N Antisera to Human Apo A-I and N High Sensitivity CRP reagent, Behringwerke AG,

Marburg, Germany) in a Dade Behring Analyzer II Nephelometer (Dade Behring, Inc., Deerfield, IL, USA).

Antigenic immunoassay methods were used for the quantification of plasminogen activator inhibitor-1 (PAI-1; Asserachrom PAI-1, Stago Diagnoses, Asnières-south-Seine, France), IL-6 (Quantikine human IL-6, RD systems, Wiesbaden-Nordenstadt, Germany), leptin (Quantikine human leptin, RD systems), and vWF (DG-EIA vWF, Diagnostic Grifols, Barcelona, Spain). sICAM-1 was measured by ELISA (IBL Immuno-Biological Laboratories, Hamburg, Germany) using a microtiter plate analyzer (PersonalLAB, Pharmacia Diagnostics). Fibrinogen was measured by quantitative assay using thrombin in an automatic analyzer (Electra 1600, Ortho Clinical Diagnostics, Madrid, Spain).

Anthropometric measurements and blood pressure

Weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. BMI was calculated as weight (kg)/height (m)². Blood pressure was measured with a mercury sphygmomanometer (Pymah Corporation, Somerville, NJ, USA) after 20 min rest, in a supine position. Three sizes of cuff were used (9 $\times$ 32, 11 $\times$ 36 and 12 $\times$ 41 cm); the cuff width was required to cover 2/3 of the length of the child's arm. One measurement was taken on each of 3 days and the mean was calculated.

Statistical analysis

Statistical assessment was performed using Microstat (Ecosoft, Indianapolis, IN, USA) or GraphPAD InStat (GraphPAD Software, San Diego, CA, USA). Abnormal values (outliers) were excluded. Results were expressed as mean $\pm$ S.E.M., with a 95% confidence interval (95% CI). The distribution of each variable was tested for departure from Gaussian distribution, and variance equality was controlled by Snedecor's *F*-test. The mean values of the groups were compared using Student's unpaired *t*-test. Statistical significance was set at $P < 0.05$.

Correlation between variables was evaluated using Pearson's correlation coefficient and regression analysis. Multivariate regression analysis was performed using the stepwise method. For each variable, potential confounding factors ($0.05 < P < 0.2$) were evaluated by an analysis of raw and adjusted regression coefficients.

Results

Table 1 shows anthropometric data and selected biochemical parameters for obese and control groups. The age range was 6–9 years. None of the children in control or obese groups had impaired glucose tolerance.

Mean sICAM-1 levels were significantly higher in obese children, at 278.13 ng/ml (95% CI 259.41–296.84)

Table 1 Descriptive study-group statistics and selected biochemical parameters.

	Control (n=46)	Obese (n=46)	P
Male/female	19/27	19/27	
Age (years)	7.74±0.11	7.79±0.16	0.797
BMI (kg/m ²)	16.73±0.18	23.67±0.32	<0.001
WHR	0.839±0.006	0.847±0.007	0.388
sICAM-1 (ng/ml)	246.82±6.30	278.13±9.74	0.008
von Willebrand factor (%)	75.71±3.62	88.49±4.70	0.034
CRP (mg/l)	0.92±0.22	2.61±0.32	<0.001
IL-6 (pg/ml)	1.67±0.24	1.89±0.16	0.698
Leptin (ng/ml)	4.68±0.62	19.80±1.54	<0.001
PAI-1 (ng/ml)	20.51±1.90	30.95±2.39	0.002
Fibrinogen (g/l)	2.25±0.06	2.83±0.08	<0.001

Value are mean±s.e.m.; BMI, body mass index; WHR, waist/hip ratio; sICAM-1, soluble intercellular adhesion molecule-1; CRP, C-reactive protein; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1.

compared with 246.82 ng/ml in the control group (95% CI 234.48–259.17) (Table 1).

Obese children displayed higher plasma vWF, CRP, leptin, PAI-1 and fibrinogen concentrations than non-obese subjects (Table 1).

Relationship between soluble intercellular adhesion molecule-1 and systemic inflammation

CRP concentrations (95% CI 1.97–3.24 mg/l obese vs 95% CI 0.488–1.356 mg/l control) were significantly higher in obese children.

Nonsignificant differences were found in IL-6 levels (Table 1) between obese and non-obese children (95% CI 1.56–2.18 pg/ml obese vs 95% CI 1.27–2.07 pg/ml control).

In the single linear correlation, for the obese group, serum sICAM-1 concentration was positively correlated with CRP and IL-6 (Fig. 1).

Multivariate regression analysis in the obese group, corrected for age and sex, showed that serum IL-6 (P partial = 0.021) and CRP (P partial = 0.025) were independent predictive factors for sICAM-1.

In the combined group (obese and non-obese together), plasma sICAM correlates positively with CRP (P < 0.001) and IL-6 (P = 0.022).

Relationship between insulin resistance, metabolic syndrome, and soluble intercellular adhesion molecule-1

Table 2 shows MS-related parameters for obese and control groups. The mean values (obese vs control) for insulin, HOMA-IR, TGs, systolic blood pressure, diastolic blood pressure and uric acid were significantly higher in obese children. Apo A-I and HDL-C were significantly lower (Table 2).

Figure 1 Serum sICAM-1 concentrations as a function of CRP (r = 0.3410; P = 0.020) and IL-6 (r = 0.3242; P = 0.023) in obese children.

The univariate correlation analysis for MS components for obese and total group (obese and non-obese together) is summarized in Table 3. In the single linear correlation, in both the obese group and the whole group, serum sICAM-1 levels correlated positively with BMI, TGs and uric acid, and negatively with apolipoprotein A-I and HDL-C.

Plasma PAI-1 levels correlated positively with sICAM-1 (r = 0.3560; P = 0.015), insulin (r = 0.4691; P = 0.001), HOMA-IR (r = 0.4671; P = 0.001), BMI (r = 0.3348; P = 0.023) and IL-6 (r = 0.2971; P = 0.045) but not with CRP (r = 0.1493; P = 0.322).

Table 2 Selected biochemical parameters related to metabolic syndrome.

	Control (n=46)	Obese (n=46)	P
Glucose (mmol/l)	4.71±0.039	4.75±0.061	0.582
Insulin (μU/ml)	5.38±0.37	7.06±0.47	0.006
HOMA-IR	1.132±0.058	1.516±0.110	0.003
Cholesterol (mg/dl)	171.65±3.21	174.56±3.54	0.544
Triglycerides (mg/dl)	52.59±2.58	71.17±3.96	<0.001
Apolipoprotein A-I (mg/dl)	160.9±2.74	146.52±3.09	<0.001
HDL-cholesterol (mg/dl)	56.46±1.34	50.34±1.44	0.002
SBP (mmHg)	88.85±1.20	95.56±3.11	0.047
DBP (mmHg)	52.21±1.06	58.82±2.63	0.020
Uric acid (mg/dl)	3.62±0.07	3.89±0.10	0.029

Value are mean±s.e.m.; HOMA-IR, homeostasis model assessment for insulin resistance; HDL-C, high-density lipoproteins cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3 Simple correlation coefficients (*r*) between sICAM-1 and different variables of the obese group (*n*=46) and the total group (obese and non-obese together) (*n*=92).

	sICAM-1	
	Obese	Obese and non-obese (together)
BMI	0.4385 [†]	0.4310*
Cholesterol	0.2095	0.0538
Triglycerides	0.4658*	0.4956*
Apolipoprotein A-I	-0.3133 [‡]	-0.3205 [†]
HDL-C	-0.3467 [‡]	-0.3506 [†]
SBP	-0.1702	-0.0839
DBP	-0.1652	-0.0468
Uric acid	0.4347 [†]	0.4279*
PAI-1	0.3560 [†]	0.3919*
Fibrinogen	-0.1776	0.0024

**P*<0.001; [†]*P*<0.01; [‡]*P*<0.05; BMI, body mass index; HDL-C, high-density lipoproteins cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; PAI-1, plasminogen activator inhibitor-1.

In the obese group (Fig. 2) and total group, serum sICAM-1 levels correlated positively with insulin and HOMA-IR. Using multivariate regression analysis, HOMA-IR (*P* partial =0.044) and insulin (*P* partial =0.044), corrected for age and sex, were independent predictive factors for sICAM-1.

Plasma vWF levels displayed no significant correlation with any of the variables analyzed.

Figure 2 Serum sICAM-1 concentrations as a function of insulin (*r*=0.3648; *P*=0.013) and HOMA-IR (*r*=0.3570; *P*=0.015) in obese children.

Discussion

The last few years have seen an alarming increase in the prevalence of childhood obesity (34). It has also been established that a number of metabolic disorders associated with obesity in adults arise at a very early age in obese children.

Insulin resistance and a range of metabolic disorders grouped under the label 'MS' are reported in obese children (27, 28, 35); a number of authors – including the present team – have detected systemic low-grade inflammation (29) and findings consistent with inappropriate fibrinolysis (36) in these children.

The onset of the MS in prepubertal children may hasten the development of diabetes and CVD.

Childhood obesity has also been associated with endothelial dysfunction (30, 37), although there has been relatively little research into the relationship between biomarkers of endothelial activation and either IR or inflammation in prepubertal obese children.

In addition to typical MS-related disorders, the present study found that obese prepubertal children had elevated vWF and sICAM-1 levels, which were significantly associated with a number of MS parameters, including IR markers. A significant correlation was also noted between sICAM levels and variables indicative of both system low-grade inflammation (CRP) and proinflammatory cytokines (IL-6). Plasma PAI-1 levels displayed a significant correlation with both sICAM levels and concentrations of insulin-resistance and inflammation markers.

Vascular endothelial dysfunction is considered the earliest stage in the atherogenic process (17, 18). One of the molecules acting on the endothelium is sICAM, elevated levels of which are indicative of endothelial dysfunction, implying enhanced leukocyte adhesion (15) which in turn is pathologically decisive in atherogenesis (16).

In obese subjects, increased sICAM levels (19, 20) prompt a pathological increase in endothelial adhesion and permeability, thus favoring atherogenesis. sICAM-1 has been associated with an increased risk of ischemic disease and peripheral artery disease (21–26, 38).

Elevated levels of sICAM-1 were found in the obese children studied here and a positive correlation was noted between these levels and BMI, a finding also reported in adults (19, 20).

It has been suggested that sICAM-mediated endothelial dysfunction is prompted by cytokines secreted in part by adipose tissue (19). Inflammation is associated with endothelial dysfunction. CRP, which appears to be a key sICAM regulator (38, 39), may be involved in this process. Liver CRP synthesis is in turn enhanced by the action of proinflammatory cytokines such as IL-6, secreted by adipose tissue.

A significant correlation was observed here between sICAM levels and both CRP and proinflammatory cytokine (IL-6) levels. Other authors report elevated

sICAM and CRP in obese children, though no correlation is noted between the two (30). However in obese children older than those studied here, an association between the two variables has been reported (37).

Improvement in endothelial function as a result of weight loss in adults has been associated with a decrease in inflammatory cytokine levels (IL-6 and tumour necrosis factor- α) (19). This would suggest that cytokines play a role in the physiopathology of obesity-related endothelial dysfunction; one such cytokine is leptin (40), elevated levels of which were also found in obese children.

Recent data suggest that CRP, IL-6 and ICAM-1 are molecular markers associated with atherosclerosis and its progression (26).

The MS has been observed in conjunction with significant IR in obese children (27, 28, 35). Insulin resistance has also been associated with endothelial dysfunction in obese patients (9, 10, 41, 42). As occurred with BMI, a positive association was noted here between sICAM levels and both basal insulin levels and the insulin-resistance index (HOMA-IR).

In the combined group (obese and non-obese together), sICAM is also correlated with BMI, HOMA-IR and inflammatory biomarker, suggesting that these parameters are the determinants of sICAM concentration in both physiological and pathological situations.

In healthy volunteers, physiopathologically elevated insulin levels similar to those observed in IR cause endothelial dysfunction (43). Although hyperinsulinemia does not directly induce endothelial activation, elevated sICAM levels may be due at least in part to IR.

An alternative explanation, which might complement the cytokine theory, is that other MS components already altered in obese children – such as low plasma HDL levels and elevated TG levels – may induce the expression of cell adhesion molecules, promoting atherogenesis and even facilitating acute atherothrombotic events (44, 45). In the present study, obese children displayed elevated TG levels and low plasma HDL-C levels, which correlated positively and negatively, respectively, with sICAM levels; similar findings have been reported in obese adults (sICAM-1).

Plasma PAI-1 levels were also significantly higher in obese children and displayed a positive correlation with ICAM concentrations, IR markers and IL-6. A positive correlation was also noted in these children between PAI-1 levels and degree of obesity as measured by BMI. Considering that PAI-1 is a cytokine that is expressed in adipose tissue and vascular endothelium, this observation could be of particular importance as it supports the postulated relationship of inflammation and endothelial activation with IR and adiposity (46).

In conclusion, prepubertal obese children displayed alterations indicative of endothelial dysfunction in addition to MS-related symptoms. An association was noted between endothelial dysfunction, IR,

inflammation and inappropriate fibrinolysis. Further research is required, particularly in this age-group, to determine the significance of these analytical data with greater precision. Confirmation of the present findings might in future provide a set of analytical variables, like biomarkers of endothelial dysfunction, IR and inflammation, which might help to assess the cardiovascular risk associated with obesity; this would naturally be of great clinical interest.

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