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Metabolism Clinical and Experimental 54 (2005) 1384–1389

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Endothelial activation and inflammation in prepubertal obese Turkish children

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Received 24 August 2004; accepted 11 May 2005

Abstract

To investigate the degree of endothelial activation and inflammation in prepubertal obese children and to determine the relationship between the markers of endothelial activation, inflammation, and cardiovascular risk factors. In 30 obese and 28 healthy prepubertal children, soluble intercellular adhesion molecule–1 and endothelial leukocyte adhesion molecule–1 (sE-selectin) as markers of endothelial activation and soluble vascular cell adhesion molecule–1 (sVCAM-1) and C-reactive protein (CRP) as markers of endothelial inflammation in addition to cardiovascular risk factors including blood lipids, glucose, insulin, hemoglobin A_{1c}, and systolic and diastolic blood pressure were investigated and compared. The tests were repeated after an oral glucose tolerance test in the obese group. Fasting CRP levels were found to be significantly higher in obese children. Vascular cell adhesion molecule–1 levels were found to be significantly increased in obese children after oral glucose tolerance test. Fasting CRP was positively correlated with body mass index (BMI) and low-density lipoprotein, whereas sE-selectin was positively correlated with total cholesterol. In the obese group, postload levels of soluble sE-selectin was positively correlated with low-density lipoprotein; sVCAM-1 was positively correlated with insulin and homeostasis model assessment values. Postload soluble intercellular adhesion molecule–1, sVCAM-1, and soluble sE-selectin levels were also positively correlated with each other. In the fasting state, BMI was the significant independent risk factor for CRP, and total cholesterol was the significant risk factor for soluble sE-selectin. Insulin resistance was the significant independent risk factor for postload sVCAM-1, and postload low-density lipoprotein stood as the significant independent risk factor for postload soluble sE-selectin. Endothelial inflammation is present in obese prepubertal children and is mainly associated with insulin resistance and lipid levels as well as BMI.

1. Introduction

Childhood obesity, a significant determinant of obesity and cardiovascular complications in later life, has become an important public health problem [1,2]. Obesity and, especially, resultant atherosclerosis are the leading causes of mortality and morbidity in adults [3]. Atherosclerosis is an immune process initiated by endothelial activation and inflammation which progresses by the involvement of the environmental and genetic factors [4,5]. Previous studies have shown that soluble intercellular adhesion molecule–1 (sICAM-1) and soluble endothelial leukocyte adhesion molecule–1 (sE-selectin) are reliable markers of endothelial activation, whereas soluble vascular cell adhesion molecule–1 (sVCAM-1) and C-reactive protein (CRP) are markers of endothelial inflammation [5-9]. In adults, it was shown that obesity causes endothelial activation and inflammation that precede atherosclerosis, independent of other risk factors such as hypertension, smoking, diabetes, and stress [10]. However, the degree of endothelial inflammation or activation in childhood obesity is not exactly known especially in the prepubertal stage. In this study, serum levels of sICAM-1, sE-selectin, sVCAM-1, and CRP levels are investigated in prepubertal obese and age-matched healthy children to clarify the presence of endothelial activation and inflammation.

2. Materials and methods

The study was conducted on 30 obese (17 girls, 13 boys; age, 7.5 ± 1.3 years) and 28 control (12 girls, 16 boys; age,

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0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.05.003

8.3 ± 1 years) Turkish children of 6 to 9 years of age. Body mass index (BMI) greater than the 95th percentile for age and sex was accepted as obesity. Centers for disease control BMI-for-age charts were used as standards. The pubic hair and breast development of all the girls [11], as well as the pubic hair and genital development of all the boys [12] in our study, were either stage 0 or 1 according to the classification of Marshall and Tanner [12]. None of the girls had menarche.

Fasting sICAM-1, sVCAM-1, sE-selectin, and CRP levels, in addition to the levels of the cardiovascular risk factors including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C), blood glucose, insulin, hemoglobin A_{1c} (HbA_{1c}) levels, and systolic and diastolic blood pressure (BP) were investigated in all children. In obese cases, sICAM-1, sVCAM-1, sE-selectin, plasma lipids, blood glucose, and insulin levels were reevaluated after an oral glucose tolerance test (OGTT).

Oral glucose tolerance test was performed with 1.75 g/kg (maximum 75 g) oral glucose, as a suspension in 250 mL of water after 12 hours of fasting. Blood glucose levels were determined just before and 120 minutes after taking the glucose solution [13,14]. All the cases received standard diet for 1 week preceding the test. Oral glucose tolerance test was not performed to the control group because of ethical reasons. Informed consent was taken from the parents of all the cases. The study was approved by the Ethical Committee of Gazi University Faculty of Medicine.

None of the cases in the obese or control groups had vascular or allergic disease; neither had a history of recent infection and alcohol or drug intake. Exercise was avoided for 5 days before sampling.

Blood samples were taken into EDTA-containing tubes at 8:30 AM after a rest of a minimum of 15 minutes. The samples were centrifugated at 4°C for 3 minutes at 3500 rpm and they were kept at −70°C until the time of the study [15,16].

Soluble vascular cell adhesion molecule–1, sICAM-1 (Biosource International kit, Camarillo, Calif), and sE-selectin (Bender Medsystems kit, Vienna, Austria) levels were determined by enzyme-linked immunosorbent assay method at Gazi University Department of Immunology.

Plasma insulin levels were determined by radioimmunoassay (Phadebas, Pharmacia Diagnostics, Piscataway, NJ). This method has a cross-reaction rate of 41% with proinsulin and a rate of less than 0.2% with C-peptide.

Plasma glucose levels were measured by colorimetric-spectrophotometric Aeroset (Abbott) autoanalyzer at 340 nm. Total cholesterol, TG, and HDL-C levels were determined by colorimetric-spectrophotometric Aeroset (Abbott, Abbott Park, Ill) autoanalyzer at 500 nm according to the trinder reaction. Very low-density lipoprotein cholesterol levels were calculated by the formula TG/5. Low-density lipoprotein levels were calculated according to the Friedwald formula [TC − (HDL-C + VLDL-C)]. Hemoglobin A_{1c} levels were determined by Shimadzu high-performance liquid chromatography device (Columbia, Md).

C-reactive protein levels were studied by nephelometric high-sensitivity assay.

Insulin resistance was assessed by homeostasis model assessment of insulin resistance (HOMA-IR), calculated as {[fasting insulin (mU/mL) × fasting glucose (mmol/L)]/22.5}.

3. Statistical analysis

The differences between the fasting parameters of the control and obese groups and also between the boys and the girls were investigated by *t* test. The differences between the fasting and postload levels of parameters in the obese group were investigated by paired *t* test. The normality of the data was checked by Kolmogorov-Smirnov test. For the correlation analysis between adhesion molecules, CRP, and cardiovascular risk factors, Pearson correlation analysis was used. Independent cardiovascular risk factors, those significantly associated with adhesion molecules or CRP levels, were investigated by multiple regression analysis. The fasting parameters of the obese and control groups were pooled for the correlation and regression studies. The correlation and multiple regression analyses were also held in obese group with only postload parameters as well as BMI and HOMA-IR. Before statistical analyses, square root transformation was applied to all parameters to provide a normal distribution [17]. $P < .05$ was accepted as significant. SPSS version 11.0 for Windows (SPSS, Chicago, Ill) was used for statistical analysis.

4. Results

Body mass index and fasting metabolic parameters of obese and control groups are shown in Table 1. C-reactive

Table 1
Body mass index and fasting metabolic parameters of obese and control groups (mean ± SD)

	Control (n = 28)	Obese (n = 30)	*P*
BMI (kg/m^2)	16.6 ± 1.3	22.3 ± 1.7	.0001[a]
TG (g/L)	0.82 ± 0.57	0.91 ± 0.39	.279
TC (mmol/L)	3.72 ± 0.61	4.42 ± 0.89	.001[a]
HDL-C (mmol/L)	1.37 ± 0.29	1.16 ± 0.23	.002[a]
LDL-C (mmol/L)	1.89 ± 0.61	2.72 ± 0.65	.0001[a]
VLDL-C (mmol/L)	0.43 ± 0.29	0.49 ± 0.21	.226
Glucose (mmol/L)	4.96 ± 0.38	4.82 ± 0.41	.178
Insulin (mU/L)	5.43 ± 2.07	10.90 ± 5.37	.0001[a]
HOMA-IR	1.20 ± 0.52	2.32 ± 1.19	.0001[a]
HbA_{1c} (%)	0.048 ± 0.0052	0.049 ± 0.010	.914
Systolic BP (mm Hg)	100.71 ± 9.88	107.83 ± 8.38	.005[a]
Diastolic BP (mm Hg)	66.07 ± 6.72	68.17 ± 10.21	.355
sICAM-1 (ng/mL)	663.56 ± 174.13	702.23 ± 137.73	.290
sVCAM-1 (ng/mL)	292.61 ± 74.52	308.12 ± 8.58	.497
sE-selectin (ng/mL)	15.16 ± 6.03	17.41 ± 7.16	.211
CRP (ng/L)	33.6 ± 62.7	125.7 ± 180.4	.0001[a]

[a] Statistically significant.

Table 2
Fasting and postload metabolic parameters of obese group (mean ± SD)

	Obese (fasting)	Obese (postload)	P
TG (g/L)	0.9150 ± 0.3908	0.8433 ± 0.3554	.034[a]
TC (mmol/L)	4.42 ± 0.89	4.18 ± 0.63	.049[a]
HDL-C (mmol/L)	1.16 ± 0.23	1.13 ± 0.21	.249
LDL-C (mmol/L)	2.72 ± 0.65	2.67 ± 0.59	.625
VLDL-C (mmol/L)	0.49 ± 0.21	0.49 ± 0.35	.921
Glucose (mmol/L)	4.82 ± 0.41	5.68 ± 0.98	.0001[a]
Insulin (mU/L)	10.90 ± 5.37	38.08 ± 23.67	.0001[a]
sICAM-1 (ng/mL)	702.23 ± 133.73	762.92 ± 221.33	.207
sVCAM-1 (ng/mL)	308.12 ± 85.81	389.91 ± 128.83	.015[a]
sE-selectin (ng/mL)	17.41 ± 7.16	16.48 ± 7.48	.633

[a] Statistically significant.

protein levels of obese children were significantly higher than the control ones. Fasting and postload metabolic parameters of obese group are shown in Table 2. Among the markers of endothelial activation and inflammation, only postload sVCAM-1 levels were significantly higher than the fasting levels in the obese group.

None of the children in control or obese groups had impaired glucose tolerance.

When the levels of the adhesion molecules and CRP were compared between boys and girls in each group, only sE-selectin levels of the girls in the control group were significantly higher than the boys (17.43 ± 6.16 and 13.45 ± 5.52, respectively; $P = 047$).

As for the correlation studies in the fasting stage, CRP was positively correlated with BMI ($r = 0.328$; $P = .012$) and LDL-C ($r = 0.268$; $P = .042$); sE-selectin was positively correlated with TC ($r = 0.275$; $P = .037$) in all children (Table 3).

In the postload correlation studies of the obese group, sE-selectin was positively correlated with LDL-C ($r = 0.388$; $P = .034$); sVCAM-1 was positively correlated with postload ($r = 0.362$; $P = .049$) insulin and HOMA-IR. Postload sICAM-1, sVCAM-1, and sE-selectin levels were also positively correlated with each other (Table 4).

Table 3
The correlations between fasting cardiovascular risk factors and markers of endothelial activation and inflammation in all cases (r values)

	CRP	sICAM-1	sVCAM-1	sE-selectin
BMI	0.328**	0.132	0.123	0.154
TG	0.095	0.081	0.063	0.142
TC	0.122	0.073	−0.071	0.275*
HDL-C	−0.221	−0.180	0.167	0.025
LDL-C	0.268*	0.086	−0.067	0.246
VLDL-C	0.114	0.038	0.030	0.161
Glucose	−0.121	0.071	−0.089	0.151
Insulin	0.145	0.064	−0.166	0.124
HOMA-IR	0.125	0.073	−0.175	0.143
HbA_{1c}	0.008	−0.003	0.022	0.101
Systolic BP	−0.091	0.016	0.011	0.082
Diastolic BP	−0.087	0.115	−0.128	0.214
sICAM-1	0.174	–	0.177	0.182
sVCAM-1	0.208	0.177	–	0.123
sE-selectin	0.176	0.182	0.123	–
CRP	–	0.174	0.208	0.176

HOMA-IR = {[fasting insulin (mU/mL) × fasting glucose (mmol/L)]/22.5}.
* $P < .05$.
** $P < .01$.

Table 4
The correlations between postload cardiovascular risk factors, markers of endothelial activation and inflammation and HOMA-IR in obese cases (r values)

	sICAM-1	sVCAM-1	sE-selectin
BMI	−0.043	0.124	−0.174
TG	−0.010	0.294	0.077
TC	0.117	0.278	0.298
HDL-C	0.159	0.130	0.332
LDL-C	0.182	0.092	0.388*
VLDL-C	0.127	0.244	0.093
HbA_{1c}	0.180	0.200	0.050
Glucose	0.165	0.251	0.086
Insulin	0.105	0.362*	0.354
HOMA-IR	0.094	0.435*	0.292
sICAM-1	–	0.704**	0.558**
sVCAM-1	0.704**	–	0.547**
sE-selectin	0.558**	0.547**	–

* $P < .05$.
** $P < .01$.

In the multiple regression analysis, markers of endothelial activation and inflammation were taken as dependent, and BMI, TC, LDL-C, and HOMA-IR were accepted as independent risk factors. In the fasting state, BMI was the significant independent risk factor for CRP ($r^2 = 0.107$; $P = .012$), and TC was the significant risk factor for sE-selectin ($r^2 = 0.076$; $P = .037$). Homeostasis model assessment of insulin resistance was the significant independent risk factor for postload sVCAM-1 ($r^2 = 0.189$; $P = .016$), and postload LDL-C stood as the significant independent risk factor for postload sE-selectin ($r^2 = 0.151$; $P = .034$).

5. Discussion

In adult studies, it was shown that blood sICAM-1, sVCAM-1, and sE-selectin levels are increased in atherosclerotic cardiovascular diseases, and these are accepted as reliable markers of endothelial activation and inflammation [18-20]. C-reactive protein is an acute phase reactant produced in the liver, which was shown to be related to the degree of endothelial inflammation and the extent of atherosclerosis in adult population [9,21]. Obesity was previously shown to trigger endothelial activation independent from the other risk factors in adults [10,22-24].

Although there are a few studies performed in obese adults, our study seems to be the first one investigating the level of endothelial activation and inflammation in obese prepubertal children in the literature. The prepubertal group was chosen to exclude the possible effects of pubertal hormones on the levels of adhesion molecules and CRP levels. The parameters of endothelial activation and inflammation except CRP were reinvestigated in the obese group after an OGTT to evaluate the possible postload

changes. In the fasting state, although an increase was noticed in all parameters of endothelial inflammation and activation, CRP levels were found to be significantly higher in the obese children, and the levels were significantly related to BMI. There are studies in the literature supporting our finding. In the 3rd National Health and Nutrition Examination Survey done between 1988 and 1994, CRP levels of 5305 children between 6 to 18 years were distributed in a wide range (2.1-93.5 mg/dL), as in our study. In that study, the highest CRP levels were found in the children whose BMI was in the upper 95% [25]. Invitti et al [26] has found CRP to be elevated in 11.5% of 710 obese Italian children. In the same study, the degree of obesity was significantly correlated with CRP levels, as in our study. However, there are also cases with puberty in both of these studies, and the finding in our study for the first time shows that obesity per se triggers endothelial inflammation in prepubertal children even before the accelerating effects of the pubertal hormones are active. In addition, Mannge et al [27] has found that high-sensitive CRP levels were significantly higher in older (86) obese children, compared with 142 age-matched controls and also with 148 patients with type 1 diabetes mellitus. It was also shown that carotis intimamedia thickness showed the best overall correlation with BMI SD score followed by high-sensitive CRP in their study.

The increase in CRP is an occasion especially in reaction to inflammation, infection, and tissue damage and triggered by cytokines. Interleukin (IL) 1 and IL-6 are the cytokines that increase the production of CRP in the liver [28,29]. About 30% of IL-6 is produced from the adipose tissue. In addition, adipocytes in obese cases produce large amounts of tumor necrosis factor α messenger RNA that supports the production of IL-6 [9]. Although BMI is an indicator of total degree of obesity, in the recent years, the type of fat distribution is also reported to be well correlated with inflammatory markers. Fried et al [30] has shown that omental adipocytes release 2 to 3 times more IL-6 than subcutaneous adipocytes in 10 severely obese patients without diabetes. Furthermore, Forouhi et al [31] showed that the CRP has correlated with the measures of central obesity such as waist girth and visceral fat area in adult South Asians. Even the associations of CRP with visceral fat area and waist girth persisted after adjustment for either BMI or percent fat. As well, Yudkin et al [9] and Festa et al [24] have found that concentration of CRP was strongly related to measures of total but particularly central obesity in adults. Although Pannacciulli et al [32] has found that CRP plasma levels positively correlated with BMI, waist, fat-free mass, and fat mass; after multivariate analyses, waist and fat mass maintained their independent association with CRP.

The levels of endothelial activation and inflammation markers were also compared between girls and boys both in obese and control groups, and only sE-selectin levels in girls of the control group were significantly higher than the levels of the boys, only with a weak significance ($P = .047$). Adipose tissue has receptors for estrogen, rogen, and progesterone, and the altered levels in puberty could create the differences in fat distribution between prepuberty and puberty and the difference between boys and girls [33]. The difference between the boys and girls could be expected to increase when they reach puberty, as the difference of the amount of adipose tissue between girls and boys are reflected differently to the levels of the inflammatory markers, especially after puberty with the effect of the sex hormones [7,24]. There are also other factors occurring during puberty, which could effect inflammation. It was previously shown that normal puberty leads to insulin resistance [34]. Elhadd et al [15] had found that endothelial inflammation was significantly higher in adolescents with type 1 diabetes mellitus compared with young adults and concluded that puberty exerts a negative effect on endothelial function. In addition, the upsurge of sex hormones and growth factors during this period of development were considered as potential factors. The increased incidence of some environmental factors in puberty, such as smoking, could also lead to increased inflammation [35].

Although it was hypothesized that there might be a significant positive effect of glucose load on the levels of the adhesion molecules in our study, only sVCAM-1 levels were found to be significantly elevated in the obese group after the OGTT. There are experimental studies in the literature that show that higher glucose concentrations increase the expression of adhesion molecules on endothelial cells and promote leukocyte adhesion to endothelium. This effect is thought to be caused either by the conformational change of collagen associated with increased glycation or by the increased concentration of advanced glycation end products [36,37]. It was also shown experimentally that insulin promotes vascular cell adhesion molecule–1 expression in human-cultured endothelial cells through a p38MAP-kinase pathway [38]. An adipocytokine resistin, known to induce insulin resistance, experimentally induced the expression of adhesion molecules in cultured human endothelial cells. [39] As the postload increase in sVCAM-1 levels also parallels the increase in blood glucose and insulin levels in our study, it could be assumed that previous experimental findings were confirmed clinically in our study. It was also seen that the relationships between sE-selectin, sICAM-1, and sVCAM-1 had gained statistical significance after OGTT. As well, in obese cases, postload sVCAM-1 was found to be significantly correlated with postload insulin and HOMA-IR. Homeostasis model assessment of insulin resistance was found to be the most significant independent risk factor for postload sVCAM-1 levels. There are contradictory reports in the literature concerning the relationship between insulin resistance and adhesion molecule levels mainly in adults. In a few previous adult studies, it was shown that insulin resistance correlate with vascular cell adhesion molecule–1 levels in type 2

diabetes mellitus [40] and also in healthy volunteers [29]. However, there exists no study done in prepubertal children. The link between the higher insulin levels and endothelial inflammation could be that higher tumor necrosis factor α levels in obese cases inhibit the function of insulin receptor and tyrosine kinase activity, which leads to peripheral insulin resistance causing higher glucose levels to activate transcriptional factor $\kappa\beta$ that leads to an increase in the expression of adhesion molecules [41,42].

Other important findings in our study are the significant correlation of fasting CRP levels with LDL-C and the correlation of sE-selectin levels with TC. Tamakoshi [43] also found CRP levels to be significantly correlated with LDL-C levels in 34- to 69-year-old adults. It was experimentally shown that hypercholesterolemia can cause endothelial damage independently [44,45]. The finding of positive correlation between sE-selectin levels and TC in our study supports the idea clinically that activation of adhesion molecules could serve as a mechanism for the endothelial damage that elevated serum cholesterol would cause. However, it is not still clear whether dyslipidemia causes endothelial dysfunction that results in increased expression and release of adhesion molecules or whether the increased levels of soluble adhesion molecules are a consequence of atherosclerosis induced by the hypercholesterolemia.

In the recent years, it was shown that acute postprandial hyperglycemia, a state which could be created by the model of OGTT, may be an independent predictor of cardiovascular disease [46,47]. In addition, macrovascular disease is more closely related to acute hyperglycemia [48]. It was also shown that postprandial low-density lipoproteins are oxidized more extensively in the presence of insulin resistance [49]. The mechanisms through which increased postprandial glucose levels and lipid concentrations may damage endothelial cells are complex. Increased expression of the adhesion molecules could be one of the mechanisms [46]. The finding of postload correlations between LDL-C and sE-selectin levels supports this hypothesis. Parallel to these, it was experimentally shown that antioxidants inhibit expression of adhesion molecules induced by oxidized low-density lipoprotein [50]. Matrix metalloproteinases produced by macrophages influence vascular remodeling and plaque disruption. Oxidized low-density lipoprotein, which is more oxidized postprandially, was experimentally shown to regulate and contribute to macrophage-mediated matrix breakdown in atherosclerotic plaques and secretion of adhesion molecules [51].

It should be stressed that a limitation of our study could be that an approximate measure of adiposity, such as BMI, may not adjust fully for fat distribution in children, and adjustments for adiposity by dual-energy x-ray absorptiometry or waist-to-hip ratio measures may give a different result.

As a conclusion, in our study, it was shown that endothelial inflammation is higher in obese prepubertal children compared with healthy ones, and this activation is mainly related to cholesterol, insulin resistance, and BMI.

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