

Some Inflammatory and Endothelial Dysfunction Biomarker levels in Obese Pre-pubertal Children

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Abstract: Childhood obesity has grown at an alarming rate, and is associated with metabolic disturbances that determine a higher risk of type 2 diabetes and atherosclerotic vascular disease in adulthood. These disturbances may arise at a very early age in obese children. These metabolic disturbances may be associated with insulin resistance (IR), a systemic low-grade inflammatory state and endothelial dysfunction.

Objective: to determine the concentration levels of some inflammatory markers in obese pre-pubertal children, and their possible relation with metabolic syndrome.

Methods: We analyzed weight (kg), height (m), body mass index (BMI; kg/m²), systolic and diastolic blood pressure (SBP and DBP/ mm Hg), fasting plasma glucose (FPG), HOMA-IR, CRP, IL-6 and sICAM-1 in 25 obese and 25 non-obese children as a control group.

Results: Obese children displayed significantly elevated values for insulin ($p < 0.001$), homeostasis model assessment for IR (HOMA-IR; $p < 0.001$), CRP ($p < 0.001$), IL-6 ($p < 0.001$) and sICAM-1 levels ($p < 0.001$). Non-significant differences were found in fasting glucose. In the obese group, sICAM-1 showed a positive correlation with insulin ($p < 0.05$), HOMA-IR ($p < 0.0001$) and CRP ($p < 0.05$) and IL-6 ($p < 0.05$).

Conclusion: Pre-pubertal obese children displayed alterations indicative of insulin resistance, endothelial dysfunction and inflammatory state which may increase dangerous of CVD and type 2 DM in the future. Thus, early identification of the inflammatory and endothelial biomarkers in obese children may assist in early interference to prevent progression and complications of type 2 DM and CVD. Moreover, longitudinal studies are needed to recognize the requirement of giving anti-inflammatory drugs to reduce inflammation in the children who are proposed to be diabetics.

Keywords: Childhood obesity, metabolic syndrome, insulin resistance and endothelial dysfunction

I. Introduction

Obesity is the most prevalent nutritional disorder among children and adolescents. Its prevalence has increased so significantly in recent years that many consider it a major health concern of the developed world [1]. Childhood obesity has more than doubled in children and quadrupled in adolescents in the past 30 years [2] and has been known as a major risk factor for insulin resistance in children with metabolic syndrome [3]. The increasing incidence of childhood obesity and the earlier onset of insulin resistance, hypertension, and dyslipidemia increased risk factors for cardiovascular disease. These metabolic abnormalities in children may predict that diabetes and cardiovascular disease complications may appear earlier than previously thought [3,4]. The pathogenesis of obesity-related insulin resistance and type 2 diabetes involve chronic low-grade inflammation and production of inflammatory cytokines contributing to vascular reactivity, endothelial dysfunction and diabetic vascular complications [5]. In presence of obesity different tissues including the adipose tissue itself are sites of inflammation. An infiltration of macrophages and other immune cells is observed in these tissues associated with a cell population shift from an anti-inflammatory to a pro-inflammatory profile. Actually these cells are endocrine cells which produces a large number of bioactive proinflammatory and pro-thrombotic adipokines [6], which interfere with insulin signaling in peripheral tissues or induce β -cell dysfunction and subsequent diabetes and metabolic syndrome [7].

The presence of inflammation in both obesity and metabolic syndrome may be caused by Toll-like receptors (TLRs) stimulation mostly by activation of TLR2 and TLR4. TLRs, especially TLR4, are activated by fatty acids released from the adipose tissue and circulating endotoxemia (a marker of gut permeability or excess circulating gut derived bacteria) [8] resulting in activation of nuclear factor- κ B and increased release of inflammatory biomediators like CRP [9], IL-6, IL-1 β , TNF- α , and monocyte chemoattractant protein-1 [10]. The metabolic syndrome and type 2 diabetes are associated with endothelial activation. Endothelial dysfunction represents an early phase of vascular changes that eventually lead to atherosclerosis with all its unfavorable complications [11]. The sICAM-1 plays an important role in the initiation of the inflammatory process and is a biochemical marker associated with atherosclerotic progression and with other inflammatory disease processes [12]. Elevated levels of this molecule are indicative of endothelial dysfunction and imply enhanced leukocyte

adhesion to the endothelium [13], a physiopathologically decisive stage in atherogenesis [14]. Diagnosis of metabolic syndrome was defined according to the National Institutes of Health (1998)[15] criteria adapted for children. As in adults, children are classified as having the metabolic syndrome if they meet three or more of the following criteria for age and gender. BMI above the 95th percentile [16], triglyceride level above the 95th percentile, HDL-cholesterol below the 5th percentile, systolic or diastolic blood pressure above the 95th percentile, impaired fasting glucose or impaired glucose tolerance (IGT) as defined by the American Diabetes Association [17]. Thus the objective of this study is to investigate the relationship of inflammatory markers and markers of endothelial dysfunction with both IR and inflammation in pre-pubertal obese children and their possible relation with metabolic syndrome.

II. Subjects And Methods

2.1 Subjects: A case-control study was carried out on 50 children of both sexes. These children were divided into two groups. The first group included 25 obese children (17 boys and 8 girls), body mass index (BMI) over percentile 95 in growth curves. These patients were attending the pediatric outpatient clinic, King AbdElaziz Hospital. The other (control group) comprised 25 non-obese children (percentile < 85) matching by age (6–10 years) and sex (13 boys and 12 girls).

An informed phrasal consent from the parent of each participant was obtained.

Exclusion criteria: The children with; symptoms of infection during the 2 weeks before the study, family history for cardiovascular diseases or diabetes, cardiovascular abnormalities, diabetes, primary hyperlipidemia and secondary obesity and any child receiving pharmacological treatment.

2.2 Collection of Blood samples: Two venous blood samples were collected. The first one (2ml) was collected over EDTA after 8 hours fasting and assayed immediately for the determination of fasting plasma glucose and plasma insulin. The other Venous blood sample (8 ml) was collected after 14 hours fasting for measuring lipid profile and inflammatory markers. Serum of the second sample was separated, divided into aliquots and preserved at -20°C until used.

2.3 Biochemical measurements:

1. Fasting plasma glucose (FPG) was determined by enzymatic method according to Trinder [18].
2. Fasting plasma insulin was quantified using radioimmunoassay system described by Buritus and Ashwood [19]. The homeostasis model assessment for IR (HOMA-IR) was used to detect the degree of insulin resistance. Resistance was assessed from fasting glucose and insulin concentrations using the formula:
 $\text{HOMA-IR} = \text{Insulin } (\mu\text{U/ml}) \times \text{blood glucose (mg/dl)} / 405$ [20].
3. C-reactive protein (CRP) was measured by the semi-quantitative latex agglutination assay (Humatec CRP, Human Gesellschaft für Biochemica und Diagnostica mbH, D-65205 Wiesbaden, Germany).
4. s-ICAM-1 was analyzed as a single determination by commercial assays using enzyme linked immune sorbent assay (ELISA) kit [17].
5. IL-6 was measured using commercially available ELISA kits according to the manufacturer's instructions (Pharmingen Inc, San Diego, Calif) for the quantitative measurement of human IL-6 in serum [18].
6. Serum total cholesterol and triglycerides [19], were measured by routine colorimetric method using commercial assay kits supplied by Bicon Co. (Germany). While HDL-cholesterol and LDL-cholesterol were measured by precipitation technique described by Lopes-Virella et al., [20] and Rifai et al., [25] respectively.

2.4 Anthropometric measurements: Body mass index [BMI (weight in kg/height² in m)] was calculated. To distinguish overweight and obese children, overweight ($\geq 85\%$ to $< 95\%$) or obese ($\geq 95\%$) according to the National Center for Health Statistics criteria. Only obese children were included in this study [16].

2.5 Statistical analysis: Results were expressed as mean \pm SD. 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.

III. Results

3.1 Anthropometric data and selected biochemical parameters: The characteristics of obese and control group children are listed in Table 1. The prevalence of obesity was higher in boys as compared to girls (56.6 vs. 43.3 %). 18 obese children (60%) were recorded to be hypertensive and 12 (40%) had hyperinsulinemia and were insulin resistant. The two groups did not differ significantly in age, gender, and height. The mean BMI was $25.46 \pm 2.77 \text{ kg/m}^2$ in the obese group and was $17.43 \pm 0.97 \text{ kg/m}^2$ in the control group; the difference was statistically significant. Between obese and non-obese children, distributions of the blood pressure readings differed significantly for systolic blood pressure ($P < 0.05$) and it showed a positive correlation with BMI ($P = 0.022$) (figure 1). Fasting glucose showed a non-significant increase in the obese group, while the mean for

insulin and HOMA for insulin resistance (HOMA-IR) index showed a significant increase in the obese compared to the non-obese group (P <0.001).

3.2 Comparison of Lipid profile and inflammatory biomarkers: Mean LDL-C and triglycerides were significantly higher in obese children (109.17±12.05, 82.07±17.78 mg/dl versus 90.4±10.77, 51.6±6.92 respectively). CRP concentrations were significantly higher in obese children (2.13±0.90 mg/dl obese versus 0.95±0.39 mg/dl control). Serum IL-6 levels were also significantly higher in the obese group than the control group (p < 0.001). Mean sICAM-1 levels were significantly higher in obese children at 267.28±31.00 ng/ml compared with 231.01±20.13 ng/ml in the non-obese group (table 2).

3.3 Correlation involving insulin and HOMA-IR with BMI and lipid profile: There were positive correlations linking insulin and HOMA-IR with BMI, systolic blood pressure, LDL-C and triglycerides. In addition a negative correlation between insulin and HOMA-IR with HDL-C was noticed (table 3).

3.4 Correlation of metabolic syndrome parameters with CRP, IL-6 and sICAM-1: In the multiple regression analysis for the obese group. Table 4 displays that BMI correlated positively with CRP, IL-6 and sICAM-1. Also sICAM-1 concentration was positively correlated with systolic blood pressure. Moreover, insulin and HOMA-IR index was positively correlated with CRP, IL-6 and sICAM-1.

3.5 regression analysis between CRP, IL-6 and sICAM-1: Figure 2 displays Serum CRP concentrations as a function of IL-6 and figure 3 displays Serum sICAM-1 concentrations as a function of CRP in obese children.

Table (1): Anthropometric data and selected biochemical parameters for obese and control groups

Parameters	Obese group(n=30)	Non-obese group(n=20)	*p- value
age	7.95±1.2	8.25±1.0	NS
Sex(M/F)	(16/14)	(12/8)	
Weight (kg)	37.45±4.75	25.29±3.06	<0.001
Height (cm)	127.61±6.65	126.8±8.5	NS
BMI (kg/m ²)	25.46 ± 2.77	17.43 ±0.97	<0.001
SBP(mm Hg)	115.04±6.11	105.2±5.92	<0.05
DBP(mm Hg)	70±9	69.9 ±9.4	NS
Fasting glucose (mg/dl)	82.5 ±8.90	80.2 ±12.60	NS NS NS
Fasting insulin (□ U/ml)	18.36±3.28	10.99±2.07	<0.001
HOMA-IR	3.97±0.67	2.28±1.23	<0.001

BMI, body mass index; SBP, systolic blood pressure ; DBP, diastolic blood pressure, HOMA-IR, homeostasis model assessment for insulin resistance

*P values <0.05 were considered as significant.

*P values <0.001 were considered as highly significant.

*P values <0.0001 were considered as very highly significant.

Table (2): Comparison of Lipid profile and inflammatory biomarkers values between obese children & non-obese children

Parameters	Obese group	Non-obese group	p value
Total cholesterol (mg/dl)	175.23±17.30	169.13±12.40	NS
HDL-C (mg/dl)	30.93±3.50	49.47 ±4.68	<0.001
LDL-C (mg/dl)	109.17±12.05	90.4±10.77	<0.001
Triglycerides (mg/dl)	82.07±17.78	51.6±6.92	<0.001
CRP (mg/dl)	2.13±0.90	0.95±0.39	<0.001
IL- 6 (pg/ml)	46.52±7.17	12.77±2.94	<0.001
sICAM-1 (ng/ml)	267.28±31.00	231.01±20.13	<0.001

HDL-C, high-density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol; CRP, C- reactive protein ; IL- 6, Interleukin 6 and sICAM-1, soluble intercellular adhesion molecule-1

Table (3): Correlation involving insulin and HOMA-IR with BMI and lipid profile in obese children.

	Insulin		HOMA-IR	
	r	p	r	p
BMI	0.381	<0.05	0.445	<0.05
SBP	0.865	<0.0001	0.760	<0.001
HDL-C	-0.799	<0.0001	-0.638	<0.0001
LDL-C	0.435	<0.05	0.539	<0.001
Triglyceride	0.365	<0.05	0.551	<0.001

Table (4): Correlation of metabolic syndrome parameters with CRP, IL-6 and sICAM in obese children.

	CRP		IL-6		sICAM	
	r	p	r	p	r	p
BMI	0.412	<0.05	0.896	<0.0001	0.632	<0.001
SBP	0.021	NS	0.165	NS	0.494	<0.0001
HDL-C	-0.786	<0.0001	-0.259	NS	-0.396	<0.05
LDL-C	0.545	<0.001	0.244	NS	0.329	<0.001
Insulin	0.885	<0.0001	0.747	<0.0001	0.374	<0.05
HOMA-IR	0.691	<0.0001	0.842	<0.0001	0.666	<0.0001

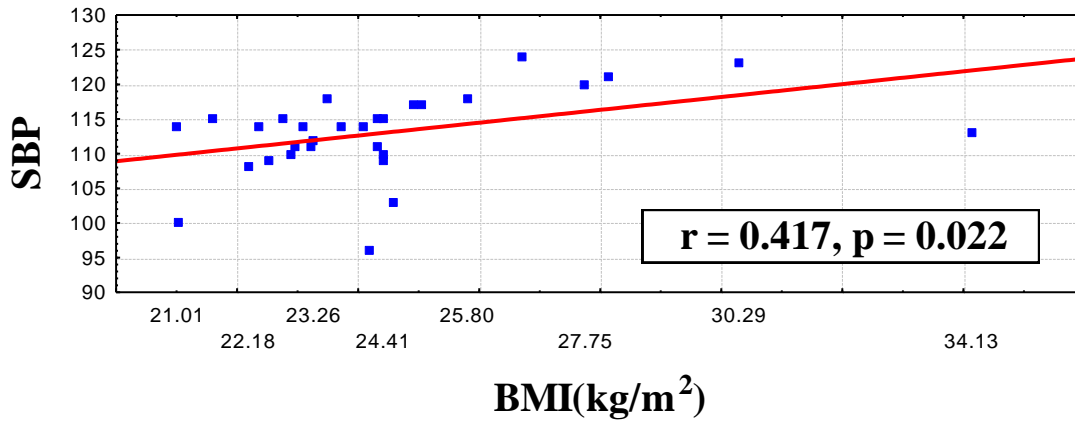


Figure 1 : Correlation between body mass index and systolic blood pressure in obese children

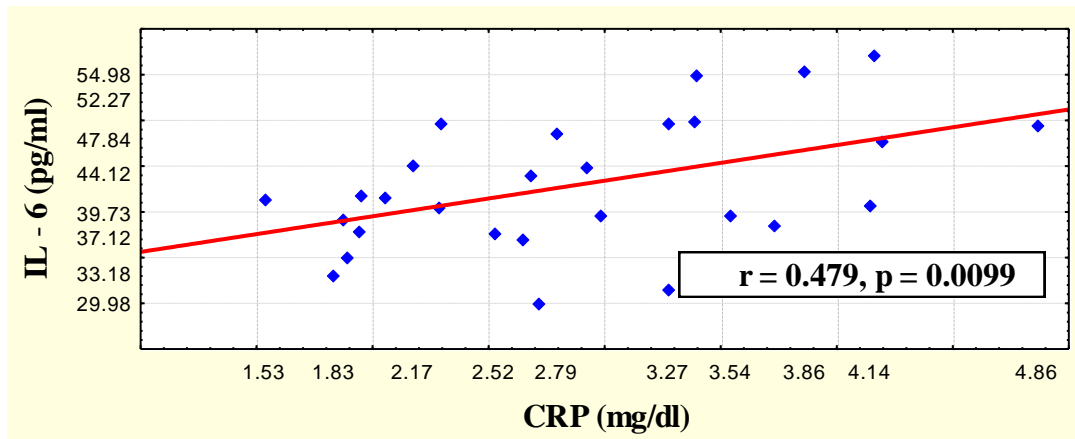


Figure 2: Serum CRP concentrations as a function of IL-6

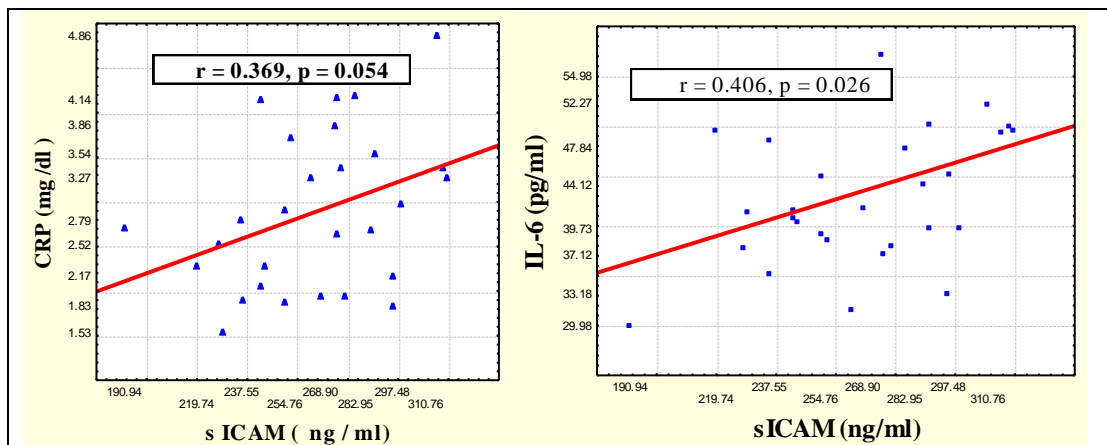


Figure 3: Serum sICAM-1 concentrations as a function of CRP in obese children.

IV. Discussion

Childhood obesity has grown at an alarming rate, and as it plays a central role in development of metabolic syndrome; it has been turned into a modern worldwide epidemic and has become a well-recognized risk factor of cardiovascular disorders and diabetes mellitus [26]. Hyperinsulinemia and insulin resistance have been involved in vascular reactivity and a growing evidence suggested the role of low grade inflammation as a link between obesity, insulin resistance and endothelial dysfunction [5]. Clustering of at least three of elevated blood glucose, obesity, high blood pressure, elevated triglycerides and low high density lipoprotein (HDL) cholesterol is defined as metabolic syndrome [15]. All of these are well established risk factors in the development of coronary artery disease [6].

This study showed a positive correlation between HOMA-IR and BMI with a highly significant difference in the two variables between obese and non-obese children. This finding validates that obesity represents a major risk factor for the development of insulin resistance in children and adolescents which can be an important link between obesity and the associated metabolic abnormalities and cardiovascular risk. Similarly Eckel et al. [27] and Morrison et al. [28] had found the same finding. Morrison et al. stated also that the interaction of BMI with HOMA-IR can open routes for intervention to reduce or even prevent the risk of metabolic syndrome later in early adulthood life [28]. Emphasizing that HOMA index is validated as a reliable measure of insulin sensitivity in children and that it is strongly associated with endothelial dysfunction and diabetes, Keskin et al. recommended to use it for identifying high-risk children and to take into consideration early preventive measures for diabetes prevention [29]. The early diagnosis and intervention for prepubertal children insulin resistance is very crucial as it might be further exacerbated by the influence of puberty, due to the physiological decrease in insulin sensitivity associated with normal pubertal development and thus leading to the greatest impairment in the parameters considered to be constituents of metabolic syndrome [30].

The presence of low-grade inflammation in obese children that appeared in this study was evaluated by measuring serum concentrations of CRP and IL-6, as both showed a highly significant difference compared to the non-obese children and a very strong positive correlation with each other and with HOMA-IR and BMI. In agreement with this Jiménez et al. [31], Sonya et al. [32], Olza et al. [33], and Valle et al. [34]; all affirmed that the levels of these inflammatory markers are elevated in prepubertal obese children and that are associated with insulin resistance with the subsequent possibility of development of diabetes and cardiovascular disease. Additionally, Oliveira et al. [35] agreed this finding and added that CRP is strongly related with metabolic syndrome and its components including insulin resistance in obese youth. Semiz et al. also approved this finding and stated that CRP correlated with BMI and BP which are risk factors for coronary heart disease, supporting the relationship between obesity, inflammation and atherosclerosis [36]. IL-6 is an inflammatory cytokine that stimulates the hepatic production of CRP; this can explain the state of inflammation associated with obesity, and could mediate, at least partially, obesity-related insulin resistance [37].

Increased circulating adhesion molecules in patients with obesity play an important role in the development of endothelial dysfunction/atherosclerosis [38]. Since sICAM is one of the molecules acting on the endothelium, therefore, elevated levels of sICAM are indicative of endothelial dysfunction, indicating enhanced leukocyte adhesion which is pathologically pivotal for atherogenesis [13]. Significantly elevated levels of sICAM-1 were found in the obese children of this study with positive correlation with BMI, SBP, HOMA-IR and LDL-C and with a negative correlation with HDL-C. A finding reported by Norata et al. agreed with this and declared that the metabolic syndrome components already altered in obese children may induce the expression of cell adhesion molecules, promoting atherogenesis and may precipitate acute atherothrombotic events [39]. The positive correlation noted here between sICAM levels and both fasting insulin levels and HOMA-IR denotes that insulin resistance has also been associated with endothelial dysfunction in obese patients.

Also, this study declared that sICAM-1 is positively correlated with both CRP and IL-6 indicating that inflammation is associated with endothelial dysfunction in obese children and suggesting that CRP, which appears to be a key sICAM regulator may be involved in this process [40]. It has been suggested that sICAM-mediated endothelial dysfunction is prompted by cytokines secreted in part by adipose tissue [41]. Valle et al. reported elevated sICAM and CRP in obese children, but they noted no correlation between the two [34]. On the other hand, Desideri et al. [42] reported an association between the two variables in obese children older than those studied here. Moreover, Jiménez et al. suggested that CRP, IL-6 and ICAM-1 are molecular markers associated with atherosclerosis and its progression [31].

The obese group in this study showed increased LDL-c levels and decreased HDL-c levels. This finding has been observed in children with central obesity; these changes are dangerous and they have been correlated with cardiovascular disease in the general population [43]. The correlation analysis in this study further revealed that insulin resistance, CRP and sICAM were positively correlated with LDL-c while negatively correlated with HDL-c. These findings are in accordance with Chang et al., and they highlighted that childhood weight should have special early attention because insulin resistance, inflammation and dyslipidemia increase the risk of cardiovascular disease in adults [44].

V. Conclusion

In conclusion, prepubertal obese children displayed alterations denoting inflammation and endothelial dysfunction, and also had several risk factors that may cluster together in different combinations defining metabolic syndrome. Thus, early identification of the inflammatory and endothelial biomarkers in obese children may assist in early interference to prevent progression and complications of cardiovascular disease and type 2 diabetes. Moreover, longitudinal studies are needed to recognize the prerequisite of giving anti-inflammatory drugs to reduce inflammation in the children who are proposed to be diabetics.

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