



Effect of Weight Loss on Markers of Inflammation and Endothelial Function in Childhood Obesity

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Abstract

Background: Obesity is associated with chronic low-grade inflammation and hyperinsulinism that may influence the progression of endothelial dysfunction and atherosclerosis already in childhood.

Methods: To study changes in metabolic profile and markers of inflammation and endothelial activation in children with primary severe obesity after weight loss we involved 14 obese children (Ob) that underwent a lifestyle intervention and 18 normal weighted subjects (C). In Ob, anthropometric data were assessed both at baseline and after intervention together with oral glucose tolerance test and fasting evaluation of cholesterol assessment, interleukin-6, endogenous secretory receptor of advanced glycation end products and endothelin levels.

Results: At baseline, serum IL-6 concentrations resulted significantly higher in Ob respect to C (12.96 ± 8.87 vs. 4.88 ± 1.19 pg/ml, $p < 0.05$). After weight loss, Ob significantly improved glucose metabolism and lipid assessment and they showed a significant reduction of all markers of inflammation and endothelial activation. In all subjects studied, BMI-SDS correlated positively with interleukin-6 ($r 0.45$, $p < 0.05$).

Conclusion: Our results demonstrated higher concentrations of inflammatory markers in obese children compared to healthy subjects. Nevertheless, an early lifestyle intervention could improve the levels of these molecules together with cholesterol and glucose metabolism and may reverse the development of premature endothelial dysfunction in obese children.

Keywords: Children; Inflammation markers; Life style intervention; Obesity; Weight loss

Introduction

Obesity is a major public health problem in industrialized countries [1]. A recent survey demonstrates that 21-24% of American children and adolescents are overweight and 16-18% obese [2]. A similar epidemic condition is undergoing in Italy, where overweight and obesity affect nearly 40% of children in Central and Southern regions [3]. Childhood and adolescent obesity is predictive of adult obesity, as previously documented [4]. Overweight and obesity are associated with hyperinsulinism and insulin resistance with an increased incidence of dyslipidemia, type 2-diabetes and cardiovascular disease already in childhood and adolescence; in particular, it has been shown that severe childhood obesity is independently associated with endothelial dysfunction and arterial wall thickening [5]. These pathological conditions can persist in adulthood regardless of the maintaining of excessive weight. Recent studies show that obesity is a status of chronic inflammation characterized by the production of cytokines by adipose cells [6]. It has been shown that adipose tissue cells (adipocytes and macrophages) secrete a cascade of pro-inflammatory cytokines, including interleukin-6 (IL-6), Tumor Necrosis Factor- α (TNF- α), interleukin-1 (IL-1) and interleukin 10 (IL-10), as well as reagents oxygen and endothelial adhesion molecules

with a paracrine and systemic effect. This up-regulation of endothelial adhesion molecules plays a basic role during the earliest phases of atherogenesis and it causes dysfunction and activation of endothelial cells, then promoting abnormal adhesion of leukocytes and platelets and forming the basis of atherosclerotic plaque [7,8]. This low-grade of persistent inflammation, associated with hyperinsulinemia may influence the progression of endothelial dysfunction and it could be responsible for the beginning of early obesity-associated atherosclerosis in childhood. Thus, obesity per se could promote atherosclerotic plaque formation since the earliest ages and it could be considered a strong predictor of coronary heart disease later in life [9]. The primary objective of the pediatrician must be to develop effective strategies to early identify and treat diseases related to obesity in children [10].

The aim of the present prospective and longitudinal study was to investigate the changes of endothelial adhesion molecules and inflammatory cytokines in severe obese patients before and after loss weight.

Materials and Methods

Study population

The study included Italian children and adolescents with severe obesity and normal weight subjects, matched according to age, gender

and pubertal stage, attending the Pediatric Metabolic Disorders Clinics of the University of Aquila and of the University of Modena and Reggio Emilia, Italy, recruited between January and September 2015.

Exclusion criteria consisted of concomitant endocrinological diseases and use of medications affecting insulin sensitivity or glucose/lipid metabolism.

Obese children underwent to a 1-year long lifestyle intervention: a gradual change in eating habits was proposed to the whole family (appropriate balance of nutrients and correct distribution of meals in 5 times of the day, avoiding snacks, and restoring importance of breakfast) and sedentary lifestyles were reduced increasing physical activity. The food portions were estimated through simple benchmarks in daily habits.

In Ob group, anthropometric data, biochemical parameters, serum concentration of IL-6, endothelin 1(ET-1) and endogenous secretory receptor of advanced glycation end products (esRAGE) were determined both at baseline (time 0) and after intervention (time 1).

Written informed consent for data collection was obtained from all subjects and parents at the moment of recruitment in the study and before the first data collection. The approval of the local Ethics Committees was obtained.

Body composition and definition of obesity

All patients underwent a complete clinical history and anthropometric measurements that were performed by well-trained examiners according to the Anthropometric Standardization Reference Manual. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Harpenden, Crymych; UK). Body weight was measured to the nearest 0.1 kg and body mass index (BMI) was calculated as weight in kg/height in meters squared and expressed as z-score with respect to chronological age using national growth charts (Cacciari 2006). Obesity was defined as BMI higher than the 95th percentile for age and sex, severe obesity when BMI was higher 99th percentile, whereas normal weight was defined as a BMI lower than the 85th percentile.

Laboratory measurements

Fasting lipid profile [total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG)] was evaluated using the commercially available kits for enzymatic test (Roche Diagnostics, Mannheim, Germany) which has international consensus values for plasma lipids, ensuring that magnitude of the potential difference is acceptable at 10%. The sensitivity of the TC, HDL-C, and LDL-C assay was 3 mg/ml. Intra and inter assay coefficients of variations (CVs) were less than 0.8% and 1.7%, respectively, for all examinations. The sensitivity of the TG assay was 4 mg/ml, while intra- and inter assay CVs were 1.5% and 1.8%, respectively.

Oral glucose tolerance test (OGTT) was performed at baseline and at the end after the beginning of the lifestyle intervention. The glucose load for OGTT was 1.75 g/Kg (maximum administered dose 75 g). The categorization of glucose tolerance status was made using the World Health Organization criteria.

Blood samples to measure inflammatory markers were obtained after a 12-h fasting period and centrifuged at 3000 g at 4°C for 10 min to obtain serum. Samples were frozen at -80°C until assayed.

ELISA (enzyme-linked immunosorbent assay) method was used to detect esRAGE (esRAGE ELISA Kit; B-Bridge International Inc., CA, USA) serum levels. The esRAGE concentrations were measured according to user manual and they were determined by interpolation of the regression curve formula. The sensitivity of the assay was 0.05 ng/ml. No significant cross-reactivity or interference was observed in these assays.

ET-1 serum concentrations were assayed using enzymatic kits (TiterZyme®, Assay Designs Inc., MI, USA). The sensitivity of the assay was 0.14 pg/ml. The intra assay CVs according to low, medium and high concentrations of ET-1 were 8.5%, 5.8%, and 4.6%, respectively; the inter assay CVs were 8.1%, 4.7%, and 3.1%, respectively.

IL-6 levels were determined by ELISA method using the Quantikine kit (R&D System), the minimum detectable concentration was 0.10 pg/ml and the interassay CV was 7.0%.

Statistical analysis

All normally distributed variables (BMI, z-score BMI) were expressed as mean ± SD. Data not normally distributed (TC, LDL-C, TG, HDL-C, glycaemia and insulin at baseline and at 120 minutes after OGTT, ET-1, IL-6, esRAGE) were log-transformed to approximate normality and expressed as mean ± SD. Between group comparison of anthropometric and biochemical data at time 0 were evaluated using Mann-Whitney's U-test. In Ob group, longitudinal changes of anthropometric, biochemical, and inflammatory data were analyzed using the Wilcoxon matched pair test. Spearman's correlation analysis was performed to assess the relationship between variables. The association between potential predictors and longitudinal changes of inflammatory markers was evaluated using the multiple linear regression model including Δ ET-1, Δ IL-6, and Δ esRAGE as dependent variables and anthropometric and biochemical data (with an association with the dependent variable in univariate analysis at 15% significance level) as independent variables. Statistical significance was inferred at a p value of <0.05.

Results

Baseline data

	Patients	Controls
Age (years)	11.00 ± 0.10	10.40 ± 1.64
BMI (kg/m ²)	28.53 ± 3.38	17.14 ± 2.34*
BMI SDS	2.90 ± 0.44	0.17 ± 0.91*
ET1 (pg/ml)	9.35 ± 4.57	6.13 ± 4.25
IL-6 (pg/ml)	12.96 ± 8.87	4.88 ± 1.19*
esRAGE (ng/ml)	0.37 ± 0.11	0.51 ± 0.28

Legend: *p<0.05 between groups

Table 1: Baseline features in study population.

Thirty two subjects entered the study: 14 children and adolescents with severe obesity [group Ob, 6 boys and 8 girls, aged 11.0 ± 0.10 years, body mass index (BMI)>99th percentile] and 18 normal weight subjects (group C, 9 boys and 9 girls, aged 10.4 ± 3.64 years, BMI <85th

percentile); baseline data of group Ob and group C patients are outlined in Table 1.

At time 0 IL-6 levels were significantly higher in Ob group than in C group (12.96 ± 8.87 vs. 4.88 ± 1.19 pg/ml, respectively; $p=0.002$; Table 1) while ET-1 and esRAGE concentrations were not significantly different.

Longitudinal data

All patients of Ob group were longitudinally evaluated at the end of the study.

Weight loss (Figure 1a) and the improvement of both glycaemic control and lipid profile were longitudinally demonstrated in our Ob group (Table 2). Specifically, the lifestyle intervention treatment from time 0 to time 1 significantly decreased z-score BMI (2.90 ± 0.44 vs. 2.22 ± 0.52 SDS, respectively; $p<0.001$), TG levels (90.92 ± 38.50 vs. 65.71 ± 17.72 mg/dl, respectively; $p<0.001$), both glycaemia (103.61 ± 7.80 vs. 86.91 ± 15.20 mg/dl, respectively; $p=0.005$) and insulin (84.77 ± 39.56 vs. 55.21 ± 24.50 microIU/ml, respectively; $p=0.01$.) at 120 minutes after OGTT. HDL-C values increased significantly from 40.78 ± 5.88 to 50.14 ± 15.67 mg/dl ($p=0.04$). The other parameters of lipid profile did not change despite intervention (Table 2).

	Time 0	Time 1
BMI (kg/m ²)	28.53 ± 3.38	$25.65 \pm 3.68^\circ$
BMI SDS	2.90 ± 0.44	$2.22 \pm 0.52^\circ$
CT (mg/dl)	160.92 ± 21.83	160.21 ± 19.02
LDL-C (mg/dl)	101.57 ± 23.45	99.71 ± 20.99
HDL-C (mg/dl)	40.78 ± 5.88	$50.14 \pm 15.67^\circ$
TG (mg/dl)	90.92 ± 38.50	$65.71 \pm 17.72^\circ$
OGTT test:		
Baseline glycaemia (mg/dl)	77.28 ± 8.52	75.85 ± 7.32
120 min glycaemia (mg/dl)	103.61 ± 14.44	$86.91 \pm 15.20^\circ$
Baseline insulin (uIU/ml)	17.92 ± 7.80	15.64 ± 7.81
120 min insulin (uIU/ml)	84.77 ± 39.56	$55.21 \pm 24.50^\circ$

Legend: $^\circ p < 0.05$ vs. Time 0

Table 2: Longitudinal analysis of anthropometric and biochemical data in obese patients.

Together with the previously described metabolic changes a significant decrease of inflammatory markers levels was found (Table 3, Figures 1b-1d). In particular, from time 0 to time 1, ET-1 (9.35 ± 4.57 vs. 5.46 ± 4.41 pg/ml; $p<0.001$) and IL-6 (12.96 ± 8.87 vs. 5.60 ± 4.91 pg/ml; $p<0.001$) concentrations were significantly decreased; moreover, a significant increase of esRAGE levels (0.37 ± 0.11 vs. 0.47 ± 0.17 ng/ml, $p<0.0001$) was demonstrated (Table 3). Figure 1 depicts the Δ of inflammatory markers (b, c, and d) along follow-up.

At baseline, in the total population, we found a significant and positive correlation between IL-6 concentrations and z-score BMI ($r=0.458$, $p<0.05$).

	Time 0	Time 1
ET1 (pg/ml)	9.35 ± 4.57	$5.46 \pm 4.41^\circ$
IL-6 (pg/ml)	12.96 ± 8.87	$5.60 \pm 4.91^\circ$
esRAGE (ng/ml)	0.37 ± 0.11	$0.47 \pm 0.17^\circ$

Legend: $^\circ p < 0.05$ vs. Time 0

Table 3: Longitudinal analysis of inflammatory markers in obese patients.

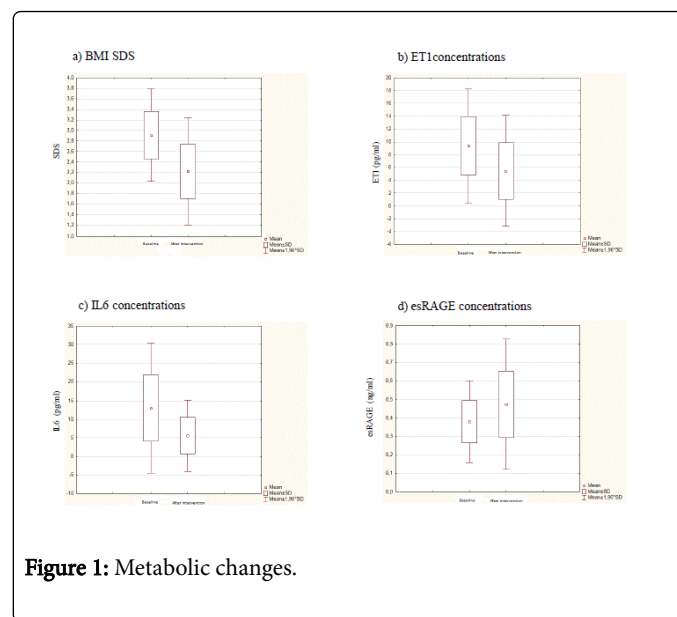


Figure 1: Metabolic changes.

No anthropometric and biochemical variable was identified as significant predictive factor for reduction of ET-1, IL-6 and increase of esRAGE in group Ob.

Discussion

In our study lifestyle modifications, including combined diet and moderate regular physical exercise, significantly reduced BMI and z-score BMI and improved insulin sensitivity and lipids profiles. Moreover, together with these metabolic changes, in our Ob group the serum levels of IL-6, ET-1, and esRAGE significantly improved along follow-up.

It has been already shown that IL-6 concentration, normally expressed in white adipose tissue and in peripheral blood mononuclear cells, increases progressively with adiposity [11] and, along with other pro-inflammatory cytokines, may substantially influence obesity development and metabolic disorders such as type 2 diabetes. IL-6 plays a significant role in energetic homeostasis and lipid-carbohydrate metabolism and it is an upstream inflammatory cytokine that plays a central role in propagating the downstream inflammatory pathway responsible for atherosclerosis. In our study, the decrease of IL-6 levels after weight loss indicates that its secretion by the adipocyte can be modulated by lifestyle changes.

The most potent vasoconstrictor peptide synthesized by the endothelial cells is ET-1 and it appears to play a central role in the pathophysiology of the vasomotor abnormalities associated with

endothelial dysfunction; moreover, it is involved in the progress of the atherosclerotic plaque [12]. It mediates host responses, including endothelial dysfunction, vasomotor contraction, leukocyte and platelet activation, and cellular proliferation. An increased activity of the ET-1 system probably may contribute to the abnormal vascular homeostasis in obese patients. Endothelial injury is the main stimulus for ET-1 secretion. Children and adolescents with obesity, hypertension or diabetes have higher ET-1 plasma concentrations than healthy subjects, and its level correlates with BMI, lipid parameters and systolic blood pressure [13]. Our data confirm published data but it is important to underlie how in our Ob subjects ET-1 levels significantly decreased after weight loss due to the only lifestyle intervention.

During these last years, both the soluble secretory receptors for advanced glycation end products (sRAGE) and the esRAGE were demonstrated to be involved in the pathogenesis of cardiovascular diseases [14]. sRAGE is a multi-ligand cell-surface protein, belonging to the immunoglobulin superfamily. RAGE interacts with other non-glycated peptide ligands, including S-100/calgranulin, amphoterin, amyloid fibrils and a leukocyte integrin, macrophage-1 antigen (Mac-1). Ligand engagement of RAGE leads to prolonged inflammation, and is also profoundly associated with macrovascular complications in diabetes due to the expression of pro-inflammatory mediators such as monocyte chemoattractant protein 1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1). Recently, numerous truncated forms of RAGE have been described; they are spliced variants of RAGE, which carry all of the extracellular domains but are lacking of the trans-membrane and intracytoplasmic domains. They bind ligands including advanced glycation end products (AGEs) and

can antagonize RAGE signaling in vitro and in vivo. A C-terminally truncated soluble form of RAGE has received much attention; it is now known to be present in human circulation and is named esRAGE. esRAGE neutralizes the effects of AGEs on endothelial cells in culture. Thus, the decoy function of esRAGE may involve a feedback mechanism by which esRAGE prevents the activation of RAGE signaling. It has also been suggested that some esRAGE isoforms that could act as decoy receptors, may be cleaved proteolytically from the native RAGE expressed on the cell surface [14,15].

In our population, we found a significant increase of esRAGE levels after the weight loss due to lifestyle treatment. The effectiveness of therapeutic lifestyle changes in improving or preventing the components of the metabolic syndrome has already been demonstrated in adults [16], but only few studies demonstrated that weight reduction resulted in an improvement of the metabolic syndrome and of the atherogenic risk-factor profile in children. A positive association between HOMA-IR, waist circumference (WC), lipid profile, and BMI was found [17], while the few studies evaluating the impact of weight loss on serum markers of systemic inflammation in obese children reported controversial results. In one study, the prevalence of metabolic syndrome decreased in adolescents if they followed a better overall Healthy Eating Index and if they trained more physical activities [18].

Furthermore, in particular, only few previous studies evaluated the impact of weight loss of markers of systemic inflammation levels in obese children (Table 4).

Authors	Molecules in study	Intervention	Age of patients (year)	Number of patients	Results
Gong I et al.	IL-6	1 year of lifestyle modification	7-11	326	Reduction of IL-6 conc.
D'adamo et al.	esRAGE	6 month lifestyle intervention with Vitamin E supplementation	8.3 ± 1.6 8.4 ± 1.3	42	Increased of esRAGE
Nemet et al.	IL-6	3 months dietary intervention and exercise program	10.4 ± 1.96	21	No change in IL-6
Roberts et al.	IL-6	2 week program of low-fat diet and daily exercise program	13.1 ± 0.5	19	Reduction of IL-6 conc.
Rynders et al.	IL-6	6 months of hypocaloric diet, exercise, and metformin	14.3 ± 2.4	16	Reduction of IL-6 conc.
Izadpanah et al.	IL-6	2 week residential lifestyle modification	13.0 ± 0.5	21	Reduction of IL-6 conc.
Roth et al.	IL-6	1 year lifestyle modification		29	No change in IL-6
Garanty-Bogacka et al.	IL-6	6-month hypocaloric diet and moderate physical activity	14.2 ± 2.6	50	Reduction of IL-6 conc.
Martos-Moreno et al.	IL-6	18 months of physical activity program and reorganization of eating habits	8.92 ± 1.80	51	Reduction of IL-6 conc.
Amati et al.	IL-6	6 month hypocaloric diet	8.9 ± 0.5	35	No change in IL-6
Balagopal et al.	IL-6	6 month lifestyle modification	adolescents	15	Reduction of IL-6 conc.

Gallistl et al.	IL-6	3 weeks hypocaloric diet and increase in physical activity	11.9 ± 1.8	49	Reduction of IL-6 conc.
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Table 4: Review of existing studies in pediatric population.

Among these studies, Gong et al. [19], Roberts et al. [20], Rynders et al. [21], Izadpanah et al. [22], Garanty-Bogacka et al. [23], Martos Moreno et al. [24], Balagopal et al. [25] and Gallistl et al. [26] showed similar change in IL-6 levels after decrease of BMI. On the other side, Nemet et al. [27], Roth et al. [28], and Amati et al. [29] found no significant change. D'Adamo et al. [30] recently demonstrated a significant increase of esRAGE in obese children after life style intervention combined with Vitamin E supplementation.

In two studies [20,21], a medical treatment was added to the diet. In five cases [19,21,24-26], the follow-up lasted only few weeks and it comprises a program of intensive physical activity and hypocaloric diet without including a gradual change in lifestyle intervention and eating habits.

To our best knowledge, no data on the role of ET-1 in children after weight loss is yet available, for these reasons our results are a new developments compared to previous literature.

In childhood, these kinds of programs need the inclusion of a multi-disciplinary team. Therefore, they are expensive and, thus, they could be usually conducted for a short period. In addition, most of the proven beneficial effects do not persist once the program is completed. Only in three published studies [17,19,24], the patients were reported to be follow for at least one year and the proposed programs was similar to the one we conducted in our study attempting to modify permanently lifestyle.

The main bias of our study is the small population sample reflecting the complexity to collect longitudinal data over a long-term follow-up period, especially among patients with severe obese that usually are not so compliant to new lifestyle suggestions. Since our study focus attention on IL-6, ET-1 and esRAGE, it could be expanded with evaluation of other chronic inflammation parameters.

Conclusion

Our study demonstrated that weight loss by lifestyle modifications can improve both low-grade inflammation status and insulin resistance in obese children. These changes appear long-term and persistent in children presenting a severe degree of obesity and experiencing a 1 years-long lifestyle intervention that includes dietary and behavioral treatment to achieve a gradual change in the eating habits of the whole family and a reduction of sedentary lifestyles.

In conclusion, we suggest that weight-reduction programme is necessary in pre-pubertal and pubertal obese children with abnormal glycemic and lipid profile as a prevention of metabolic syndrome later in life, since we demonstrated that a non-intensive long-term weight management program may significantly improve the degree of obesity together with glucose metabolism, abnormal lipid profile and some cardiovascular risk factor in childhood.

Informed Consent

Written informed consent for data collection was obtained from all subjects and parents at the moment of recruitment in the study and before the first data collection.

Ethical Considerations

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation.

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