Low-grade systemic inflammation, hypoadiponectinemia and a high concentration of leptin are present in very young obese children, and correlate with metabolic syndrome

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Summary

Objective: To determine the concentration levels of C-reactive protein (CRP), leptin and adiponectin in obese pre-pubertal children, and their possible relation with metabolic syndrome, fibrinogen and plasminogen activator inhibitor-1.

Methods: A study was carried out in 51 obese children (aged 6 to 9 years) and the same number of non-obese children (control group), matched by age and sex. (Cross-sectional study of obese children). Body mass index (BMI), waist/hip ratio (WHR) and blood pressure were determined for each child. Serum CRP, leptin, adiponectin, glucose, insulin, lipid profile, plasminogen activator inhibitor-1 (PAI-1) and fibrinogen were all measured.

Results: The levels of CRP serum (1.67 ± 0.222 vs 0.92 ± 0.16 mg/l) and leptin (15.56 ± 1.27 vs 4.68 ± 0.62 ng/ml) were significantly higher in obese children. The adiponectin level was significantly higher in non-obese children (11.58 ± 0.63 vs 9.64 ± 0.49 ng/dl). In the obese group, log. CRP showed a positive correlation with BMI, insulin, homeostasis model assessment (HOMA), triglycerides, alanine aminotransferase (ALT), uric acid, PAI-1, fibrinogen and interleukin 6 (IL-6), and correlated negatively with apolipoprotein A-I and high-density lipoprotein cholesterol (HDL-C). The leptin was positively correlated with BMI, insulin, HOMA, triglycerides and PAI-1 and negatively with Apo A-I and HDL-C. Adiponectin correlated negatively with BMI, insulin, HOMA, and triglycerides.

Conclusions: Low-grade systemic inflammation, elevated leptin concentration and low adiponectin level are described in very young obese children, correlating with a range of variables of metabolic syndrome. Inflammation and adipocytokines can play an important role in the etiopathogenesis of metabolic syndrome.

Key-words: C-reactive protein · Obesity · Children · Metabolic syndrome · Cardiovascular risk.

Résumé

Inflammation systémique de faible intensité, hypoadiponectinémie et hyperleptinémie sont observées chez de très jeunes enfants obèses, en corrélation avec le syndrome métabolique

Objectifs : Déterminer les concentrations de C-reactive protéine (CRP), leptine et adiponectine chez des enfants obèses prépubères, et leurs liens possibles avec le syndrome métabolique, le fibrinogène et le plasminogén activator inhibitor-1.

Méthodes : L’étude a été réalisée chez 51 enfants obèses (âgés de 6 à 9 ans) et un nombre identique d’enfants non obèses (groupe témoin), appariés selon âge et sexe. (Étude transversale d’enfants obèses). L’indice de masse corporelle (BMI), le ratio taille/hanche (WHR) et la pression artérielle ont été déterminés pour chaque enfant. Ont été mesurés : CRP sérique, leptine, adiponectine, glycémie, insulinémie, profil lipidique, PAI-1 et fibrinogène.

Résultats : Les concentrations plasmatiques de CRP (1,67 ± 0.222 vs 0.92 ± 0.16 mg/l) et de leptine (15,56 ± 1.27 vs 4,68 ± 0.62 ng/ml) étaient significativement plus élevées chez les enfants obèses. Les concentrations d’adiponectine étaient significativement plus hautes chez les enfants non obèses (11,58 ± 0.63 vs 9,64 ± 0.49 ng/dl). Dans le groupe obèse, le log. CRP était positivement corrélé avec BMI, insulinémie, l’homeostasis model assessment (HOMA), les triglycérides, l’alanine aminotransferase (ALT), l’acide urique, le PAI-1, le fibrinogène et l’interleukine 6 (IL-6), et négativement corrélé avec l’apolipoprotéine A-I et le cholestérol-HDL (HDL-C). La leptine était positivement corrélée avec le BMI, l’insulinémie, le HOMA, les triglycérides et le PAI-1, et négativement avec l’apo A-I et le HDL-C. L’adiponectine était corrélée négativement avec le BMI, l’insulinémie, le HOMA et les triglycérides.

Conclusions : Une inflammation systémique de faible intensité, des concentrations élevées de leptine et des concentrations basses d’adiponectine sont observées chez des enfants obèses très jeunes, en corrélation avec plusieurs variables du syndrome métabolique. L’inflammation et les adipocytokines peuvent jouer un rôle important dans l’étiopathogénie du syndrome métabolique.

Mots-clés : C-reactive protéine · Obésité · Enfants · Syndrome métabolique · Risque cardiovasculaire.
Obesity is a chronic pathology with a high morbidity-mortality rate which, due to eating habits and Western lifestyle, may become a veritable epidemic in coming years. It is associated with hypertension and various metabolic disorders such as high fasting glucose, dyslipemia, hyperuricemia, inappropriate fibrinolysis and hyperinsulinemia [1-4]. The syndrome known as metabolic syndrome (MS) is associated with high risk for diabetes and cardiovascular events [5, 6].

Obesity has been described as associated with a low degree of systemic inflammation and high plasma CRP concentrations [7, 8]. C-reactive protein is a prototypic marker of inflammation and has been shown in several prospective studies to predict cardiovascular events [9, 10]. A variety of components of MS are associated with increased CRP levels [7, 8, 11]. CRP levels also correlate with impaired fibrinolysis [12] and are an independent predictor of both incident diabetes and incident cardiovascular disease [13-16]. This measurement of CRP adds clinically important prognostic information to metabolic syndrome in adults [17].

Some of the disorders related to this syndrome have been described in children, suggesting that it may have a pre-pubertal origin [3, 18], including high levels of PAI-1, t-PA, fibrinogen and insulin [2].

Adiposity is the major determinant of CRP levels in children [19]. An increase of CRP is described in obese children. CRP correlated with several cardiovascular risk factors (fibrinogen, HDL cholesterol, systolic blood pressure) in children aged 10-11 years [19].

Adipose tissue secretes a variety of biologically active molecules, the adipocytokines, that interact with metabolic, endocrine and immune functions and may contribute to the development of obesity-related disorders [20]. These adipocytokines include leptin and adiponectin.

Adiponectin is an anti-inflammatory and antiatherogenic hormone synthesized in adipose tissue [21]. In adults and children, the plasma adiponectin concentration negatively correlates with the degree of body fat and insulin resistance [22-24]. Adiponectin is considered to be an important factor in the pathogenesis of metabolic and cardiovascular disease [25, 26].

Obesity is frequently associated with high plasma leptin concentrations and leptin resistance. The concentration of leptin in serum is correlated with insulin resistance and metabolic syndrome [27-29] and it has been shown as an independent risk factor for coronary heart disease [30].

The strong relationship between CRP and adipocytokines with these cardiovascular risk factors suggests a role for inflammation and the secretional products of adipose tissue in the development of atherosclerosis and cardiovascular disease.

The aim of this study is to analyze the CRP, leptin and adiponectin values in obese pre-pubertal children (6 to 9 years), and the possible correlation of these values with the concentration of PAI-1, fibrinogen and the various components of metabolic syndrome.

Material and methods

Subjects

A case-control study was carried out in obese children of both sexes. One group was formed of 51 obese children [body mass index (BMI) over percentile 90 in growth curves for the study population [31], and another with the same number of non-obese children paired by age and sex (aged 6-9 years), as the control group. All subjects were at Tanner stage 1.

Several schools in the area were informed of the study to be carried out. All parents submitted written consent, and the study was authorized by the ethical-investigation committee of our hospital.

Children with primary hyperlipidemia, hypertension, diabetes or glucose intolerance, and secondary obesity were excluded from the study. Any child receiving pharmacological treatment was also excluded. All children had similar lifestyles, with no significant physical training program.

Blood sampling and analysis

Blood samples were collected after 12 hours of fasting from a vein in the antecubital fossa, without venous occlusion. All collections were made between 8:00 and 9:00 am. Whole blood specimens were collected in different tubes to obtain serum and plasma. The samples were separated in aliquots and frozen immediately at -45°C until determination could be performed.

The C-reactive protein concentration was determined for all the children, as well as adiponectin, leptin and different variables related to metabolic cardiovascular syndrome (insulin, lipids, blood pressure, hydrocarbonate metabolism, hemostasis, uric acid).

Glucose, uric acid, alanine aminotransferase (ALT), cholesterol and triglycerides (TG) were determined in a random access analyzer (Axon, Bayer Diagnostics, Tarrytown, NY, USA) with Bayer Diagnostics reagents. Homeostasis model assessment (HOMA) was used to detect the degree of insulin resistance. The resistance can be assessed from the fasting glucose and insulin concentrations by the formula: resistance (HOMA) = [insulin (mU/l) x glucose (mmol/l)]/22.5.

High-density lipoprotein cholesterol (HDL-C) was determined after precipitation of chylomicrons, very low-density lipoproteins and low-density lipoproteins, with phosphotungstic acid and magnesium ions. The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [32].

Insulin was quantified by a microparticle immunoassay (IMx system Insulin, Abbott Laboratories, Chicago, IL, USA) in an IMx automatic analyzer (Abbott Laboratories).
Apolipoprotein A-I (Apo A-I), apolipoprotein B (Apo B) and C-reactive protein were measured by nephelometry (N Antisera to Human Apolipoprotein A-I, Apolipoprotein B and N High Sensitivity CRP reagent, Behringwerke AG, Marburg, Germany) in a Dade Behring Analyzer II Nephelometer, with an interassay coefficient of variation (CV) of 3.1%, 3.8% and 3.4% for concentrations of 0.5, 1.1 and 2.1 mg/l.

Antigenic immunoassay methods were used for the quantification of plasminogen activator inhibitor-1 (PAI-1) (Asserachrom PAI-1, Stago Diagnoses, Asnieres-Southern-Seine, France), tissue-plasminogen activator (t-PA) (Coliza t-PA; Chromogenix, Mölndal, Sweden), IL-6 (Quantikine human IL-6, RD systems, Wiesbaden-Nordenstadt, Germany), leptin (Quantikine human leptin, RD systems, Wiesbaden-Nordenstadt, Germany) and adiponectin (Quantikine human adiponectin, RD systems, Wiesbaden-Nordenstadt, Germany) carried out in a microtiter plate analyzer (Labotech, Cormédica, Barcelona, Spain). The fibrinogen was measured by quantitative assay using thrombin in an automatic analyzer (Electra 1600, Ortho Clinical Diagnostics, Madrid, Spain).

**Anthropometric measurements**

Weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. Body mass index was calculated as weight (kg)/height (m)². Waist circumferences were measured at the level of the umbilicus, and hip circumferences at the level of greater trochanters and pubic symphysis to the nearest 0.1 cm.

**Blood pressure**

The blood pressure of all the children was measured with a mercury sphygmomanometer (Pymah Corporation, Sommerville, NJ, USA) after 20 minutes of rest and in a supine position. One measure was taken on each of three days, and the mean was calculated.

**Statistical analysis**

Statistical assessment was conducted 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 ± SEM, 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.

In both groups (obese and non-obese), we determined the percentage of children with values of CRP < 1, 1 to 3, and > 3 mg/l, indicating low, moderate, and high risk, even when applied to those already defined as having metabolic syndrome [20].

The detection limit for CRP was 0.175 mg/l, and in the statistical evaluation all values < 0.175 mg/l were treated as 0.10 mg/l. Additional statistical analyses were performed excluding individuals with CRP levels > 10 mg/l, indicating clinically relevant inflammatory conditions, but this did not alter our results. One obese child was eliminated for this reason.

Correlation between variables was evaluated using Pearson’s correlation coefficient and regression analysis. CRP concentrations were skewed and were transformed onto a logarithmic scale before 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 I* shows the anthropometric data of both groups. The median age was 7.64 (obese) and 7.74 (control), with a range of 6-9 years.

*Table III* shows the results from the control and obese children, both male and female.

Four obese and 3 non-obese children were excluded from the study (abnormal values).

**C-reactive protein and metabolic syndrome**

Mean C-reactive protein concentration was significantly higher in the obese children, at 1.67 mg/l (95% CI

**Table I**

<table>
<thead>
<tr>
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<th>Control n = 51</th>
<th>Obese n = 51</th>
<th>p =</th>
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</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>20/31</td>
<td>20/31</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>7.74 ± 0.12</td>
<td>7.63 ± 0.14</td>
<td>0.547</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>16.75 ± 0.19</td>
<td>22.29 ± 0.29</td>
<td>&lt; 0.001</td>
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<tr>
<td>WHR</td>
<td>0.834 ± 0.005</td>
<td>0.851 ± 0.006</td>
<td>0.031</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.79 ± 0.041</td>
<td>4.81 ± 0.042</td>
<td>0.730</td>
</tr>
<tr>
<td>Insulin (mU/mL)</td>
<td>6.06 ± 0.34</td>
<td>8.32 ± 0.58</td>
<td>0.001</td>
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<tr>
<td>HOMA</td>
<td>1.29 ± 0.073</td>
<td>1.80 ± 0.114</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.92 ± 0.222</td>
<td>1.67 ± 0.16</td>
<td>0.008</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.81 ± 0.242</td>
<td>2.32 ± 0.236</td>
<td>0.134</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>4.68 ± 0.62</td>
<td>15.56 ± 1.27</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adiponectin (mg/dl)</td>
<td>11.58 ± 0.631</td>
<td>9.64 ± 0.487</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Value are mean ± SEM; BMI = body mass index; WHR = waist/hip ratio, HOMA = homeostasis model assessment; CRP = C-reactive protein, IL-6 = interleukin-6.
Adipocytokines and metabolic syndrome

Mean plasma leptin concentration was significantly higher in the obese children, at 15.56 ng/ml (95% CI 12.96-18.16 ng/ml), in comparison to the control group, at 4.68 ng/ml (95% CI 3.37-5.99 Tab I). In the obese and control group, sex-related differences in plasma leptin and adiponectin concentration were evaluated, with no significant differences found for these variables (Tab III).

Tab V shows the single linear correlation of leptin, adiponectin and adiponectin/leptin ratio with metabolic parameters. In the obese group, the study with multivariant regression analysis showed (corrected for age and sex) that plasma leptin is an independent predictive factor for basal insulin (R = 0.4165; p < 0.001), HOMA (R = 0.4125; p < 0.001), BMI (R = 0.4081; p < 0.001), triglycerides (R = 0.1814; p = 0.024) and PAI-1 (R = 0.2273; p = 0.008).

Adiponectin level was significantly higher in non-obese children 11.58 ng/ml (95% CI 10.12-13.04) in comparison to the obese group 9.64 ng/ml (95% CI 8.51-10.77). Tab III shows sex-related differences in plasma adiponectin concentration.

In the obese group, corrected for age and sex, plasma adiponectin concentration is an independent predictive factor for basal insulin (R = 0.2683; p = 0.0107) and HOMA (R = 0.2547; p = 0.0132).

Discussion

Elevated serum levels of acute-phase proteins, indicating chronic subclinical inflammation, have been associated with cardiovascular disease and metabolic syndrome. High leptin and hypoadiponectinemia may contribute to insulin resistance and accelerated atherogenesis associated with obesity [22, 29, 30, 33]. This work describes hypoadiponectinemia and high levels of leptin and CRP in very young obese (vs non-obese children of the same age and sex), which correlate significantly with the main variables of metabolic syndrome.

Obesity is a chronic pathology with high morbidity-mortality rates, frequently associated with various metabolic disorders (metabolic syndrome) [1-4]. The syndrome is associated with high CRP levels in a systemic low-grade inflammatory state [7].

Metabolic syndrome has also been described in children [3, 18]. As well as the disorders traditionally related with this syndrome, we have described (in obese children in the same age group as those studied here, and in comparison with non-obese children) significantly high levels of PAI-1, t-PA, fibrinogen, insulin and leptin [2]. Patients with metabolic syndrome are at an increased risk of diabetes and cardiovascular events [5, 6].

Obesity is associated with high plasma CRP and leptin concentrations, hypoadiponectinemia and a variety of MS components are associated with these parameters [7, 8, 24, 28, 29].

1.348-1.997), in comparison with the control group, at 0.92 mg/l (95% CI 0.88-1.356) (Tab I). In the obese group, 33.3%, 51.8% and 14.9% of children had values of CRP < 1, 1 to 3, and > 3 mg/l, respectively. For the non-obese group these percentages were 79.6%, 11.1% and 9.3% respectively.

Comparison between the biochemical parameters related to metabolic syndrome is summarized in (Tab II).

In the single linear correlation, for the obese group, log. CRP was positively correlated with insulin (r = 0.2963; p = 0.049) and BMI (r = 0.4483; p = 0.001), but not with the rest of the variables analyzed.

Using multivariant regression analysis, in the obese group, corrected for age and sex, log. CRP is an independent predictive factor for triglycerides (P partial = 0.015), HDL-C (P partial = 0.008), Apo A-I (P partial = 0.046), ALT (P partial = 0.001), uric acid (P partial = 0.009), PAI-1 (P partial = 0.008) and t-PA (P partial = 0.002).

In the control group, log. CRP correlates with insulin (r = 0.2965; p = 0.049) and BMI (r = 0.4483; p = 0.001), but not with the rest of the variables analyzed.

<table>
<thead>
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<th>Table II</th>
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<tr>
<td>Selected biochemical parameters related to metabolic syndrome.</td>
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<td></td>
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<tr>
<td>Cholesterol (mg/dl)</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
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<tr>
<td>Apolipoprotein A-I (mg/dl)</td>
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<td>Apolipoprotein B (mg/dl)</td>
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<tr>
<td>HDLcholesterol (mg/dl)</td>
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<tr>
<td>SBP (mmHg)</td>
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<tr>
<td>DBP (mmHg)</td>
</tr>
<tr>
<td>ALT (U/l)</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
</tr>
<tr>
<td>t-PA (ng/ml)</td>
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<tr>
<td>Fibrinogen (g/l)</td>
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</table>

Value are mean ± SEM; SBP = systolic blood pressure; DBP = diastolic blood pressure; ALT = alanine aminotransferase; PAI-1 = plasminogen activator inhibitor-1; t-PA = tissue-plasminogen activator.
C-reactive protein is a prototypic marker of inflammation, and has been shown in several prospective studies to predict cardiovascular events [9, 10], although recent data indicate that CRP is a relatively moderate predictor of coronary heart disease [34].

In children aged 8 to 16 years, obesity is associated with higher CRP levels [35]. Other works have described this association, in a wide series (6 to 18 years), without finding differences relating to age or sex (for the method used, the lower detection limit for CRP was 3.0 mg/l, with the authors assigning a value of 2.1 mg/l to values below the detection limit) [36].

In this work we describe significantly high levels of CRP in obese pre-pubertal children (mean 1.67 mg/l) with regard to non-obese children (mean 0.92 mg/l), we found no differences by sex for this age (the detection limit for CRP was 0.175 mg/l). The elevated CRP concentrations in obese subjects might be explained by the expression of the cytokine interleukin-6 in adipose tissue [37] and its release into the circulation [38]. Interleukin-6 is a proinflammatory cytokine that stimulates the production of CRP in the liver [39].

In both the obese and non-obese groups that we studied, CRP correlated positively with BMI. Furthermore, in obese children CRP was significantly associated with both waist/
hip ratio and IL-6 concentration, which suggests the participation of adipose tissue in this metabolic disorder, even from prepubescent ages.

For some authors, CRP levels of < 1, 1 to 3, and > 3 mg/l correspond to low-, moderate- and high-risk groups for future cardiovascular events [17]. In accordance with these criteria, in the group of obese children studied here, 14.9% are at a high risk, and 51.8% a moderate risk of developing future cardiovascular events, whereas in non-obese children the risk is low in 79.6% of cases.

In adult subjects, various components of MS [7, 8] are associated with high CRP levels. In pre-pubertal children, we found similar data to those described in adults. For this age group (6-9 years), CRP was positively correlated with triglycerides, fasting insulin OMA and uric acid, as well as with BMI and waist/hip ratio, and negatively with HDL-C and Apo-A.

The CRP levels also correlated positively with the concentration of PAI-1. Plasminogen activator inhibitor-1 levels have shown to correlate with many variables of metabolic syndrome [40, 41], and CRP levels are significantly increased in patients with features of metabolic syndrome [42, 43].

CRP induces PAI-1 expression and activity in humans [44], and both variables are associated with the development of atherosclerosis and type-2 diabetes [12]. Equally, we found a significant correlation between CRP and fibrinogen.

Adiponectin, is an anti-inflammatory and antiatherogenic hormone exclusively synthesized in adipose tissue [21], with lower levels reported in obese subjects [22-24, 45]. It is considered to be an important factor in the pathogenesis of metabolic and cardiovascular disease [25, 26]. In our results, adiponectin was significantly lower in the obese children, correlating with both the BMI and the degree of resistance to insulin (HOMA). As described by other authors [24], we found no differences by sex for this age.

Obesity is frequently associated with high plasma leptin concentrations. The concentration of leptin in serum is correlated with insulin resistance and metabolic syndrome [27-29] and it has been shown as an independent risk factor for coronary heart disease [30]. The obese children we studied showed significantly higher levels of leptin, which were significantly associated with different MS variables. No sex-related differences were found.

The adiponectin-to-leptin ratio also correlated with different metabolic syndrome variables. For some authors, the adiponectin-to-leptin ratio may provide additional help in differentiating between type 1 and type 2 diabetes [46].

The strong relationships between CRP levels and metabolic syndrome, fibrinolysis and coagulation in obese children suggest that inflammation plays a role in the development of this syndrome. The production of proinflammatory cytokine by the adipose tissue (IL-6), together with other adipocytokines as leptin and adiponectin, may play a part in the origin of metabolic syndrome.

Resistance to insulin and hyperinsulinism, which are associated with obesity, may be responsible for the increased production of cytokines [47]. Chronic insulin increase, described in obesity, favours an increase in leptin. In our case, both basal insulin and the HOMA ratio correlated significantly with the concentration of C-reactive protein, leptin and adiponectin.

Low-grade systemic inflammation, decrease of anti-inflammatory factors and antiatherogenic (adiponectin) and elevated leptin concentration are described in very young obese children, and correlates with a range of variables of metabolic syndrome.

Inflammation and adipocytokines can play an important role in the etiopathogenesis of metabolic syndrome. Measuring CRP, leptin and adiponectin, even at pre-pubertal ages, may provide clinical information on the definition of metabolic syndrome and add important prognostic information in terms of future cardiovascular risk.

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References


