New factors of cardiometabolic risk in severely obese children: influence of pubertal status

P. Codoñer-Franch1,2, R. Murria-Estal3, M. Tortajada-Girbés3, C. del Castillo-Villaescusa1 y V. Valls-Bellés2, E. Alonso-Iglesias4

1Department of Pediatrics, Dr Peset University Hospital, Valencia, Spain. 2Department of Pediatrics, Obstetrics and Gynecology, Faculty of Medicine and Odontology, University of Valencia, Valencia, Spain. 3Clinical Biochemistry Laboratory, Dr Peset University Hospital, Valencia, Spain. 4Department of Biochemistry and Molecular Biology, Faculty of Medicine and Odontology, University of Valencia, Valencia, Spain.

Abstract

The aim of this prospective study was to evaluate the utility of new biochemical markers to assess cardiometabolic risk in severely obese children and adolescents. A total of 107 subjects aged 7 to 14 years, were clinically assessed and anthropometric measures and percentage of fat mass by single frequency bioimpedance analysis were recorded. Of these, 44 were non-overweight and 63 severely obese (body mass index Z-score >2.5) which were stratified by Tanner stages. To estimate the metabolic risk the following variables were considered for analysis: Waist circumference/height >0.5, fasting glucose >100 mg/dL, triglycerides >110 mg/dL, HDL-C <40 mg/dL, and systolic or diastolic blood pressure >95th percentile for age and gender. Fasting insulinemia, apoprotein A1 and B, high-sensitive C-reactive protein, alanine aminotransferase, homocysteine, and folic and uric acids were determined. In severely obese children, metabolic risk was present more frequently in mid puberty. The normalized anthropometric parameters with respect to 50th percentile for age and gender did not differ in the presence of metabolic risk. Insulin resistance was an independent determinant of metabolic risk, adjusted by Tanner stages. Elevated high-sensitive C-reactive protein was noted without any effect of metabolic risk or pubertal stage. Homocysteine, apoprotein B, and alanine aminotransferase values increased with metabolic risk and were not influenced by puberty. Although insulin resistance remains the main factor influencing metabolic risk, biochemical markers as homocysteine, apoprotein B, and alanine aminotransferase, may be useful for identif-

Correspondence: P. Codoñer-Franch.
Departamento de Pediatría.
Hospital Universitario Dr. Peset.
Avenida Gaspar Aguilar, 90.
46017 Valencia.
e-mail: pilar.codoner@uv.es

Abbreviations

ALT: Alanine aminotransferase.
BMI: Body mass index.
CVD: Cardiovascular disease.
Hcys: Homocysteine.
HDL-C: High-density lipoprotein cholesterol.
hs-CRP: High-sensitivity C-reactive protein.
HOMA-IR: Homeostasis model assessment index.
LDL-C: Low-density lipoprotein cholesterol.
MetS: Metabolic syndrome.
MRFs: Metabolic risk factors

Introduction

Obesity has an adverse impact on health populations that is a consequence of comorbid conditions. Its presence in childhood significantly increases the risk of adult obesity and the development of related morbidities that ultimately lead to atherosclerosis and cardiovascular disease (CVD). Although the clinical manifestations of atherosclerosis occur in adulthood, the process begins in childhood and is accelerated in the presence of metabolic risk factors (MRFs).

Metabolic syndrome (MetS), a constellation of anthropometric, physiologic, and biochemical abnormalities, is a well-established risk factor for CVD in adults. However, uniform and objective MetS criteria do not exist for the youth. In fact, many different MetS criteria have been employed in children and adolescents, and it has been suggested that MetS cannot be diagnosed at all in children under 10 years old. Moreover, MetS develops in stages, and components such as anthropometric variables and visceral obesity, blood pressure, lipid levels, and insulin sensitivity may change with age and pubertal development. In addition to the previously known risk factors to CVD, new factors have emerged, such as inflammatory markers [high-sensitivity C-reactive protein (hs-CRP)] and plasma elevation of the sulphur amino acid homocysteine (Hcys). Although these factors have been implicated in the development of complications, data in pediatric patients remain scarce.

Concerning the methodology for identification of metabolic risk in obese children candidates to intensive preventive measures some debate exists. The present study was conducted with the aim of providing new insights on the clinical tools to evaluate the metabolic risk in young severely obese subjects. We analyzed known factors implicated in MetS such as hypertension, dyslipidemia, and impaired glucose tolerance, in addition to hypothesized new factors related to cardiovascular risk such as hs-CRP, Hcys, apoproteins A1 and B, alanine aminotransferase (ALT), folic acid, and uric acid. The results were also examined regarding pubertal stage in order to identify the influence of puberty.

Subjects and methods

Subjects

This prospective study was conducted on 107 children and adolescents (58 boys), aged from 7 to 14 years. Of these, 44 were non-obese (19 boys) who were recruited by primary care physicians as healthy controls and who gave their consent to participate in the study. The other 63 children (39 boys) were suffering from severe obesity [body mass index (BMI) >2.5 standard deviations standardized by age and gender, according to Spanish BMI data] and were referred to our pediatric endocrinology outpatient clinic for investigation and treatment of their obesity. All subjects were Caucasian and of Spanish origin. None had chronic or hereditary diseases, or endocrinologic disorders. Infectious and/or inflammatory illness was ruled out by medical histories and physical examinations. Pubertal stage was assessed in each patient by the same pediatrician according to the criteria of Marshall and Tanner, by means of inspection and palpation. All the subjects were in stage ≤3, and female subjects had not yet begun menstruating. We considered the following groups: prepuberty (stage 1), early puberty (stage 2), and mid-puberty (stage 3). Written informed consent was obtained from all parents, and oral consent from all children. The study was approved by the Ethical Committee of the University Hospital Dr Peset (Valencia, Spain).

Measurements

Weight and height measurements were taken with the child lightly dressed and barefoot by standardized methods. The degree of obesity was determined using the BMI Z-score calculated with the lambda, mu, sigma (LMS) method. The fat mass percentage was obtained via bioelectrical impedance using the BC-418MA Tanita Segmental Body Composition Analyzer (Tanita Europe BV, Hoofddorp, The Netherlands). Waist circumference (WC) was obtained over...
the unclothed abdomen at the narrowest point between the rib cage and the superior border of the iliac crest. Hip circumference was measured over light clothing at the level of the widest diameter around the buttocks using a non-elastic flexible tape, and measurements were recorded to the nearest 0.1 cm. Anthropometric measures were converted to normalized parameters compared to the 50th standard percentile for age and gender for the fat mass percentage (relative fat mass)*, and for WC (relative WC)*. We also used the indices WC/hip circumference and WC/height for analysis.

Blood pressure was measured using an automated sphygmomanometer (Dinamp 200; GE medical Systems Information Technologies, Inc., Milwaukee, Wisconsin, USA). To find the age-specific height percentile level for each case, the Spanish growth curves were used*. Elevated blood pressure (≥95th percentile for height) was determined using tables provided by the Task Force Report†.

Laboratory procedures

After overnight fasting, blood samples were taken from the antecubital vein. Biochemical characterization tests (performed by the Clinical Biochemistry Laboratory of Dr. Peset University Hospital) included serum glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, uric acid, and alanine aminotransferase (ALT), and were measured using direct methods (Aeroset System®). Insulin determination, Hcys, and folic acid were measured using the automated electrolyoluminescence immunoassay (Architect c8000®), both (Architect c800® and the Aeroset System®) from Abbott Clinical Chemistry (Wiesbaden, Germany). We used the homeostasis model assessment index (HOMA-IR) to determine insulin resistance by employing the following formula: fasting insulin levels (IU/L) × fasting glucose (mmol/L) / 22.5. hs-CRP and apoproteins A1 and B were measured using kinetic nephelometry (Immage Nephelometer®, Beckman Coulter Inc. Brea, California, U.S.A).

Definitions

To estimate the metabolic risk the same risk factor variables that were used for the adult definition were included*. Thus, there were considered for analysis: WC/height >0.5, fasting glucose >100 mg/dL, triglycerides >110 mg/dL, HDL-C <40 mg/dL, and systolic or diastolic blood pressure >95th percentile for age and gender.

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). Differences among groups were assessed by the Kruskal-Wallis one-way analysis of variance and post hoc comparisons using the Dunn test correction. Values were expressed as the mean ± standard deviation. Categorical variables were analyzed using the Chi-square test. Correlations between variables were evaluated by calculating Spearman’s correlation coefficients. Significantly correlated variables as determined by the univariate analysis were included in the multivariate regression analysis using a stepwise method. Logistic regression was used to examine the relationship between ≥3 MRFs and different variables. A P-value < 0.05 was considered to be significant.

Results

Table I presents the descriptive characteristics of the 107 subjects enrolled in the study. The average age of obese children with a higher number of MRFs was significantly older than the other groups. All obese subjects presented with abdominal obesity (WC >90th percentile and WC/height >0.5), and 78% of them had a BMI Z-score >3. The presence of MRFs was not different with respect to the anthropometric characteristics of the obese subjects, when normalized by the relation to the 50th percentile. The prevalence of elevated blood pressure (Z-score) was significantly different between children with ≥3 MRFs (68%) and children with <3 MRFs (22%). Dyslipidemia and significant differences in the level of apoproteins (A1 and B) were only noted in the group with ≥3 MRFs. Fasting insulin and HOMA-IR results were higher in obese children and significant differences were found after categorizing the subjects according the presence of MRFs; subjects with ≥3 MRFs displayed showed the highest levels of both variables. Fasting glucose was only significantly higher in the group of subjects with ≥3 MRFs, 45.2% of them had values greater than 100 mg/dL. Variance analyses revealed that the obese children had higher levels of hs-CRP than the non-obese children, but there were no significant differences between the groups with regard to their metabolic risk. Concentrations of Hcys and ALT were elevated significantly in the group of severely obese children with ≥3 MRFs, as compared to the non-obese children. Folic acid was significantly lower in obese subjects with metabolic risk as compared to those without metabolic risk and to the non-obese children. Uric acid increases in all severely obese children, particularly in the group with ≥3 MRFs.

Severely obese subjects (n = 63) were divided into three groups according the pubertal Tanner stage for comparison by analysis of variance (Table II). The prevalence of subjects with ≥3 MRFs was higher for subjects at Tanner stage 3 (70.8%) in comparison to stage 2 (35.0%) or stage 1 (36.8%) subjects. The mean BMI Z-score was not different among the three Tanner stage groups considered, and a similar result was found for all normalized anthropometric variables measured and blood pressure. Modifications in lipid profiles were
observed; in mid-puberty, HDL-C, apoprotein A1, and LDL-C levels were significantly lower and triglyceride levels were significantly higher. Fasting glucose was not modified, but hyperinsulinism was found in subjects in early or mid-puberty. Consequently, the HOMA-IR index was elevated, mainly in adolescents at Tanner stage 3. Variance analysis did not show any significant differences in the concentrations of hs-CRP and Hcys in children stratified according to Tanner stage. In contrast, folic acid was lower in adolescents in mid-puberty. Higher uric acid levels were observed in subjects from early to mid puberty.

A correlation analysis on the new variables studied (Hcys, hs-CRP, ALT, and folic and uric acid) that could affect the metabolic risk was performed in the 63 severely obese subjects. No associations were found between these factors and normalized anthropometric variables or blood pressure levels in this group of subjects. The Hcys concentration was positively correlated with HOMA-IR (r = 0.39, P < 0.01) and uric acid (r = 0.29, P < 0.05), and negatively with folic acid (r = -0.44, P < 0.001). Uric acid levels and HOMA were also positively related (r = 0.43, P < 0.001). The hs-CRP, and ALT values were not associated with any anthropometric or biochemical variables. A multivariate regression analysis using the stepwise method with Hcys as the dependent variable, and clinical, anthropometric and biochemical parameters (age, BMI Z-score, relative WC, SBP Z-score, DBP Z-score, HDL-C, triglycerides, HOMA-IR, hs-CRP, and folic acid) as independent variables was performed. The only significant variables that remain related to Hcys levels were folic acid (β = -0.36, t = -3.03, P = 0.004) and HOMA-IR (β = 0.25, t = 2.06, P = 0.044) with a determination coefficient adjusted of R² = 0.26 (P < 0.0001).

To examine the relationship between metabolic risk and puberty (Tanner stage), anthropometric (relative WC), and biochemical (HOMA-IR, folic acid, ALT, Hcys) parameters, a binary logistic regression analysis using the criterion of <3 or ≥3 MRFs as the dependent variable was performed. The HOMA-IR was the only independent variable related to the presence of metabolic risk (OR 1.562, IC 95% 1.196 to 2.040, P < 0.001), irrespective of pubertal stage.

Discussion

Obesity in children is associated with increased cardiovascular risk, which may be clinically sympto-
matic later in life. Identification of severely obese children at higher risk for co-morbidities in a clinical context poses a significant challenge for pediatricians in order to initiate vigorous preventive measures, including the use of medication or, in certain instances, solely in teenagers, the bariatric surgery. In the present study, both known MRFs and newer factors implicated in CVD risk were assessed in a group of severely obese children and adolescents and compared with findings obtained in a group of non-overweight subjects. The results were also examined in terms of pubertal status because physiological modifications that occur in this period can influence the biochemical parameters.

The BMI is generally used to evaluate obese subjects, and it is considered a reliable indicator of adiposity. However, the use of this index alone has some limitations in children because the relationship between the fat, and fat-free mass varies at different ages. In this sense, some studies suggest that the WC is superior to the BMI for explaining obesity-related health risks as it quantifies abdominally accumulated fat, but it must be remembered that the WC is related to both subcutaneous abdominal fat and intra-abdominal fat. Other measures such as the WC/height and WC/hip circumference ratios have also been used to indicate abdominal obesity, and it has been emphasized that these parameters are more reliable because they are not age-dependent. In the current study, nearly 78% of the obese subjects had a BMI Z-score >3, and all presented with abdominal obesity (WC percentile >90th, with a WC/height >0.5); therefore, they exhibited a theoretically high metabolic risk. However, we did not found differences between these anthropometric measures, when normalized through conversion to a Z-score or relative to the 50th percentile for age and gender of normal weight children, or WC indices, in the obese children which exhibited <3 or ≥3 traditional MRFs. In the same way, MRFs in logistic regression were not related to anthropometry in the presence of other factors. Thus, the anthropometric parameters do not allow us the identification of specific children with higher risk for comorbidities in the case of severe obesity.

Obesity is related to adverse blood lipid profiles with low HDL-C and high triglyceride levels. Most subjects with atherogenic dyslipidemia also have higher levels of apoprotein B and lower concentrations of apoprotein A1. The concentrations of these apoproteins may be correlated with the development of atheroma more than their equivalent lipoproteins. We have found that puberty caused changes with de-

<table>
<thead>
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<th>Table II</th>
<th>Clinical and biochemical data of 63 subjects with severe obesity (BMI Z-score ≥ 2.5) distributed according to pubertal status</th>
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<tbody>
<tr>
<td></td>
<td>Prepuberty (n = 19)</td>
</tr>
<tr>
<td>Gender (boys/girls)</td>
<td>11/8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>8.1 ± 1.1</td>
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<tr>
<td>MRFs (≥3/&lt;3)</td>
<td>7/12</td>
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<tr>
<td>BMI Z-score</td>
<td>4.02 ± 1.13</td>
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<tr>
<td>Relative fat mass (%)</td>
<td>194.7 ± 36.0</td>
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<tr>
<td>Relative WC (%)</td>
<td>139.7 ± 14.8</td>
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<tr>
<td>WC/hip</td>
<td>0.95 ± 0.07</td>
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<tr>
<td>WC/height</td>
<td>0.61 ± 0.06</td>
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<tr>
<td>SBP Z-score</td>
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<tr>
<td>DBP Z-score</td>
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<td>Total cholesterol (mg/dL)</td>
<td>182.3 ± 36.7</td>
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<tr>
<td>HDL-C (mg/dL)</td>
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<tr>
<td>LDL-C (mg/dL)</td>
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<td>Triglycerides (mg/dL)</td>
<td>83.8 ± 25.2</td>
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<tr>
<td>APOE</td>
<td>131.1 ± 27.7</td>
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<tr>
<td>APOB</td>
<td>85.5 ± 23.3</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>95.1 ± 6.5</td>
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<tr>
<td>Insulin (µIU/mL)</td>
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<tr>
<td>HOMA-IR index</td>
<td>3.42 ± 1.86</td>
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<td>hs-CRP (mg/L)</td>
<td>3.31 ± 2.33</td>
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<tr>
<td>Uric acid (mg/dL)</td>
<td>3.74 ± 0.54</td>
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<tr>
<td>Homocysteine (µmol/L)</td>
<td>7.01 ± 1.69</td>
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<tr>
<td>Folic acid (ng/mL)</td>
<td>9.54 ± 3.95</td>
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<tr>
<td>ALT (U/L)</td>
<td>22.8 ± 11.1</td>
</tr>
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</table>

BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; hs-CRP: high-sensitivity C-reactive protein; ALT: alanine aminotransferase; MRFs: metabolic risk factors. Values are means ± standard deviation. (*) statistically different versus prepuberty subject, (†) statistically different versus prepuberty subjects, and early puberty subjects.
crease in HDL-C, LDL-C, and apoprotein A1, and increase in TG levels, but not in the levels of apoprotein B. Thus, apoprotein B may be an important discriminatory factor to evaluate metabolic risk in pubertal subjects.

Abnormal fasting glucose, a classical metabolic risk factor, is scarcely evident among children. In the present study, however, 14 of 31 (45.2%) children with ≥3 MRFs had abnormal fasting glucose levels. Levels of insulin and the surrogate marker of insulin resistance, HOMA-IR, were different significantly between non-obese and obese subjects, the highest levels were related to an increased number of MRFs. With respect to pubertal stages, there was an increase in insulin levels and in HOMA-IR values (between Tanner stages 1 and 2) that remained stable through Tanner stage 3. Puberty causes a decrease in insulin sensitivity simultaneously with a complementary increase in insulin secretion\(^1\), and the majority of children (71%) with ≥3 MRFs were in mid puberty, which complicates the assessment of the role of each condition (puberty or obesity) on the metabolic risk; however, in the logistic regression analysis, insulin resistance as determined by HOMA-IR was the main determinant of metabolic risk, independent of Tanner stage. This finding is consistent with other studies\(^1\), highlighting the importance of insulin resistance in MetS. Therefore, severe obese children are a population particularly prone to the metabolic risks when they reach the pubertal age, because there is a synergy of the metabolic changes occurring naturally during puberty and the insulin resistance linked to obesity.

Aside from the classical related MRFs, there have been new risk factors proposed that may provide new insight into the pathogenesis of CVD\(^1\). Obesity is associated with inflammation which plays an important role in atherosclerosis. In this way, hs-CRP measurement could aid to refine risk assessment. In our study we found that obese children have an increased level of hs-CRP than non-obese that was different according to the presence of ≥3 classic cardiovascular risk factors, in contrast with the data of Soriano-Guillén et al.\(^1\). One possible explanation for this discrepancy is that the metabolic derangement could be more advanced in that study. Anyway, the measurement of hs-CRP seems to have little value as an early discriminating factor for the identification of severe obese children prone to metabolic risk. Moreover, we found no differences in hs-CRP levels with respect to pubertal stage, implying that puberty does not influence the values.

Uric acid levels were elevated in obese patients, mainly in those with high metabolic risk. However, there was a progressive increase in levels throughout the pubertal development. Recently, a study in prepubertal children shows the association of this parameter with some factors of metabolic risk, mainly hypertension, adverse lipid blood profile, and insulin resistance\(^1\). Undoubtedly, this biochemical parameter may be a reliable indicator of an early disorder.

An elevated plasma Hcys level has recently been established as a casual independent factor for thrombosis and vascular disease\(^1\). In the present study, obese subjects with ≥3 MRFs had significant higher Hcys levels as compared to non-obese subjects. Similarly to the data of other studies\(^1\), Hcys concentrations were not related to indices for obesity (BMI, fat mass or other anthropometric variables studied). The absence of an influence of the Tanner pubertal stage on Hcys levels suggests the utility of this parameter as a metabolic risk marker in this period. We also found a significant positive correlation of Hcys levels with HOMA-IR, similar to other studies\(^1\). Both are factors that point to the risk of complications, and have a great utility in the evaluation of severe obese children. It is of note that Hcys levels can be influenced by genetic and environmental factors such as dietary folate. The decrease of plasma folate levels in the group of obese children (with MRFs and in mid-puberty) deserves further attention. It is not likely that these children eat an unbalanced diet, as they eat fruits or vegetables every day. Nevertheless, it is possible that there was an increase in body’s requirements due to accelerated growth\(^1\). This is a factor that should be kept in mind, as suboptimal levels of folate favor the accumulation of Hcys and could increase cardiovascular risk in these subjects. Also, similarly to other studies\(^1\), ALT elevation that can underlie hepatic steatosis in obese subjects is associated with features of metabolic risk. This is another factor not influenced by pubertal stage.

We must recognize that there is at least one limitation to our study: the relatively reduced number of subjects may be considered a limitation of the ability to generalize the data to the entire population. However, the sample was consisted only of children with severe obesity and was very homogeneous with regard to lifestyle and social condition. On the other hand, a major strength of our study was that it was conducted in obese subjects because risk factors may be increased in this period.

In conclusion, is necessary to take into account the fact that in children and adolescents, the characteristics and consequences of obesity are also influenced by growth and maturation. Although metabolic risk factors increase with increasing Tanner stage, insulin resistance remains as an independent determinant of metabolic risk. Biochemical markers as apoprotein B, Hcys, and ALT levels were not influenced by puberty and can identify the subjects prone to complications. Special attention should be given to obese pubescent subjects because risk factors may be increased in this period.

**References**

Metabolic risk factors and pubertal status


