Steatosis and Insulin Resistance: Prevalence and Association With Cardiovascular Risk in Obese Adolescents

Sofia Laura Rodrigues Sousa, Ines Margarida Araujo Soares Costa Tomada Marques, Emidio Santos Carreiro, Carla Maria Barreto da Silva de Sousa Rego

Abstract

Background: Steatosis is strongly associated with obesity, even in childhood. Insulin resistance (IR) is one of the comorbidities of pediatric obesity, associated with cardiovascular risk (CVR). The goals of this study were to characterize steatosis and IR presence in the context of pediatric obesity and to assess the connection between them and with CVR markers.

Methods: It was a retrospective study including 184 adolescents (10.6 ± 1.9 years) with primary obesity. All data refer to the first evaluation. Based on a study protocol (www.gneiop.pt), anthropometric parameters, body composition (InBody™), lipid profile, basal glucose and insulin, homeostatic model assessment of insulin resistance (HOMA-IR) and liver ultrasonography were collected. The value of P ≤ 0.05 was considered significant.

Results: It is a young population with a considerable obesity (BMI z score = 2.29 ± 0.76). From those who were evaluated, one-third present steatosis or IR. Regardless of gender and chronologic age, there is a significant positive association between IR and cardiovascular risk factor (CVRF) occurrence. IR is associated with a probability of more than three times and with a relative risk 2.5 times higher for the aggregation of two or more CVRFs (P = 0.014).

Conclusion: A non-negligible prevalence of steatosis and of IR was observed in obese young adolescents. IR and steatosis are associated with an increased aggregation of CVRF. The presence of IR, but not of steatosis, shows a strong predictability concerning the risk of aggregation of two or more CVRFs. For this reason, it should be part of the clinical assessment of the obese adolescent.

Keywords: Adolescents; Cardiovascular risk factors; Hepatic steatosis; Insulin resistance; Obesity

Introduction

Obesity is a metabolic and energetic disorder, where excessive storage of energy as triglycerides in the fatty tissue occurs [1]. According to the World Health Organization (WHO), obesity is considered a chronic disease, whose occurrence has been increasing in the last years and has presently reached epidemic proportions, not only in adulthood but also in childhood [2]. Actually, according to the report of the International Obesity Task Force (IOTF) - Childhood Obesity Group, 155 million people of the world population aged between 5 and 17 years old are overweight, of whom 30 - 40 million are obese (2-3%) [3]. Child obesity has also been increasing in Europe, with a stronger incidence in Mediterranean countries such as Portugal than in northern and central Europe (COSI, SPEO, and EPACI) [4].

Together with this growing obesity epidemic, there has been a significant increase in obesity-related comorbidities, mainly non-alcoholic fatty liver disease (NAFLD), insulin resistance (IR), diabetes mellitus type 2 (DM2) and cardiovascular disease (CVD) [5].

The NAFLD is a chronic liver disease that has gained interest in the last years and is associated with the presence of obesity in 90% of the cases, even in children and adolescents. It is characterized by the accumulation of fat in the liver greater than 5% of its weight, in the absence of chronic alcohol consumption and other causes of liver pathology [6-8]. NAFLD was described for the first time in 1980 by Ludwig and collaborators when analyzing adult patients who presented a histological situation of alcoholic hepatitis, in spite of having no previous history of alcohol ingestion [9]. Steatosis is the most benign form of NAFLD, and its true incidence and prevalence are unknown, depending on the population studied and on the definition used, being estimated a world prevalence that varies from 6.3% to 33%, with an average of 20% [10]. In the pediatric population, its prevalence varied from 2.6% to 9.8% in different studies and this number increases to 70-80% among obese individuals [11]. The most serious and clinically significant form of NAFLD is the non-alcoholic steatohepatitis (NASH), less common and with a prevalence of 15-20% [12]. In the absence of treatment, it is possible to observe a progression from NAFLD to NASH, cirrhosis and hepatic...
Steatosis and IR

From children and adolescents with overweight or primary obesity followed in a reference outpatient obesity clinic (n = 418), inclusion criteria were taken into account the following parameters: chronological age ≥ 10 years old and the simultaneous existence of nutritional status evaluation, biochemical markers and blood pressure (n = 184).

Study protocol

All data are reported to the first evaluation, based on a previously defined evaluation protocol (www.gneiop.pt). Among several parameters that are in the protocol, the items that follow were considered.

Anthropometric parameters and nutritional status characterization

Data related to weight, stature and waist circumference were collected and evaluated according to the internationally recommended methodologies and technics [26]. BMI values were calculated (kilograms per square-meter) and z score values for BMI (BMI z score) were obtained with WHO AnthroPlus® software (WHO anthro). Excess weight and obesity were defined as a z score value ≥ 1.0 (85th percentile) and 1.6 (95th percentile) respectively [27, 28]. Waist-to-height ratio (WtHR) was calculated and a value higher than 0.5 was defined as predictor of increased cardiometabolic risk [29].

Body composition: total body fat

Body composition was characterized using InBody®. Total body fat was collected and the results are presented in body fat mass percentage (%BF).

Blood pressure

According to the recommendations set by the American Heart Association and by the British Hypertension Society [30, 31], the blood pressure values were determined by the oscillometric method, resorting to Dinamap Criticon® [32]. High-normal blood pressure was defined for systolic blood pressure (SBP) and diastolic blood pressure (DBP) values between the 90th and 95th percentiles and HBP was considered for values higher than 95th percentile [32].

Biologic parameters assessment

Blood samples were collected by venipuncture in EDTA containing tubes after an overnight fasting (8 - 10 h) and processed within 2 h after collection. The hepatocyte function was determined through the dosage of transaminases (ALT/TGO and AST/TGP). It was considered hepatocyte dysfunction in the presence of higher values to the maximum limit of reference for the laboratory (AST > 37 U/L and ALT > 65 U/L).
In order to characterize the lipid profile, the dosages of TG, total cholesterol (TC) and HDL and LDL cholesterol (HDL-c and LDL-c) were included. CVR was defined based on the 95th percentile for the TG (≥ 150 mg/dL), TC (≥ 200 mg/dL) and LDL-c (≥ 130 mg/dL) and on the 5th percentile for the HDL-c (≤ 35 mg/dL) [33].

As for the characterization of the glucose and insulin metabolism, baseline values of glucose < 100 mg/dL were considered normal, being the “impaired fasting glucose” (IFG) defined for the glucose values ≥ 100 and < 120 mg/dL and the DM2 for the glucose values ≥ 126 mg/dL in fasting. The IR was evaluated resorting to the homeostatic model assessment of insulin resistance (HOMA-IR) [34], being considered normal with an HOMA-IR value between 1.21 and 1.45, normal to high with a value between 1.46 and 2.60 and insulin resistant with values > 2.61 [35].

### Cardiometabolic risk classification

Values of TC or LDL-c or of TG higher than the 95th percentile, HDL-c lower than the 5th percentile, SBP or DBP higher than the 95th percentile, BMI or LDL-c or of TG higher than the 95th percentile, HDL-c lower than the 5th percentile, SBP or DBP higher than the 95th percentile and the presence of IFG or DM2 or HOMA-IR values ≥ 2.61 were considered for the cardiometabolic risk classification.

### Statistical analysis

Statistical analysis was conducted resorting to the software Statistical Package for the Social Sciences (SPSS®) versao 20 para Windows®. The comparative analysis between the different population groups bearing in mind the age group and gender was made through the variance analysis (ANOVA). The associations between anthropometric variables and biochemical parameters were tested by linear regression models, having been adjusted to chronologic age and gender variables. The significance value considered was of 5%.

### Results

This is a retrospective cross-sectional study that includes 184 children and adolescents with chronological ages between 10 and 20 years. The characteristics of the study population according to gender and age group were described in Table 1.

<table>
<thead>
<tr>
<th>Total (n = 184)</th>
<th>Male (n = 77)</th>
<th>Female (n = 107)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronologic age (years)</td>
<td>12.6 ± 1.9 (11.0 - 13.8)</td>
<td>12.4 ± 1.6 (11.1 - 13.5)</td>
<td>12.8 ± 2.1 (10.9 - 14.1)</td>
</tr>
<tr>
<td>BMI z score</td>
<td>2.29 ± 0.76 (1.78 - 2.68)</td>
<td>2.53 ± 0.79 (2.12 - 2.90)</td>
<td>2.12 ± 0.69 (1.64 - 2.53)</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>38.2 ± 7.7 (33.0 - 43.2)</td>
<td>37.0 ± 8.4 (31.3 - 42.9)</td>
<td>39.1 ± 7.0 (34.8 - 43.3)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90.0 ± 11.4 (82.5 - 96.0)</td>
<td>92.1 ± 12.0 (83.5 - 97.0)</td>
<td>88.4 ± 10.4 (88.1 - 95.4)</td>
</tr>
<tr>
<td>WtHR</td>
<td>0.59 ± 0.09 (0.54 - 0.61)</td>
<td>0.60 ± 0.10 (0.54 - 0.62)</td>
<td>0.59 ± 0.09 (0.54 - 0.61)</td>
</tr>
</tbody>
</table>

**BMI: body mass index; WtHR: weight-to-height ratio; HDL-c: high-density lipoprotein-cholesterol; TC: total cholesterol; LDL-c: low-density lipoprotein-cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotrasferase. Data are expressed in average and standard deviation for variables with symmetric distribution and in median for variables with asymmetric distribution (†). In both cases, values of the first and third quartiles (25th percentile and 75th percentile, P25-P75) are presented. *Statistical significant value for a level of significance of 5%. **Statistical significant value for a level of significance of 1%.**
and 18 years (12.6 ± 1.9), from which 107 are female (58%). All data report to the first assessment and are based on a study protocol (www.gneiop.pt).

Table 1 describes the characterization of the population. It is a young population with a considerable obesity scope (BMI z score = 2.29 ± 0.76), significantly higher in males (BMI z score < 0.001) who also register a higher deposition of intra-abdominal fat (P = 0.041), a more unfavorable TC/HDL-c ratio (P = 0.034) and a higher value of SBP (P < 0.001).

No cases of IGF, IGT and of DM2 were registered.

The prevalence of overweight and obesity, IR (HOMA-IR) and liver steatosis can be seen in Table 2. It is important to refer that HOMA-IR was only calculated in 59.8% (n = 110) and then ultrasound for hepatic morphology evaluation was done in 53.3% (n = 98) of all population. It should be noted that two-thirds of the population is obese and that more than one-third of those who were evaluated present with steatosis or IR.

The study of the association between IR and hepatic steatosis with the antropometric variables, the nutritional status and the biochemical parameters can be observed in Table 3. Regardless of gender and chronologic age, it can be observed that there is an association between basal insulin value (P = 0.006), WtHR (P = 0.033) and TG (P = 0.004) and the occurrence of IR, while higher glucose values (P = 0.026) and lower HDL-c values (P = 0.001) were independently associated with the risk of steatosis.

The occurrence of steatosis and IR based on the presence and aggregation of CVRF can be observed in Table 4. It is necessary to refer that only 12% (n = 22) of the studied children/adolescents did not show any CVRF and, even in these, four presented with steatosis. Moreover, we observe an increase in the occurrence of steatosis and IR in the dependence of the presence of only one or if aggregation of two or more CVRFs is observed.

In Table 5, we can observe the influence of steatosis or IR in the probability (OR) and the relative risk (RR) of CVRF aggregation. The presence of IR is associated with a probability of more than three times and to an RR 2.5 times higher for the aggregation of two or more CVRFs (P = 0.014). It is worth referring that if WtHR is included in the model, the RR of this association stands but the probability of its occurrence goes up to 30.4 (P < 0.001) (data not presented).

**Discussion**

Obesity is considered the most prevalent nutritional disease worldwide and is defined by WHO as the 21st century epi-
demic. A trend between pediatric and adult obesity was described and the concern with its high prevalence is heightened by the acknowledged association of obesity with an early risk of cardiometabolic disease, compromising life quality and expectancy.

Literature has documented an association between the presence of visceral adiposity and the increase in the prevalence of steatosis and IR, both considered risk factors for the development of CVD.

The aims of this work were to study in what way the prevalence of steatosis and IR in obese children and adolescents can be associated with CVR markers.

The studied population (n = 184) is a young adolescent population (chronologic age = 12.6 ± 1.9 years old) and not only does it present a severe obesity (z score IMC: 2.29 ± 0.76) but also a high cardiometabolic risk (WtHR: 0.59 ± 0.09) (Table 1). The male gender shows a higher prevalence (79.2%) (Table 2), a higher magnitude of obesity (z score BMI, P < 0.001) and of central adiposity (waist circumference, P = 0.041) (Table 1). Besides total adiposity, the elective deposition of intra-abdominal fat has been a more and more used and valued marker for the definition of cardiometabolic comorbidity risk of obesity, being the cutoff point of 0.50 for the reason WtHR adopted in the definition of cardiometabolic comorbidity occurrence in early age [4].

The fact that the female gender presents a higher total adiposity (%fat mass), though with no statistic meaning (Table 1), can be explained by the sexual dimorphism of puberty, characterized by a higher relative percentage of fat mass in the girl. It should be noted the presence of significantly higher values of SBP for the male gender, probably dependent of the higher magnitude of obesity registered (Table 1).

The worldwide increase of the prevalence of DM2 in children and adolescents, paralleled with the increase of prevalence of child obesity, is consensual [4]. As it has widely been described in literature, the fasting glucose levels are not a good marker of the alteration of glucose/insulin metabolism in pediatric age [4, 37] a fact that can be corroborated in our population where neither a value higher than 100 mg/dL (Table 1) nor any case of IFG and IGT is registered (non-presented data).

It should be assumed, that, more than the magnitude of obesity, it will take time for the “diabetic march” to translate itself by alterations of these two fasting markers [4] and the young age of our population can justify the absence of results. However, 42 from the 110 assessed adolescents (38.2%) present IR criteria (Table 2). Although the HOMA-IR calculation has only been carried out in the course of the clinical evaluation for about half (59.8%) of the assessed population and, for this reason, this prevalence should be carefully interpreted, it actually

Table 4. CVRF Aggregation in the Total Population and by Gender, the Presence of Insulin Resistance and of Hepatic Steatosis

<table>
<thead>
<tr>
<th>CVRF</th>
<th>Without CVRF, n (%)</th>
<th>1 CVRF, n (%)</th>
<th>≥ 2 CVRF, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population total (n = 184)</td>
<td>22 (12.0)</td>
<td>78 (42.4)</td>
<td>84 (45.7)</td>
</tr>
<tr>
<td>Male gender (n = 77)</td>
<td>7 (9.1)</td>
<td>30 (39.0)</td>
<td>40 (51.9)</td>
</tr>
<tr>
<td>Female gender (n = 107)</td>
<td>15 (14.0)</td>
<td>48 (44.9)</td>
<td>44 (41.1)</td>
</tr>
<tr>
<td>Steatosis (n = 32)</td>
<td>4 (12.5)</td>
<td>9 (28.1)</td>
<td>19 (59.4)</td>
</tr>
<tr>
<td>Insulin resistance (n = 42)</td>
<td>0</td>
<td>2 (4.8)</td>
<td>40 (95.2)</td>
</tr>
<tr>
<td>Steatosis + insulin resistance (n = 9)</td>
<td>0</td>
<td>0</td>
<td>9 (100.0)</td>
</tr>
</tbody>
</table>

Data are presented in number of cases and respective percentage for the total of assessed cases. It was considered CVRF, regardless of the nutrition status, the following parameters: waist-to-height ratio > 0.5; total cholesterol ≥ 200 mg/dL; HDL-c ≤ 35 mg/dL; LDL-c ≥ 130 mg/dL; triglycerides ≥ 150 mg/dL; systolic or diastolic blood pressure > 95th percentile.

Table 5. Probability (OR) and Relative Risk (RR) of the Presence of Cardiovascular Risk Factors (Glucose Metabolism, Lipidic Profile and Blood Pressure) Excluding Waist to Height Ratio, According to the presence of steatosis and Insulin Resistance

<table>
<thead>
<tr>
<th>Presence of two or more cardiovascular risk factors</th>
<th>Chi-square (P-value)</th>
<th>OR (95% CI)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis (n = 7)</td>
<td>0.188 (P = 0.665)</td>
<td>1.26 (0.44 - 3.58)</td>
<td>1.20 (0.52 - 2.76)</td>
</tr>
<tr>
<td>Insulin resistance (n = 12)</td>
<td>6.070 (P = 0.014*)</td>
<td>3.49 (1.25 - 9.76)*</td>
<td>2.77 (1.18 - 6.49)*</td>
</tr>
<tr>
<td>Steatosis and insulin resistance (n = 4)</td>
<td>3.255 (P = 0.071)</td>
<td>3.60 (0.84 - 15.44)</td>
<td>2.44 (1.00 - 5.96)</td>
</tr>
<tr>
<td>Steatosis or insulin resistance (n = 15)</td>
<td>3.934 (P = 0.047*)</td>
<td>2.61 (0.99 - 6.91)</td>
<td>2.24 (0.98 - 5.14)</td>
</tr>
</tbody>
</table>

*Statistically significant value.
points to a prevalence of about one-third in this population of young adolescents. In the meantime, the positive association registered between the obesity magnitude (z score BMI) and the visceral adiposity (WtHR) allows inferring the increase of the occurrence of IR in the dependence of the increase of these somatic markers, particularly of the central adiposity as well as an increase in triglyceridemia (Table 3). In effect, higher adiposity is associated with a lower peripheral sensitivity (smooth muscle) to insulin, while fat visceral deposition results in an increase of lipidic rate, leading to a higher amount of free fatty acids in the bloodstream, inducing IR [38].

Finally, a higher aggregation of cardiometabolic risk factor (CMRF) in the presence of IR (Table 4) is observed, being its presence responsible for the 3.49 times probability of occurrence of CMRF with a predictability of 2.77 (P = 0.014) (Table 5). The evaluation of IR based on the HOMA-IR, by the predictability in defining RR of CMRF, should be part of obese adolescents follow-up as well as be seen as an alert sign relatively to future risk of cardiometabolic pathology.

As far as steatosis is concerned and although its research has occurred only in about half the population studied (n = 98, 53.3%), a prevalence of 32.7% (n = 32) was registered for this subsample (Table 2). As referred for the HOMA-IR, not all the adolescents did an ultrasound throughout the clinical follow-up of their obesity, seeing that these exams are not part of the initial assessment protocol of obesity in pediatric age in our country. Even though steatosis is silent in its early stage, the clinical evolution of NAFLD translates itself through the gradual increase of hepatic enzymes (ALT and AST). From the 116 (63%) adolescents with hepatic profile assessment, 7.8% and 4.3% presented high values of AST and ALT, respectively, with predominance in the male gender. From the 80 individuals with ultrasound and hepatic profile, the individuals with steatosis (36%) presented a higher increase of hepatic enzymes, working as indicators of fat liver (non-presented data). Associations with statistical significance between the presence of steatosis and somatic risk factors, or blood pressure, or IR were not registered. A positive association with hipo-HDL (P < 0.011) was only found (Table 3).

However, the presence of steatosis is associated with an increase of the aggregation of CMRF (Table 4), showing a probability for its aggregation of 1.26 and an RR of 1.2 (P = 0.014) (Table 5), lower than those of IR. One can postulate that, in pediatric age, the process of liver fat infiltration will be latter than the occurrence of IR, presenting a lower sensitivity to predict the aggregation risk of CMRF. Nevertheless, it should be pointed out that if one considers the presence of IR and steatosis simultaneously, the total of the adolescents studied present aggregation of two or more CMRFs (Table 4), even though the predictability and the RR do not present statistical significance (Table 5).

**Conclusion**

The results of the study suggest a non-negligible prevalence (about one-third) of steatosis and of IR in obese young adolescents, registering an association between the presence of IR, the visceral deposition of fat and the occurrence of hypertriglyceridemia.

The increase of the aggregation of CMRF in the dependence of the presence of IR and steatosis is important, being their simultaneous presence associated to the aggregation of two or more RF for the total of the population.

The presence of IR, but not of steatosis, shows a strong predictability as far as the risk of aggregation of two or more CMRF is concerned. For this reason, it should be part of the clinical assessment of the obese adolescent.

**Abbreviations**

ALT: alanine aminotransferase; AST: aspartate aminotransferase; %BF: body fat percentage; BMI: body mass index; CVD: cardiovascular disease; CVR: cardiovascular risk; CVRF: cardiovascular risk factor; CMRF: cardiometabolic risk factor; DBP: diastolic blood pressure; DM2: diabetes mellitus type 2; FFA: free fatty acids; HBP: high blood pressure; HDL-c: high-density lipoprotein-cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; IGF: impaired fasting glucose; IGT: impaired glucose tolerance; IOTF: International Obesity Task Force; IR: insulin resistance; LDL-c: low-density lipoprotein-cholesterol; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; OR: odds ratio; pc: percentile; RR: relative risk; SBP: systolic blood pressure; SPSS: Statistical Package for the Social Sciences; TC: total cholesterol; TG: triglycerides; WHO: World Health Organization; WtHR: waist-to-height ratio

**References**


