Biochemical Characteristics and Risk Factors for Insulin Resistance at Different Levels of Obesity

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KEY WORDS: metabolically obese normal-weight children, metabolically normal obese children, risk factors, birth weight, family history of type 2 diabetes, family history of hypertension

ABBREVIATIONS
CI—confidence interval
FHD—family history of diabetes
FHH—family history of hypertension
HBW—high birth weight
HOMA-IR—homeostasis model assessment for insulin resistance
LBW—low birth weight
MS—metabolic syndrome
NO-IR—nonobese, insulin resistant
NO-NIR—nonobese, not insulin resistant
O-IR—obese, insulin resistant
O-NIR—obese, not insulin resistant
OR—odds ratio
0w-IR—overweight, insulin resistant
0w-NIR—overweight, not insulin resistant
WC—waist circumference

OBJECTIVE: To establish the biochemical characteristics of nonobese, overweight, and obese children as well as to determine the risk factors associated with insulin resistance in nonobese children and with non–insulin resistance in obese children in the age strata of 6 to 11 years.

METHODS: A total of 3512 healthy children were enrolled in a cross-sectional study. In the absence of obesity, fasting hyperinsulinemia and hypertriglyceridemia defined nonobese, insulin-resistant (NO-IR) children. In the absence of metabolic abnormalities of fasting insulin and triglycerides levels, obese children were defined as obese, not insulin-resistant (0-NIR) children.

RESULTS: The gender- and age-adjusted prevalence of NO-IR and 0-NIR was 6.6% and 21.3%, respectively. In the age-, gender-, and birth weight–adjusted analysis, family history of hypertension (FHH) in both maternal and paternal branches (odds ratio [OR]: 1.514; 95% confidence interval [CI]: 1.2–3.9; P = .04) was associated with NO-IR children. In the analysis adjusted by gender, age, waist circumference (WC), BMI, FHH, and family history of diabetes, high birth weight was associated with NO-IR children (OR: 1.319; 95% CI: 1.2–2.1; P = .04). Finally, in the gender-, age-, family history–, and birth weight–adjusted analysis, a WC lower than the 95th percentile was associated with a lower odds of insulin resistance among obese children (OR: 0.96; 95% CI: 0.91–0.98; P < .0005).

CONCLUSIONS: FHH and high birth weight are associated with NO-IR children, and a low WC is associated with lower odds of O-IR children.

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The concept of metabolically obese, normal-weight individuals was originally introduced to define those individuals who are not obese according to standard weight tables or other readily available criteria but who have metabolic disorders such as hyperglycemia, hyperinsulinism, and/or hypertriglyceridemia. In addition, the concept of metabolically normal, obese individuals was introduced to characterize obese individuals who have insulin sensitivity and a metabolic profile within normal range values.

Although characterization of these individuals at childhood is an important public health issue, reports in children are scarce. In this regard, Pacifico et al reported that ghrelin excess negatively modulates insulin action in nonobese children, contributing to development of insulin resistance and metabolic syndrome (MS); Kelishadi et al emphasized the need for additional investigation of cardiovascular risk factors in normal-weight children with ethnic predisposition to chronic diseases. In addition, Kelishadi et al highlighted the presence of obese children without hyperinsulinemia or metabolic disorders.

To find differences in the presence of insulin resistance between children at different levels of obesity, we studied the biochemical characteristics of nonobese, overweight, and obese children in the age strata of 6 to 11 years. In addition, we determined the risk factors that are associated with insulin resistance in nonobese children and with non–insulin resistance in obese children.

**METHODS**

With the approval of our protocol by the Ethics Committees of the Faculty of Medicine of the University of San Luis Potosi and the Mexican Social Security Institute at Durango, Mexico, and after obtaining the appropriate written informed consent of the children and at least 1 of their parents, a cohort study was carried out.

The sampling strategies and target population of this cohort have been described elsewhere. In brief, a sample of elementary schools representative of different social, economic, and cultural characteristics from the cities of San Luis Potosi and Durango in central and northern Mexico, respectively, was randomly obtained. Children aged 6 to 11 years and with Tanner stage 1 development were randomly selected from the schools and invited to participate in the study. All of the participants were Latin American Mexican children.

Diabetes, hypertension, chronic illnesses, and hormonal replacement were exclusion criteria. Height and weight were assessed with a stadiometer by using a fixed scale. The increments of the height and weight measurements were 0.1 kg and 0.01 m, respectively. Waist circumference (WC) was measured to the nearest centimeter with a flexible steel tape while the children were in a standing position; the anatomic landmarks used were midway between the lowest portion of the rib cage and the superior border of the iliac crest. BMI was calculated as weight (kg) divided by height (m) squared. All measurements were performed with the children standing in light clothing without shoes and under fasting conditions, which was confirmed by a direct interview with the parents of children.

Obesity was defined by a BMI ≥95th percentile, overweight was defined by a BMI ≥85th to <95th percentile, and normal weight was defined by a BMI <85th percentile. BMI cutoff values used were age- and gender-specific according to the childhood international BMI cutoff charts.

The presence of fasting hyperinsulinemia and hypertriglyceridemia defined insulin resistance. In addition, the homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated.

Children were allocated to the following groups according to BMI and insulin-resistance status: (1) nonobese, not insulin resistant (NO-NIR); (2) nonobese, insulin resistant (NO-IR); (3) overweight, not insulin resistant (OW-NIR); (4) overweight, insulin resistant (OW-IR); (5) obese, not insulin resistant (O-NIR); and (6) obese, insulin resistant (O-IR).

Serum fasting triglyceride levels ≥110 mg/dL and serum fasting insulin levels ≥95th percentile of distribution according to age and gender defined the criteria for hypertriglyceridemia and hyperinsulinemia. The HOMA-IR was calculated as (fasting insulin [μU/mL] x fasting glucose [mmol/L]) / 22.5.

Family history of type 2 diabetes (FHD) and hypertension (FHH) were defined by the presence of type 2 diabetes or hypertension in at least 1 parent or grandparent of the children. Family history was ascertained by a self-administered questionnaire to the parents and by verification of clinical records.

Low birth weight (LBW) and high birth weight (HBW) were defined by a body weight <2400 and ≥4000 g for newborns delivered at term and without congenital malformations.

MS was defined by the presence of at least 3 of the following features: WC ≥90th percentile according to age and gender, fasting plasma glucose levels ≥110 mg/dL, blood pressure ≥90th percentile for height and gender, triglyceride levels ≥110 mg/dL, and HDL cholesterol levels ≤40 mg/dL (in both genders).

Whole-blood samples were collected from the antecubital vein after an 8- to 10-hour overnight fast. Serum glucose
was measured by using the glucose-oxidase method (Sigma Diagnostics, St Louis, MO), and the intra- and interassay coefficients of variation for the glucose measurements were 1.1% and 1.5%, respectively. Insulin levels were measured by microparticle enzyme immunoassay (Abbott AxSYM Systems, Alameda, CA), and the intra- and interassay coefficients of variation were 4.5% and 6.9%, respectively. Triglycerides were enzymatically measured and HDL cholesterol fraction obtained after precipitation by phosphotungstic reagent. The intra- and interassay coefficients of variation were 1.6% and 3.0% for triglycerides and 1.3% and 2.7% for HDL cholesterol, respectively.

Samples were frozen at −20°C until their analysis, which was performed in the Central Laboratory of the Biomedical Research Unit at Durango. All measurements were performed in a Data Pro Plus random access clinical analyzer (Thermo Electron Corporation, Arlington, TX).

Numeric values are reported as means ± SD, and categorical variables as proportions. Bivariate analysis was performed by using an unpaired Student’s t test. Differences between >2 groups were analyzed by 1-way ANOVA test with Bonferroni post hoc test. Skewed data were log, transformed for the purpose of statistical analysis.

Unadjusted logistic regression analysis was performed to compute the odds ratios (ORs) between FHD, FHH, and FHD by age, gender, WC, BMI, and birth weight. In the overall population, 42.5%, 29.2%, and 10.8% of participants had 0, 1, and ≥3 components of MS, respectively. All of the NO-IR, Ow-IR, and O-IR groups. The prevalences of MS were 0.7% for the NO-NIR, 6.0% for the NO-IR, 0.94% for the Ow-NIR, 3.3% for the O-NIR, 0.8% for the O-IR groups.

In the overall population, 42.5%, 29.2%, and 10.8% of children had at least 1 component of MS (Table 1). There were progressive increases in WC and birth weight from NO-NIR to O-IR children. FHH and birth weight showed a significant correlation with HOMA-IR and insulin levels in nonobese children (Supplemental Table 7).

Unadjusted logistic analysis showed that FHD and FHH in both maternal and paternal branches are related to the risk of developing NO-IR; in addition, HBW, but not LBW, showed a significant association with NO-IR (Table 2).

In the age-, gender-, WC-, BMI-, and birth-weight-adjusted logistic analysis, FHH in both branches (OR: 1.514; 95% CI: 1.2–1.9; P = .04) remained significantly associated with the presence of NO-IR
children; however, FHD in both branches (OR: 1.214; 95% CI: 0.9–2.6) were not associated with NO-IR. In addition, in the gender-, age-, WC-, BMI-, FHH-, and FHD-adjusted logistic regression analysis, HBW remained associated with NO-IR (OR: 1.319; 95% CI: 1.1–2.4; P = .04).

With regard to O-NIR in children, we did not find associations with family history or with birth weight (Table 2). The gender-, age-, BMI-, family history-, and birth weight–adjusted logistic regression analysis showed that WC lower than the 95th percentile was associated with lower odds of insulin resistance among obese children (OR: 0.96; 95% CI: 0.91–0.98; P < .0005).

**TABLE 2** Unadjusted ORs Between FHD, Family History of Hypertension, and Birth Weight (Independent Variables) and NO-IR and O-NIR Children (Dependent Variables)

<table>
<thead>
<tr>
<th></th>
<th>NO-IR</th>
<th>O-NIR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI P</td>
<td>OR 95% CI P</td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal branch</td>
<td>1.983 (1.2–3.1)</td>
<td>0.03 0.87 0.5–1.5 0.60</td>
</tr>
<tr>
<td>Paternal branch</td>
<td>1.621 (0.99–2.6)</td>
<td>0.52 0.71 0.4–1.3 0.25</td>
</tr>
<tr>
<td>Both branches</td>
<td>1.364 (0.74–2.5)</td>
<td>0.32 0.88 0.5–1.6 0.67</td>
</tr>
<tr>
<td>Family history of hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal branch</td>
<td>0.911 (0.5–1.5)</td>
<td>0.74 1.46 0.8–2.6 0.37</td>
</tr>
<tr>
<td>Paternal branch</td>
<td>1.400 (0.87–2.3)</td>
<td>0.16 1.30 0.9–1.9 0.18</td>
</tr>
<tr>
<td>Both branches</td>
<td>2.141 (1.3–3.5)</td>
<td>0.003 1.55 1.0–2.4 0.53</td>
</tr>
<tr>
<td>High birth weight</td>
<td>1.316 (1.1–1.7)</td>
<td>0.04 0.49 0.4–1.7 0.49</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>0.83 (0.4–1.7)</td>
<td>0.78 0.72 0.2–2.5 0.61</td>
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**DISCUSSION**

The major findings of our study are as follows: (1) among phenotypically obese children, there is an elevated prevalence of O-NIR (21.3%); (2) among nonobese children, the prevalence of NO-IR was 6.6%; (3) FHH and HBW are related to the presence of NO-IR children; (4) a lower WC is associated with lower odds of being O-NIR; and (5) the first report about metabolic abnormalities and prevalence of NO-IR and O-NIR in prepubertal children.

Because policies for the early detection of metabolic and cardiovascular risk factors are usually focused on obese children, nonobese children might not receive the benefits of screening for metabolic and cardiovascular diseases. Our results, which reveal that among the 2259 nonobese children 149 (6.6%) exhibited cardiovascular and metabolic risk factors similar to those in O-IR children, support the abovementioned statement.

Given that NO-IR individuals usually are not detected, our results suggest that HBW and FHH should be considered as criteria for screening metabolic and cardiovascular risk factors in the prepubertal population. In this regard, although there are no previous reports in children, recently it has been shown that nonobese, young-adult offspring of at least 1 hypertensive parent had significantly higher serum insulin and triglyceride levels than offspring of normotensive parents.

In addition, although NO-IR children exhibited lower BMI and WC than O-IR children, the HOMA-IR and triglyceride and insulin levels were similar, supporting the statement that nonobese children also could have increased cardiovascular risk. Given that 6.6% of nonobese children are NO-IR, our results also suggest that obesity-related morbidity is not totally revealed by the usual anthropometric measures. In addition, given that hyperinsulinism correlates closely with metabolic and cardiovascular diseases.
an increase in fat cells,\textsuperscript{18–20} it is not unreasonable to expect that, with aging, NO-IR children will develop central obesity.

We earlier reported that children aged 10 to 14 years with positive FHD exhibited significant higher fasting insulin and triglyceride levels as well as a higher HOMA-IR index than children with a negative family history;\textsuperscript{21} however, because we did not stratify the population according to BMI and metabolic status, it is possible that overlapping of O-IR, O-NIR, and NO-IR children influenced the results of this previous study.

Interestingly, our results, which revealed a strong association of FHH, but not of FHD, with NO-IR, agree with previous reports in middle-aged adults that showed no reduction in insulin secretion in obese subjects with FHD compared with controls\textsuperscript{22} and with the report by Grill et al\textsuperscript{23} showing that increases in insulin levels are induced by hyperglycemia and insulin resistance with no discernible influence of FHD.\textsuperscript{24} These findings suggest that the cluster of cardiovascular risk factors associated with hyperinsulinemia could be independent of FHD.\textsuperscript{24} Additional research in the field is necessary to determine the underlying mechanisms involved in the familial predisposition to metabolic abnormalities among nonobese individuals.

Because there is no consensus on the definition of normal-weight individuals who display obesity-related phenotypic characteristics, we chose the original description by Ruderman et al and Conus et al\textsuperscript{1,2,25} to identify NO-IR children; however, is necessary to keep in mind that other definitions have been proposed. In this regard, we also analyzed the biochemical characteristics of NO-IR children according to the definitions of (1) Lee,\textsuperscript{26} Succurro et al,\textsuperscript{27} and Tsai et al;\textsuperscript{28} (2) Kelishadi et al;\textsuperscript{26} and (3) Shea et al.\textsuperscript{29} All definitions consistently support that NO-IR children exhibited elevated WC, BMI, blood pressure, and HOMA-IR index as well as higher fasting glucose, triglyceride, and insulin levels and decreased HDL cholesterol levels compared with NO-NIR children; the prevalence of NO-IR was higher based on definitions used in this study (BMI <95th percentile by age and gender, hyperinsulinemia, and hypertriglyceridemia) (Supplemental Tables 8, 9, 10, and 11).

The prevalence of obesity and differences in the metabolic profile between boys and girls in the nonobese and obese population in our study closely mirrors that reported in Mexican children (26.8%),\textsuperscript{30} however, among the 948 obese children, 202 (21.3%) exhibited a cardiovascular and metabolic profile similar to that of NO-NIR children. Although there are no previous reports on the prevalence of O-NIR children, our findings suggest that a significant proportion of resources might be unnecessarily used in the screening for metabolic risk in obese children. In this regard, our results suggest that a WC lower than 95th percentile is associated with lower odds of insulin resistance in obese children, according to BMI criteria.

The criterion used for diagnosis of obesity was based on the percentile of BMI, according to gender and age, as it has been suggested for recognition of NO-IR and O-NIR in adults.\textsuperscript{1,15,29} The use of BMI as a criterion diagnosis of obesity allowed us to evaluate the role of WC (as a nonrelated variable with the criterion for classification of groups) on the presence of metabolic parameters among different levels of obesity; whereas a low WC is related to lower odds of O-IR, neither family history nor birth weight showed a significant association. In the absence of previous reports in children, our results agree with the results by Succurro et al\textsuperscript{27} showing that O-NIR adults exhibited lower WC compared with O-IR subjects.

These results strongly suggest that adipose depots seem to play a major role in the pathophysiology of developing an obesity phenotype without metabolic alterations; thus, it is rational to review the obesity diagnosis criteria in children to facilitate the recognition of O-NIR children.

Our results did not reveal significant associations between LBW and NO-IR or O-NIR children. In this regard, we identified a total of 86 (2.4%) children with a history of LBW; of these, 66 (76.7%) were in the group of O-IR children, a finding that supports previous observations that highlight that early catch-up growth, after fetal growth restriction, modifies the organism on its growth trajectory and exerts a strong influence on developing cardiovascular and metabolic disease in adulthood.\textsuperscript{31,32} Therefore, the lack association between LBW and NO-IR or O-NIR children in this study could be related to the fact that the vast majority of children with a history of LBW developed obesity and metabolic disturbances and were classified as O-IR children.

Several limitations of this study deserve to be mentioned. First, although diet-recall tests and tests of physical activity were applied, an important interassay variation and a serious information bias were identified. To report reliable data, we decided not to include information about diet and exercise. Given that some dietary factors could promote the increase of insulin levels, and physical activity is strongly related with energy expenditure and decrease in insulin resistance, diet and exercise are important contributing factors in the development of metabolic abnormalities. Taking into account the sampling strategy and random selection of the population in study, this limitation does not exert a significant influence on the determination of prevalence and biochemical characteristics of NO-IR and O-NIR children. In addition,
Kelishadi et al.33 recently reported the insulin sensitivity and an adverse adipose tissue mass, which promotes lifestyle modification would be beneficial at the population level. Additional research is necessary in the field. Second, we did not measure adipose tissue mass, which promotes insulin sensitivity and an adverse lipid profile.19 Finally, we did not assess history of gestational diabetes or maternal diabetes, which is strongly related to HBW. Given that fetal growth, in normal as well as in diabetic pregnancies, is far more complex than previously thought,34 additional research is needed in the field to clarify if the HBW could determine differences in the development of metabolic abnormalities later in life.

The main strengths of our study were the sampling strategy, sample size, and the representative sample of children aged 6 to 11 years.

Additional research focused on the early identification of NO-IR and O-NIR children that facilitates the successful prevention of type 2 diabetes and cardiovascular disease in adolescence and/or adulthood is needed.

CONCLUSIONS

This is the first study, to our knowledge, to report the prevalence of NO-IR (6.6%) and O-NIR (21.3%) in prepubertal children. In addition, our results reveal that FH and HBW are related to NO-IR, whereas low WC is associated with lower odds of O-IR.

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