Prevalence of Insulin Resistance in adolescents in the city of Posadas. 
Recommended diagnostic criteria

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ABSTRACT

Introduction: There is a growing recognition of the important role of insulin resistance (IR) in the pathogenesis of type 2 diabetes in children and adolescents. The aim of this study was to obtain the reference percentiles and the prevalence of fasting insulin and index of IR, HOMA and QUICKI, in adolescents in the city of Posadas, Misiones.

Materials and Methods: A descriptive study of 420 high-school students was performed. Anthropometric parameters, waist circumference and blood pressure were evaluated. Blood samples were taken after 12 hours fasting for biochemical measurements: fasting glucose, lipid profile and insulin. The samples were processed with in-house and external quality control.

Results: Percentile 95 of Insulin ≥ 12 mU/lt, HOMA ≥ 2.5 and percentile 5 for QUICKI was ≤ 0.33. Comparison between genders showed no significant differences in HOMA and QUICKI values (U = 3077, p = 0.058), but significant differences were found for insulin: females = 8.59 ± 2.93 mU/l, males = 6.50 ± 2.55 mU/l (U = 2929, P = 0.019), However, when applying the z test and 1.5 of SD, calculation for groups separated by gender would not be necessary (calculated z = 2.63, critical z = 2.47, 1.5 SD = 3.82 mU/l). 11.7% were hyperinsulinemic and the prevalence of IR by HOMA was 10.5% and 9.8% for QUICKI, with no statistical differences by sex and age group. Insulin and two indexes of IR were significantly higher in overweight or obese adolescents.

Conclusions: These values may be used as a guide in the diagnostic algorithm of IR by the medical community.

No financial conflicts of interest exist.

Key words: insulin-resistance, adolescents, risk factors

INTRODUCTION

Disturbed glucose and insulin metabolism associated with dyslipidemia and hypertension are related to an increased risk for developing cardiovascular diseases (1). This clustering of risk variables has been found to persist from childhood into adulthood (2,3).

Studies in obese children and adolescents have shown that insulin resistance (IR) is associated with abnormal glucose metabolism (4,5). Therefore, there is a growing recognition of the important role of IR in the pathogenesis of type 2 diabetes in this population (6,7).

Baseline insulin concentrations have been considered as an accurate measurement for assessment of insulin resistance in epidemiologic studies, as there is a good correlation between IR and the results reported using the hyperinsulinemic-euglycemic clamp. The Homeostasis Model Assessment (HOMA) (8) and the Quantitative Insulin Sensitivity Check Index (QUICKI) (9) are used for IR assessment because of their convenience, low-cost and good correlation with the gold standard method (10-15).
There is no internationally accepted cut-off value for these indexes, as it varies according to the population studied. Therefore, it is essential to establish cut-off points, with valid and reliable methods, for each region.

It is necessary to identify children and adolescents with IR, in order to prevent risk factors for developing cardiovascular disease and to institute specific intervention measures. This is related to the fact that habits associated with an increased risk of atherosclerotic disorders are acquired in late adolescence.

Based on the above, our aim was to obtain the reference percentiles and the prevalence of fasting blood insulin and IR, HOMA and QUICKI indexes, in high-school attending adolescents from the city of Posadas, province of Misiones.

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted in students attending public and semipublic high-schools in the city of Posadas, province of Misiones, Argentina.

Adolescents aged 12-18 years in the city of Posadas totaled 35,000 in 2004, with 30,000 enrolled in secondary level, Educación General Básica or EGB III (Spanish for “Basic General Education, third cycle) and “Polimodal” (three-year high-school cycle replacing the traditional secondary school system in some provinces). There are 113 schools in Posadas (74 public and 39 private schools); 61 only have EGB III level, 9 only “Polimodal” and 43 offer both levels.

For this study, schools and then courses were randomly selected. Stratification was performed according to geographic location (urban or peri-urban), age and gender. We selected eight schools that had both levels of education and over 1000 students, 2 located at the heart of the city and 6 in the peripheral urban area. Four courses were selected among all school courses: two per each level of education, with an expected enrollment of 25 to 30 students per course and an expected agreement to participate in the study of 60% (based on a pilot test performed in one school), which would imply 60 to 70 students per school. In order to achieve an increased participation and a better subsequent educational work, all students from selected courses were invited to take part in the study.

Based on an expected prevalence of 9.2%, a maximum acceptable level of 12.0% and a 95% confidence interval, using the Epi Info 6 software version 6.04 d, we calculated that for an adequate representation of the studied population, the sample size should be 400 students. Data for this calculation were obtained from a pilot test conducted in one school.

A survey conducted by 4 qualified individuals collected personal data, information about diseases at the time of the survey or previous conditions and treatments. Weight, height, waist circumference and blood pressure measurements were performed. Blood samples were subsequently obtained, following students' preparation considering preanalytical variations.

All students who voluntarily accepted to participate and had the written informed consent signed by their parents or guardians were included in the study. Students were excluded if they were less than 12 years old, older than 19, had diabetes, were pregnant, had a less than 12-hour fast or had hemolysis in their serum samples.

For determination of percentiles and measures of central tendency and dispersion of fasting blood insulin levels and HOMA and QUICKI indexes, only apparently healthy adolescents were considered, defined as: normal blood weight, with blood triglycerides below 110 mg/dL, HDL cholesterol above 40 mg/dL, fasting blood glucose levels below 110 mg/dL, waist circumference and systolic and diastolic blood pressure below the 90th percentile, not taking lipid-lowering drugs, oral contraceptives or any other type of
medication and with no previous or current known inflammatory, hepatic, renal, hypertensive, hormonal or coronary heart disease (18).

The definitions and methodologies of the National Committee for Clinical Laboratory Standards (NCSSL) (19) guidelines were considered for determining percentiles and measures of central tendency and dispersion.

Values at or above the 95th percentile were considered as elevated fasting blood insulin levels and IR by HOMA, while for QUICKI, IR was considered when values were at or below the 5th percentile.

Biochemical measurements were performed in venous blood serum collected in primary tubes with coagulation accelerators and phase separation. Samples were stored at 4°C until assayed, which was done within 24 hours (17). Measurements performed included: blood glucose (coefficient of variation [CV] = 2.38 %) triglycerides (CV = 2.42 %), both by colorimetric enzymatic methods, HDL cholesterol (CV = 1.24 %) by direct colorimetric assay without previous hydrolysis. For processing these analytes, a Targa BT3000 automated analyzer (Biotecnica Instruments, S. p. A., Italy) was used with high-quality reagents (Wiener Lab, Rosario, Argentina). In-house quality control was performed with commercially available pathologic and normal control sera (Standatrol S-E 2 levels, Wiener Lab, Rosario, Argentina) and a serum pool prepared at our laboratory. External Quality Control was carried out with controls provided by the Argentine Biochemical Foundation (Buenos Aires). Insulin was manually measured by a competitive solid-phase radioimmunoassay using 125 iodine-labeled Coat-A-Count Insulin (DPC, USA.) (CV = 8.00%) with low, normal and high level commercially available quality controls.

A previously trained operator carried out weight and height measurements using a balance beam scale with height rod accurate to 0.1 kg and 1 cm, respectively (20). These data were used to calculate the body mass index (BMI) – weight in kilograms divided by the square of the subject's height in meters—and classified as students with: low weight, normal weight, overweight or obesity; according to Cole et al tables and the age and weight charts according to gender of the Argentine Society of Pediatrics (20, 21).

Blood pressure was measured in mmHg after a 15-minute rest with a mercury sphygmomanometer with an adequate cuff size for the adolescent’s arm circumference. For classification, the average of two measurements taken on the right arm at a 5-minute interval was used (22). For students with elevated blood pressure levels, measurements were repeated on two different days and the average of the six measurements performed was taken as final value. Measurements were performed by a physician and three advanced students of the nursing course at the National University of Misiones, after training, and following the Pan American Health Organization recommendations (23).

Waist circumference measurement in centimeters was performed by a single specifically-trained operator to the nearest 0.5 cm (24). Increased waist circumference was considered when the student had a waist circumference measurement above the 90th percentile for gender and age (25).

Results were analyzed using the Epi Info 6 v. 6.04 d software. The Kolmogorov-Smirno test was used for assessing distribution of the quantitative variables. The χ² test, Mann Whitney test and Kruskal-Wallis test were used, according to the type of variable and the number of groups compared. For determination of percentiles and measures of central tendency and dispersion of apparently health adolescents, we used the Z-test and the smallest 1.5 standard deviation (SD) value to determine the usefulness of the calculation of percentiles per separate group in those variables with statistically significant differences (19). A significance level p < 0.05 was used.
The study was approved by the Ministry of Public Health and the Ethics Committee of Hospital Dr. Ramón Madariaga in Posadas. The ethical principles stated in the Declaration of Helsinki were followed.

RESULTS

From July to October 2005, 420 students with a mean age of 15.3 years were studied. The main characteristics of the sample are shown in tables I and II.

Table III shows the total values and percentiles of insulin and IR indexes obtained from an apparently healthy group of adolescents, following strict screening criteria (n = 184). The cut-off values found for insulin were ≥ 12 mU/lit, HOMA ≥ 2.5 and QUICKI ≤ 0.33.

When variables were compared according to age group, no statistically significant differences were found for blood insulin levels, HOMA and QUICKI (U = 3704, p = 0.149; U = 3619, p = 0.094 and U = 3619, p = 0.094; respectively).

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When making comparisons between genders, no significant differences were found in HOMA and QUICKI values (U = 3077, p = 0.058, for both cases). Significant differences were found in insulin values in females = 8.59 ± 2.93 mU/l versus males = 6.50 ± 2.55 mU/l (U = 2929, p = 0.019); however, when evaluating these differences with the z-test and the smallest 1.5 SD, we were able to establish that the calculation by separate groups per gender would not be necessary (calculated value of z = 2.63, critical z = 2.47; 1.5 SD = 3.82 mU/l).

Prevalence of increased blood insulin levels was 11.7%, with no statistical differences according to gender (χ² = 1.09 p = 0.297) and according to age group (χ² = 1.80 p = 0.179). Prevalence of IR according to HOMA index was 10.5% and according to QUICKI index 9.8%, with no statistically significant differences according to gender (χ² = 0.006, p = 0.938; χ² = 3.62, p = 0.057, respectively) and according to age group (χ² = 0.457, p = 0.499; χ² = 0.555 p = 0.456 respectively). When considering the four groups stratified according to gender and age, no statistical differences were found either (Table IV).

No statistical differences were found in prevalence of low weight, normal weight, overweight or obesity between genders (χ² = 3.106, p = 0.376), according to age group (χ² = 0.804, p = 0.848) or among the four groups classified according to gender and age (χ² = 7.851, p = 0.549).

Table V shows the variables blood glucose insulin and both IR indexes in groups classified according to BMI and age charts for weight according to gender, independently of gender and age group. The variables blood glucose levels and both IR indexes were significantly higher in adolescents with overweight or obesity than in those with normal or low weight.
TABLE I: General characteristics of the sample of adolescents in the city of Posadas, Misiones, Argentina (n = 420)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total women</td>
<td>260</td>
<td>61.9</td>
</tr>
<tr>
<td>Total men</td>
<td>160</td>
<td>38.1</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-14 years</td>
<td>192</td>
<td>45.7</td>
</tr>
<tr>
<td>15-18 years</td>
<td>228</td>
<td>54.3</td>
</tr>
<tr>
<td>a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low weight</td>
<td>38</td>
<td>9.0</td>
</tr>
<tr>
<td>Normal weight</td>
<td>309</td>
<td>73.6</td>
</tr>
<tr>
<td>Overweight</td>
<td>56</td>
<td>13.3</td>
</tr>
<tr>
<td>Obese</td>
<td>17</td>
<td>4.0</td>
</tr>
</tbody>
</table>

a. Classification according to body mass index and age chart for weight according to gender.

TABLE II: Prevalence of risk factors for cardiovascular disease evaluated in adolescents in the city of Posadas, Misiones, Argentina. (n = 420)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated systolic and/or diastolic blood pressure a</td>
<td>74</td>
<td>17.6</td>
</tr>
<tr>
<td>Increased waist circumference a</td>
<td>82</td>
<td>19.5</td>
</tr>
<tr>
<td>Elevated triglycerides b</td>
<td>90</td>
<td>21.4</td>
</tr>
<tr>
<td>Low HDL cholesterol c</td>
<td>73</td>
<td>17.4</td>
</tr>
<tr>
<td>Elevated fasting blood glucose</td>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a At or above the 90th percentile
b At or above 110 mg/dl
c Below 40 mg/dl.

TABLE III: Percentiles, measures of central tendency and dispersion of fasting blood insulin and insulin resistance indexes in the subset of apparently healthy adolescents (n = 184)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD(^a)</th>
<th>95% CI(^b)</th>
<th>P5</th>
<th>P10</th>
<th>P25</th>
<th>P50</th>
<th>P75</th>
<th>P90</th>
<th>P95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (mU/l)</td>
<td>5.5</td>
<td>2.8</td>
<td>5.1-5.9</td>
<td>1.6</td>
<td>2.3</td>
<td>3.2</td>
<td>5.0</td>
<td>7.0</td>
<td>9.5</td>
<td>11.0</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.13</td>
<td>0.63</td>
<td>1.04-1.22</td>
<td>0.31</td>
<td>0.44</td>
<td>0.62</td>
<td>1.03</td>
<td>1.46</td>
<td>2.00</td>
<td>2.50</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.39</td>
<td>0.04</td>
<td>0.38-0.40</td>
<td>0.33</td>
<td>0.34</td>
<td>0.36</td>
<td>0.38</td>
<td>0.42</td>
<td>0.44</td>
<td>0.48</td>
</tr>
</tbody>
</table>

a Standard deviation
b 95% confidence interval

DISCUSSION
Cut-off values found for fasting insulin and HOMA and QUICKI indexes in our population were 12 mU/l, 2.5 and 0.33, respectively, similar to findings reported by some authors (27, 28) and below those reported by others (29, 30).

In a study conducted in USA where IR was assessed with an euglycemic clamp, the authors found a cut-off value of 12 mU/l for insulin for the same age group (31).
It should be noted that most studies conducted to obtain percentiles and cut-off values that are subsequently used as reference are based on findings in the general population or obese patients. As recommended, a selected population was used, following strict exclusion criteria (19).

Even if the coefficients of analytical variation of all biochemical measurements performed were acceptable, intraindividual and interindividual biological variations should be considered. Therefore, statistically significant differences in means should not always imply a need for cut-off values for different groups. In addition to the above, and evaluating the variable fasting insulin, which had shown a statistically significant difference in relation to gender, with the z-test and the smallest 1.5 SD to determine the usefulness of calculation of percentiles by separate group (19), it was established that in the sample used to determine the percentiles it was not necessary to calculate percentiles for groups separated by gender, i.e., clinical significance in these cases had little relevance. Data obtained from this study may be used in clinical practice or epidemiologically, independently of the age group and gender, which from the practical viewpoint facilitates diagnosis.

The rate of IR found in our population was below that reported by Lee et al, in a population of adolescents in the USA; these authors reported that 29.1% of the population had IR (27). It was also below that reported in a study conducted in India by Vikram et al, who found 29% of fasting hyperinsulinemia (31) and 37% in Venezuela (32). These discrepancies might be due to the fact that 4.0% of our population is obese, while in the other studies, obesity has a higher prevalence (27,31,32), considering obesity as an important factor for the development of IR.

It should be noted that using the QUICKI index, prevalence of IR in men was lower than that found with the HOMA index, which could indicate that this index might be underestimating IR in this group.

Our results, in consistency with those from the studies conducted by Lee et al (27), Carlos Juárez-López et al (33) and others (4, 5, 12) provide scientific evidence of increased fasting insulin values and IR, HOMA and QUICKI indexes in adolescents with overweight and obesity. For this reason, adolescents with overweight and obesity were excluded for calculating percentiles.

Limitations of the study might include the type of cluster sampling, which in turn was selected from schools with the largest number of students with both levels of education and not from all the schools in the city, which might imply some bias in the prevalence found. However, a simple random sampling would have made the logistics of the study more difficult, as the rate of agreement to participate in the study would have considerably decreased and the costs for this type of sampling would have made it impossible to conduct the study.

Even if the fasting insulin levels and HOMA and QUICKI indexes reported in this study correspond to a single city, they were obtained using strict exclusion criteria, and therefore they might be taken into account for further studies or considered for the definition of individual patterns.

It should also be noted that values found were obtained from a cross-sectional study and their prospective implication should be evaluated.

Furthermore, for logistic and acceptability reasons, pubertal stage—a potential confounder—was not evaluated in this study.

Undoubtedly, a highly reproducible insulin assay with no cross-reactivity with proinsulin and split products, with low intra-individual variability, would provide a parameter more closely linked to IR, but in the absence of a standardized insulin assay, it is necessary to use secondary indicators with reasonable clinical usefulness to define interventions for higher cardiovascular risk.

Clearly, chronic diseases such as type 2 diabetes may be delayed or prevented by identifying subjects prone to IR and subsequently reducing risk factors with changes in
lifestyle and/or the use of pharmacologically agents. Simple, low-cost measures—albeit difficult to maintain over time—such as a healthy diet, physical exercise and a greater knowledge of atherogenic risk factors and non-transmissible diseases at this age could contribute in the long term to the reduction of deaths from cardiovascular diseases.

We conclude that the cut-off values found for fasting insulin and the HOMA and QUICKI indexes in our population were 12 mU/L, 2.5 and 0.33, respectively, independently of age and gender. These values may be used in further studies in adolescents and used as a guide in the diagnostic algorithm for IR by the medical community.

The prevalence of increased fasting blood insulin levels was 11.7%, and for IR 10.5% according to HOMA and 9.8% according to QUICKI, which makes it possible to estimate that 3000 to 3600 school-attending adolescents in the city of Posadas would be experiencing these alterations.

Insulin values and IR indexes were significantly higher in adolescents with overweight or obesity as compared to those found in adolescents with normal or low weight.

Screening for IR in adolescents is a strategy to identify individuals at risk that may require intervention to prevent the future development of cardiovascular disorders.
### TABLE IV: Prevalence of fasting hyperinsulinemia and insulin resistance according to HOMA and QUICKI indexes in adolescents of the city of Posadas, classified according to gender and age. (n = 420)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Females</th>
<th>Males</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total N</td>
<td>Total</td>
<td>12-14 years</td>
<td>15-18 years</td>
</tr>
<tr>
<td>Fasting Hyperinsulinemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N 420</td>
<td>% 95% CI</td>
<td>% 95% CI</td>
<td>% 95% CI</td>
<td>% 95% CI</td>
</tr>
<tr>
<td>HOMA ≥ 2.5</td>
<td>49 11.7</td>
<td>7.1-14.9</td>
<td>10.4</td>
<td>7.1-14.9</td>
</tr>
<tr>
<td>IR-QUICKI ≤ 0.33</td>
<td>40 9.8</td>
<td>7.2-13.1</td>
<td>11.9</td>
<td>8.4-16.6</td>
</tr>
</tbody>
</table>

*95% Confidence Interval
X² for each variable comparing four groups (females 12 to 14 years old, females 15 to 18 years old, males 12 to 14 years old and males 15 to 18 years old)

### TABLE V: Distribution of fasting blood insulin levels and insulin resistance indexes in adolescents classified as low weight, normal weight, overweight and obese in the city of Posadas, province of Misiones, Argentina. (n = 420)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low weight (n = 38)</th>
<th>Normal weight (n = 309)</th>
<th>Overweight (n = 56)</th>
<th>Obese (n = 17)</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD*</td>
<td>Median</td>
<td>95% CI</td>
<td>Mean</td>
<td>SD*</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>5.86</td>
<td>4.95</td>
<td>4.00</td>
<td>4.23-7.49</td>
<td>6.00</td>
<td>3.43</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.20</td>
<td>1.05</td>
<td>0.84</td>
<td>0.85-1.54</td>
<td>1.21</td>
<td>0.73</td>
</tr>
<tr>
<td>QUICKI index</td>
<td>0.40</td>
<td>0.05</td>
<td>0.39</td>
<td>0.38-0.42</td>
<td>0.38</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* SD: standard deviation
95% CI: 95% confidence interval of the mean.
X²: contrast statistics of the Kruskal-Wallis test for each variable comparing four groups (low weight, normal weight, overweight and obese).
REFERENCES


