Diagnostic methods of insulin resistance in a pediatric population
Azucena Martínez Basila, Jorge Maldonado Hernández and Mardia López Alarcón

ABSTRACT

Obesity is the main risk factor for insulin resistance (IR) in the pediatric population. IR represents a link between obesity and other metabolic complications such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Therefore, accurate diagnosis and early intervention may reduce the incidence of T2DM and CVD in at-risk individuals. In this study we describe the techniques used to assess insulin sensitivity in pediatric populations. We also describe in detail three diagnostic tests: the glucose clamp technique, which represents the gold standard to determine tissue insulin sensitivity and insulin secretion; HOMA and QUICKI, which are indexes obtained from fasting glucose and insulin concentrations; and ISI-Composite, obtained from an oral glucose tolerance test, which provides additional information on glucose metabolism after an oral glucose load. In conclusion, the glucose clamp technique is an invasive procedure that is difficult to use in routine clinical settings. Because the cut-off points to diagnose IR with values derived from ISI-Composite have not been established for pediatric populations, HOMA and QUICKI, despite their lack of precision, remain the most used in clinical practice.

Key words: insulin resistance, diabetes mellitus, glucose.

INTRODUCTION

Obesity is the main risk factor for insulin resistance (IR) in the pediatric population.1 In Mexico, the national combined prevalence for overweight and obesity in children between 5 and 11 years old reaches 26%. Similarly, 1/3 adolescents between 12 and 19 years of age present overweight or obesity.2 This situation is significant from a public health perspective because childhood obesity has been associated with an increased risk for developing type 2 diabetes mellitus (T2DM) in adulthood.3 Significantly, T2DM is preceded by an IR period that also constitutes a cardiometabolic risk factor.4 This situation has aroused interest to determine insulin sensitivity in the pediatric population5,6 because early diagnosis may reduce risks and delay onset of irreversible pathological entities.

Insulin is a peptide hormone composed of 51 amino acids coded on the short arm of chromosome 11 and synthesized in the pancreas within β-cells in the islets of Langerhans.7 Insulin production in response to food intake is carried out in a rhythmic, two-phase fashion. The first phase (or quick secretion phase) begins within the first minute after food intake and reaches its maximum in 3-5 min. This phase lasts about 10 min and releases insulin that was already synthesized. The second phase (or slow secretion phase) starts 10 min after food intake. Secretion of insulin becomes apparent after 10 min of food ingestion. Duration of this phase is proportional to the time circulating glucose levels remain high. Under normal conditions this period extends up to 120-180 min.8 Insulin is an anabolic hormone that plays an essential role in carbohydrate metabolism by maintaining euglycemia. Its main functions include glucose uptake of muscle and adipose tissue by favoring translocation of glucose transporter 4 (GLUT-4) to cell membrane, synthesizing hepatic and muscular glycogen, suppressing hepatic glucose synthesis, activating Na/K-ATPase pump in adipose and muscular tissue, synthesizing proteins, uptake of amino acids, and gene expression.9,10 When there is interference of insulin action, a resistance state initiates that will affect functions associated with this hormone.
Therefore, appropriate insulin sensitivity is based on the efficiency of this hormone to reduce glycemia by promoting glucose uptake by muscle and adipose tissue, increasing hepatic glycogen production and reducing hepatic glucose production. On the other hand, insulin resistance (IR) is a metabolic dysfunction characterized by a reduced biological response to this hormone with the following consequences: decreased glucose uptake by muscle and adipose tissue cells, reduced hepatic glycogen production and increased production of hepatic glucose. In most cases, this leads to an increased release of insulin to compensate for progressive elevation of circulating glucose (compensatory hyperinsulinemia). This explains why an increased level of insulin is the most characteristic feature in IR either while fasting or as a challenge response.7

In this study we present the available diagnostic methods to measure insulin sensitivity in a pediatric population and describe in detail certain techniques that are particularly important for diagnosing IR. These techniques are as follows: 1) glucose clamp technique (hyperinsulinemic-euglycemic), which is the gold standard to measure tissue insulin sensitivity and insulin release; however, because of its complexity it does not have clinical utility; 2) indexes such as HOMA (Homeostasis Model Assessment) and QUICKI (Quantitative Insulin Check Index), which are the simplest and most frequently used methods to assess IR and 3) the Matsuda-DeFronzo insulin sensitivity index (ISI-M), which is calculated using data obtained from an oral glucose tolerance test (OGTT), which provides additional information regarding glucose metabolism after stimulus.

**Insulin Resistance Diagnostic Methods**

Insulin resistance can be determined directly by evaluating the physiological response to an exogenous insulin infusion that promotes glucose uptake in insulin-dependent tissues or indirectly through estimating the glucose-insulin ratio while fasting or after receiving a stimulus, either orally or intravenously.12 Table 1 presents the different diagnostic alternatives for IR and their main characteristics.

*Hyperinsulinemic and hyperglycemic clamp*

The clamp technique developed by DeFronzo et al. in 1979 has become the gold standard to diagnose IR.13 It is a very complex and invasive technique that has almost no clinical application.14 However, because it allows the determination of tissue insulin sensitivity (both hepatic and muscular) as well as response of β-cells to glucose, it is frequently used in research environments. Two variants of this technique have been described: 1) hyperinsulinemic clamp that allows measurement of overall glucose disposal under a stimulus and 2) hyperglycemic clamp that allows measurement of pancreatic response to glucose under hyperglycemia conditions.

Hyperinsulinemic clamp (hyperinsulinemic-euglycemic) is based on the concept that under constant hyperinsulinemia conditions, glucose uptake by insulin-dependent tissues will be proportional to the exogenous glucose infusion rate required to keep a constant circulating glucose concentration. The goal of the clamp is to increase insulin concentration by 100 µU/ml over base value and maintain a constant glucose concentration in blood ~90 mg/dl through periodic adjustments using a glucose infusion.13 During the clamp procedure, it is essential to reach a period of at least 30 min where variation between glucose levels is <5%; this is usually accomplished during the last 30 min of the clamp and this time frame is known as “steady state.” Before this technique is carried out, two intravenous catheters are placed, one antecubital and one distal. The distal catheter is used to collect blood samples; for this, the arm must be placed inside a warming box in order to arterialize venous blood. The antecubital catheter is used to administer a constant insulin infusion and a variable glucose infusion. Once catheters are in place and we have three basal glucose measurements, we can begin insulin infusion. During the first 10 min, two insulin dosages are infused and, later on, infusion is maintained at a constant rate. Insulin infusion is calculated based on the patient’s body surface as proposed by DeFronzo et al. (40 µU/m²/min).13 Glucose measurements are carried out every 5 min during the clamp period and glucose infusion is adjusted based on such measurements to keep glucose concentration ~90 mg/dl (Figure 1).

Clamp results are analyzed using measurements obtained during the “steady period” to calculate two values: 

\[ M = ISI \]  

\[ ISI = \frac{M}{I} \]

where \( M \) is a measure of glucose tolerance given by the glucose infusion rate administered during this period (mg/kg/min) and \( ISI \) (insulin sensitivity index, also known as M/I ratio). The latter specifies the amount of metabolized glucose (M) by plasma insulin unit (I) and represents a tissue insulin sensitivity index (mg/kg/min per µU/ml). Hyperinsulinemic-euglycemic clamp is the gold standard to diagnose
IR because it provides the most reliable measurement of tissue insulin sensitivity (M/I ratio) because all insulin administered to the patient is biologically active. However, no cut-off point has been reported for diagnosing IR using the clamp because this is a technique used primarily in investigation and not in clinical practice. Therefore, M and M/I values are used for its interpretation, for instance, higher M and M/I values reflect a better insulin sensitivity and secretion. A study carried out in preadolescents found $M$ value experienced no changes when the clamp was carried out with a 2-year window using the same cohort (8.9 ± 3.3 and 8.3 ± 3.3 mg/kg/min, respectively). Interestingly, $M$ was different in overweight children when compared to children with an appropriate BMI (10 ± 3.1 vs. 6.9 ± 2.8 and 9.3 ± 3.0 vs. 6.7 ± 3.1 mg/kg/min in the first and second measurement, respectively).  

Hyperglycemic clamp allows measurement of the pancreatic response to glucose under hyperglycemia. Its purpose is to increase glucose plasma concentration to 125 mg/dl over basal concentration and maintain it during a period of ~2 h (Figure 2). This technique challenges the pancreas and allows evaluation of the two-phase release of insulin in vivo where an alteration of the first phase will reflect pathology of β-cells. The hyperglycemic clamp is easier to carry out than the hyperinsulinemic clamp because it does not require exogenous insulin administration. Although results obtained by both methods correlate strongly, each measures different variables of glucose metabolism. It is necessary to identify the research goal to decide which clamp method will be used. For instance, a study by Uwaifo et al. used a hyperinsulinemic clamp and a hyperglycemic clamp in 31 children with an interval of 2-6 weeks. Values reported for $M$ were 14.7 ± 8.2 and 14.1 ± 6.5, respectively. Although $M$ values were similar, there was a better correlation between the hyperglycemic clamp and insulin sensitivity levels from measurements during fasting.

### Insulin sensitivity indexes based on fasting

The Homeostasis Model Assessment Index (HOMA) proposed by Mathews et al. in 1985 is the most widely used method to diagnose IR in a pediatric population. It is estimated from interaction between β-cell function and insulin sensitivity using a mathematical model where glucose and insulin levels are measured during fasting. The model is calibrated using a β-cell function at 100% and a normal insulin resistance = 1 according to the following formula:

$$\text{HOMA-IR} = \frac{\text{fasting plasma insulin (µU/ml)} \times \text{fasting plasma glucose (mmol/L)}}{22.5}$$

HOMA index can be used to evaluate pancreatic β-cell function using the following mathematical model:

Table 1. Insulin resistance diagnostic methods

<table>
<thead>
<tr>
<th>Method type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indirect methods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma insulin during fasting</td>
<td>Simpler than direct methods</td>
<td>Moderate clamp correlation</td>
</tr>
<tr>
<td>HOMA index</td>
<td>Moderate to good clamp correlation</td>
<td>Variable cut-off points according to studied population</td>
</tr>
<tr>
<td>QUICKI</td>
<td>Good clamp correlation</td>
<td>Multiple blood samples, placement of IV catheter</td>
</tr>
<tr>
<td>Matsuda-DeFronzo index</td>
<td>More reliable IR measure</td>
<td></td>
</tr>
<tr>
<td><strong>Direct methods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperinsulinemic-euglycemic clamp</td>
<td>Gold standard to assess insulin sensitivity</td>
<td>Complex, invasive, difficult to carry out in a pediatric population: unsuitable for use in large populations or in daily clinical practice</td>
</tr>
<tr>
<td>Hyperglycemic clamp</td>
<td>Gold standard to assess insulin release</td>
<td></td>
</tr>
<tr>
<td>FSIVGT minimal model</td>
<td>Assess tissue sensitivity and insulin release</td>
<td></td>
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</tbody>
</table>

HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index, FSIVGT, frequently sampled intravenous glucose tolerance test.
Figure 1. Hyperinsulinemic-euglycemic clamp simulation. (A) Plasma glucose concentration is maintained at ~90 mg/dl for 120 min and remains stable between 75 and 120 min. Solid line shows required adjustments in glucose infusion to maintain glucose levels according to initial goal. (B) Plasma insulin concentrations during clamp; a burst occurs during the first 10 min (produced by two initial dosages), a gradual decrease in insulin concentration and finally a steady state during the remainder of the test (insulin ~100 μU/L).

Figure 2. Hyperglycemic clamp simulation. An increased plasma insulin level is observed as a response to exogenous glucose administered through infusion. Two-phase insulin release is presented with an initial burst followed by a constant increase in insulin concentration.

HOMA—%\(\beta\) = \([20 \times \text{fasting plasma insulin (μU/ml)}]/[\text{fasting plasma glucose (mmol/L)} - 3.5]\]

Another widely used method to determine IR is the use of the Quantitative Insulin Check Index (QUICKI), which is based on a logarithmic model calculated from glucose and insulin concentrations during fasting as follows:

QUICKI = \(1/[(\log \text{fasting plasma insulin (μU/ml)}) + \log \text{fasting plasma glucose (mg/dl)}]^{21}\)

It is interesting to observe that these models do not differentiate between hepatic and peripheral insulin sensitivity. The ratio between glucose and insulin concentrations during fasting reflects the balance between hepatic glucose use and insulin release maintained by β-cells and liver feedback.\(^{22}\)

Variations have been reported when correlating HOMA, QUICKI and clamp results. In general, the best associations have been observed when these indexes are estimated using three or more glucose and insulin measurements from consecutive samples obtained within 5- to 10-min intervals. Correlation coefficients have been reported with clamp ranging from 0.43 to 0.91 for QUICKI and -0.53 to -0.91 for HOMA.\(^{4,6,17,18}\) Although HOMA and QUICKI indexes correlate similarly with the gold standard for IR, HOMA has been used more widely in clinical practice.\(^{6}\) It is possible that this has led several authors to establish cut-off points to diagnose IR through this index. Although some studies in adults have suggested cut-off points starting at 2.5 to diagnose IR, HOMA is usually higher in the pediatric population, especially in preadolescents. A 3.16 cut-off point suggested by Keskin et al. to diagnose IR in children is more frequently used and widely accepted among several authors.\(^{23}\) However, several studies have observed HOMA index increases with age and preadolescent stage in children and adolescents;\(^{3}\) therefore, some authors prefer to use higher reference values.\(^{24}\) The study carried out by García-Cuartero et al. obtained an overall index of 3.43 considering several preadolescent stages.\(^{25}\)
**Oral glucose tolerance test**

Oral glucose tolerance test (OGTT) is used mainly to evaluate glucose tolerance and not to diagnose IR. Carrying out OGTT in a pediatric population requires the administration of 1.75 g of anhydride glucose/kg without exceeding 75 g. Plasma glucose concentrations are then measured at different intervals, usually 30, 60 and 120 min after administration. Subjects with glucose ≥140 mg/dl at 120 min are diagnosed with glucose intolerance. However, OGTT has a clear disadvantage for determining the risk of diabetes in adults or children when combined with overweight and obesity. According to American Diabetes Association (ADA) guidelines, OGTT should be used only to screen obese children with associated risk factors. Several studies have reported intraclass correlation coefficients of 0.34, 95% CI 0.14-0.57 and coefficient variation (CV) of 16.7% per person for glucose 2 h after administration. Similar results (CV = 14.96%) have been reported in studies carried out by our group in obese adults when two OGTT measurements are carried out within a 1-week interval (unpublished data).

Interestingly, insulin sensitivity indexes have been developed using measurements obtained from OGTT. In 1999, Matsuda and DeFronzo proposed an insulin sensitivity index based on glucose and insulin measurements obtained during an OGTT. This method is known as the Matsuda-DeFronzo index or insulin sensitivity index M (ISI-M), which is calculated according to the following formula:

\[
ISI-M = \frac{10,000}{\sqrt{(PIF*PGF)(xPGC * xPIC)}}
\]

where PIF is fasting plasma insulin (µU/ml), PGF is fasting plasma glucose (mg/dl), xPGC is average plasma glucose concentration in all curve points and xPIC is average plasma insulin concentration in all curve points.

ISI-M has reported acceptable correlation levels vs. hyperinsulinemic clamp in adults (r = 0.73). Abdul-Ghani et al. proposed a 4.5 cut-off point in adults, which is useful to predict future onset of T2DM. So far, no cut-off points have been proposed for pediatric populations.

Preliminary results from a study carried out in our laboratory confirm ISI-M usefulness to identify subjects with IR. This study describes glucose and insulin behavior during OGTT in adults classified according to two groups, one with ISI-M ≥4.5 and a second group with ISI-M <4.5. Although glucose concentrations were no different between groups throughout the curve, insulin concentrations were significantly higher in subjects with ISI-M <4.5, demonstrating a low sensitivity of this group to hormone activity after a challenge and suggesting that this index appropriately identifies IR subjects (Figure 3).

Finally, an alarming proportion of children and adolescents are now at risk of becoming diabetic or they have already been diagnosed. This is associated with an epidemic of increasing overweight and obesity. From this perspective, it is essential to carry out an appropriate and optimal examination to detect at-risk patients. In fact, the ADA recommends screening for T2DM in overweight and obese children >10 years old every 2 years when they present with one or more of the following: 1) T2DM family history, 2) belong to an at-risk ethnic group (Amerindian, Afro-Americans, Hispanic, Asians/persons from the South Pacific region) and 3) patients with IR signs or associated conditions (acanthosis, polycystic ovary syndrome, arterial hypertension, or dyslipidemia). Screening should be carried out according to glucose concentrations during fasting of after OGTT. Because no diagnostic guidelines or algorithms have been proposed to detect IR in children, we may consider using the same criteria proposed by the ADA to identify T2DM in a pediatric population. Therefore, the most appropriate method may be the HOMA index because it is a relatively simple technique with several suggested cut-off points, although we should emphasize that there is still no clear and reliable method to screen IR in the pediatric population. On the other hand, when IR is diagnosed, physicians should guide patients on risk reduction for development of T2DM by modifying their lifestyle: increasing physical activity and achieving and maintaining a healthy weight. Up to now, there are no pharmacologic alternatives for the management of IR in children. In adults who are at risk for the development of diabetes, lifestyle changes have proven more effective than metformin to reduce T2DM incidence. Childhood obesity has been accompanied by an increase in the incidence of T2DM when reaching adulthood. However, as demonstrated by previous studies, an appropriate and timely intervention may reduce T2DM incidence in high-risk patients. This fact highlights the importance of determining IR in the pediatric population; however, until now, no appropriate methods have been defined.
The gold standard for diagnosis of IR is a very complex technique with multiple limitations to be applied in the pediatric population. Other diagnostic methods such as ISI-M from the OGTT have the disadvantage of requiring multiple blood samples and complex calculations (even though they have a better correlation with clamp results). In addition, the OGTT has shown a poor replication in overweight adults and children for diagnosing diabetes risk. Other viable alternatives for testing such as HOMA and QUICKI have a limited precision, suggesting that these tests should be used only in at-risk subjects.

It would be interesting to carry out studies to seek new noninvasive diagnostic techniques or cut-off points that adjust to phenotypic and genotypic characteristics of our population. A non-invasive 13C-glucose breath test with stable isotopes is being developed in our laboratory. This is a promising test because if it is validated and reliable, it will serve to carry out community-based screening tests. However, HOMA remains the most widely used method both in clinical and scientific environments because we do not have comprehensive information about reference values for other methods such as QUICKI and OGTT indexes to diagnose IR in the pediatric population.

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