Estimation of reference intervals of insulin resistance (HOMA), insulin sensitivity (Matsuda), and insulin secretion sensitivity indices (ISSI-2) in Polish young people

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article

Abstract

INTRODUCTION

Insulin resistance is becoming an increasingly widespread problem worldwide. For its determination indirect indices of insulin resistance or insulin sensitivity have used. Those indices based on simultaneous measurements of blood glucose and insulin concentrations under fasting conditions or during the Oral Glucose Tolerance Test. Insulin resistance (IR) is defined as an impaired glucose homeostasis, in the course of which the sensitivity of target tissues to insulin is decreased. This phenomenon affects mainly the skeletal muscles (peripheral IR) and/or liver and adipose tissue cells (central IR) [1]. Current studies indicate that insulin resistance affects large population groups of working-age, and is particularly associated with overweight and obesity [2, 3]. However, an increasing number of reports show that insulin resistance may also affect young people and insulin secretion sensitivity indices (ISSI-2) in particular populations. The early detection of metabolic disorders allows for introduction of effective preventive action.

Materials and method.
191 selected participants, aged 18 – 31, were enrolled into the reference population. 130 participants from the reference population with fasting glucose ≤ 5.5 mmol/L, BMI<25 kg/m², and without metabolic syndrome, were finally included in the reference group. The following insulin resistance indices were calculated: HOMA1-IR, HOMA2, HOMA2 C-pep., and QUICKI, respectively. For insulin sensitivity, the value of the Matsuda Index was established as ≥3.19 and for beta cell pancreatic function ISSI-2 as ≥206.

Results. The reference intervals for indirect insulin resistance indices examined according to CLSI protocol were: ≤ 4.00, ≤ 2.27, ≤ 4.10, ≥ 0.31 for HOMA1-IR, HOMA2, HOMA2 C-pep., and QUICKI, respectively. For insulin sensitivity, the value of the Matsuda Index was established as ≥ 3.19 and for beta cell pancreatic function ISSI-2 as ≥ 206.

Conclusions. Establishing a reference intervals for these indices enable the proper identification and differentiation of the types of insulin resistance in particular populations. The early detection of metabolic disorders allows for introduction of effective preventive action.

Key words
insulin resistance, young adult, Oral Glucose Tolerance Test, Reference Interval

INTRODUCTION

Insulin resistance (IR) is defined as an impaired glucose homeostasis, in the course of which the sensitivity of target tissues to insulin is decreased. This phenomenon affects mainly the skeletal muscles (peripheral IR) and/or liver and adipose tissue cells (central IR) [1]. Current studies indicate that insulin resistance affects large population groups of working-age, and is particularly associated with overweight and obesity [2, 3]. However, an increasing number of reports show that insulin resistance may also affect young people with normal body weight, with no overt metabolic disorders or positive family history of metabolic disturbances [4].

Even though intravenous glucose and insulin infusion (i.e. the glucose clamp test) is still referred to as the “gold standard,” and the intravenous glucose tolerance test is recommended, these methods are very rarely used because of their high onerousness for the patient [5]. For this reason, in clinical practice, indirect indices of insulin resistance have been used, based on simultaneous measurements of blood glucose (FG) and insulin concentrations under fasting conditions or after oral glucose administration – Oral Glucose Tolerance Test (OGTT) [6]. The most widely used method for IR recognition is the measurement of fasting glucose and insulin concentration, followed by the calculation of appropriate indicators according to the relevant formulas, such as the Insulin Glucose Index, or indices derived from Homeostasis Model Assessment (HOMA) [7]. Detecting abnormalities in these parameters may be the first sign of metabolic disturbances, especially impaired glucose homeostasis, and an indication for future detailed diagnosis by performing additional tests (i.e. OGTT). Measurement of glucose and insulin during the oral glucose tolerance test and calculation of the Matsuda Index enables estimation of the ability to
utilize large amounts of exogenous glucose by peripheral tissues, mainly muscle cells, whereas the Insulin Secretion-Sensitive Index-2 (ISSI-2) measures the ability of β-cells to compensate for changes in whole-body insulin sensitivity by a change in insulin secretion [1]. All these indices enable the identification and differentiation of types of insulin resistance. Indices calculated in the fasting state describe the balance between liver production of glucose and pancreatic insulin under this condition, while the ability of glucose uptake through muscle tissue, and the ability of beta cells to compensate for peripheral insulin resistance, are described by indicators determined by a functional test (OGTT), which mimics the post-prandial state [8].

In the scientific literature, the reference values for indirect indices used for insulin resistance diagnosis differ according to the studied population, age, gender and ethnicity, as well as the laboratory methods used for glucose and insulin concentration determination [3, 9, 10]. Especially confusing and ambiguous is IR recognition concerning young people, on whom such studies are not routinely performed. Very little data is available on either the prevalence of insulin resistance or cut-off values for the above-mentioned indices for the young Polish population [11, 12]. The most common values of insulin resistance indices used for identification of these disturbances extant in the scientific literature are established as the upper 75th (95th) or below the 25th (5th) percentile values observed in different analysed populations. This is not exactly in accordance with current laboratory guidelines, but is very widely applied in epidemiological studies [1, 10, 13]. For this reason, it is important to define reliable reference intervals (RIs) for the indices used in insulin resistance diagnosis in this age group, which may enable the early detection of metabolic disorders and the introduction of preventive action.

**OBJECTIVE**

The aim of this study was to estimate the reference intervals for the most common insulin resistance indices, such as HOMA1-IR, HOMA2 and HOMA2 C-pep. [7], QUICKI [14], Matsuda Index [15] and ISSI-2 [16], using the standard method based on the procedure of determining reference interval (RI) recommended by the Clinical and Laboratory Standards Institute (CLSI) in document C28-A3 from 2008 [17]. Moreover, the cut-off values of the 25th or 75th percentile, respectively, for these indices were established in the same study group in order to facilitate comparison of the analyzed indices with other studies and literature data.

**MATERIALS AND METHOD**

349 participants (260 females and 89 males) in an apparently good state of health were initially enrolled in the study "Determination of the reference intervals for the HOMA Index, Matsuda Index and Disposition Index in young people", financed by a grant from Wroclaw Medical University and conducted in 2016–2017 [18].

Participants, the majority of whom were current or former university students, were given information about recruitment from advertisements distributed at the universities of Wroclaw. After being informed about the purpose and procedure of the study, all took part in the research voluntarily and gave their written consent. The study protocol was approved by the Bioethics Committee of Wroclaw Medical University (537/2018). The criteria for inclusion in the study were: age 18–31, willingness to participate in the study and good overall health. Exclusion criteria were a history of diabetes, liver or kidney failure, past cancer, acute infections during the 2 weeks preceding the study, or taking anti-allergic drugs during the 3 months preceding the study, as reported by the participants. Before the study, all participants completed a specific author's questionnaire, in which data about smoking status, physical activity, health condition (subjectively assessed), current medication intake and family history of metabolic disorders were collected. After completing the questionnaire, participants underwent a physical examination which included anthropometric measurements, such as height, weight, and waist circumference.

Although the current study is generally based on the same set of participants as a previous study [18], the aim and results take into consideration a completely different research issue. The research problem presented in this study has been resolved strictly in accordance with global guidelines, and applies only to the participants who agreed to perform OGTT, which was obligatory for the Matsuda Index and ISSI-2 calculations. Among the total number of 349 participants, 191 (138 females and 53 males) performed OGTT, and only these participants were selected as the reference population in the presented study. Before fasting blood samples were taken and OGTT performed, each participant had their glucose concentration in capillary blood checked by a glucometer, and further collection of venous blood was performed if the capillary glucose was ≤ 7.0 mmol/L. Three-point OGTT was then performed with 75 glucose ingestion in 250 ml of water. Finally, 130 participants (106 females and 24 males) from the reference population with fasting glucose ≤5.5 mmol/L, BMI<25 kg/m², and without metabolic syndrome diagnosis, were included in the reference group. Venous blood samples were drawn from each participant using the S-Monovette system (Sarstedt, Nümbrecht, Germany) in the fasting state (0' start point) at 60th min. (middle point) and 120th min. (end point) during OGTT and placed into 2 tubes, the first one containing a clotting activator and the second containing an anticoagulant (K3EDTA), to obtain serum and plasma, respectively.

At all study points, plasma glucose was determined by the GOD/POD method (Thermo Electron Oy, Vantaa, Finland), Lipid parameters: total cholesterol (TC) by the CHO/POD method (DiaSys, Holzheim, Germany), high-density lipoprotein (HDL-C), by the AB-Wako method (DiaSys, Holzheim, Germany), triglycerides (TG) by the GPO/POD method (DiaSys, Holzheim, Germany) in the blood serum were determined in the fasting state. All biochemical parameters were measured using the Konelab 20i (ThermoScientific, Vantaa, Finland) biochemical analyser. Intra- and inter-assay coefficients of variation for FG were 1.13% and 1.99%, for TC 1.72% and 2.27%, for HDL-C 1.33% and 2.42%, for TG 1.74% and 4.08%, respectively. Low-density lipoprotein (LDL-C) was estimated by the Friedewald equation. Insulin at all OGTT points was determined by the enzyme-linked immunosorbent assay (ELISA), using a DRG Instruments GmbH (Marburg, Germany) reagent kit (standards calibrated against international WHO approved...
reference material NIBSC 66/304). Serum C-peptide in the fasting state was determined by the enzyme-linked immunosorbent assay (ELISA), using a DRG Instruments GmbH (Marburg, Germany) reagent kit (standards are calibrated against international WHO approved reference material IRR C-peptide, code 84/510). Multiscan GO microplate reader (Thermo Fisher Scientific, Oy, Finland) was used for insulin and C-peptide measurement, and intra- and inter-assay coefficients of variation for insulin and C-peptide were 5.5%, 8.7% and 7.1%, 11.4%, respectively. On the basis of glucose and insulin concentrations under fasting conditions, the following indices were calculated: HOMA1-IR, HOMA2, HOMA2 from C-peptide, QUICKI, as described previously [18]. Moreover, Matsuda Index and ISSI-2 were calculated on the basis of OGTT glucose and insulin results by on-line calculator [18] and mathematical formula [16], respectively. Determination of reference limits for selected indirect indices of insulin resistance was conducted in accordance with the procedure recommended by CLSI and published in the C28-A3 document in 2008 [17]. The process for establishing reference ranges was carried out in accordance with the protocol shown in Figure 1.

Even though protocol C28-A3 is mainly dedicated to 2-sided reference interval determination, it was decided to establish in this study 1-sided reference limits for the indirect insulin resistance indices analyzed. In the authors’ opinion, this is a more appropriate goal for clinical practice purposes, and although it is less frequently used, this method is in line with C28-A3 protocol.

**Statistical analysis.** The normality of the anthropometric, clinical and biochemical parameters in the male and female groups was checked by the Shapiro-Wilk test. Based on the results, the Mann-Whitney test was applied for comparison of these parameters between females and males. The Reed test was applied to truncated reference values from outliers, but none were revealed. The one sided reference limits for HOMA1-IR, HOMA2, HOMA2 C-peptide as 95th
percentile with 95% CI were established, when for QUICKI, Matsuda Index and ISSI-2 the one sided reference limit as the 5th percentile with 95% CI were established. This procedure was in accordance with CLSI protocol and carried out using the MedCalc 18.6 applications (Ostende, Belgium).

RESULTS

The results of the anthropometric, clinical and biochemical parameters of the reference group, with comparative analysis between females and males, are presented as medians with a 25–75 percentile range (Q1-Q3) in Table 1. When developing the research plan, it was not assumed that the participants were divided into groups with relation to gender, because there was no information on the differences in values of insulin resistance indicators in the available literature. Moreover, the values for glucose concentration, BMI and metabolic syndrome considered in the exclusion criteria were the same for males and females. Nonetheless, analysis results revealed physiological differences in values of waist circumference and HDL cholesterol between males and females. Moreover, levels of BMI, SBP, FG and TG were significantly higher in males compared to females, while glucose and insulin concentration at the 120th minute of OGTT was higher in females (Tab. 1). However, no significant differences were observed for the analyzed insulin resistance, insulin sensitivity or insulin secretion-sensitivity indices. Due to the absence of significant differences between females and males for comparison values, it was possible to designate the RIs for the analyzed indices, taking into account the fact that the whole reference group consisted of 130 participants without gender partitioning. Table 2 shows the 5th, 25th, 50th, 75th and 95th percentiles for analyzed indices. Values were established of 1-sided reference intervals, according CLSI as equal and lower than 95th percentile (number of participants: 123) for all HOMA indices, and equal and higher than the 5th percentile (number of participants: 124) for QUICKI, Matsuda and ISSI-2 indices. The 1-sided reference limits for the insulin resistance indices were: ≤ 4.00, ≤ 2.27, ≤ 4.10, ≥ 3.19 and ≥ 206 for HOMA1-IR, HOMA2, HOMA2 C-pep., and QUICKI, respectively, when for insulin sensitivity Matsuda Index this value was established as ≥ 3.19 and for beta cell pancreatic function ISSI-2 as ≥ 206. The cut-off values for insulin sensitivity Matsuda and ISSI-2 indices. The 1-sided reference limits for the insulin resistance indices were: ≤ 4.00, ≤ 2.27, ≤ 4.10, ≥ 3.19 and ≥ 206 for HOMA1-IR, HOMA2, HOMA2 C-pep., and QUICKI, respectively and <4.31 and <261 for Matsuda and ISSI-2, respectively. The values of the upper 75th percentile (number of participants: 97) for HOMA indices and below the 25th percentile (number of participants: 32) for QUICKI, Matsuda and ISSI-2 indices are presented for comparison with results obtained in Polish young adults with data from the other publications in which such values are commonly used for IR recognition (Tab. 2).

DISCUSSION

Insulin resistance indices are useful not only for IR recognition in high-risk individuals, but also in apparently healthy people, especially those with normal or near-normal glycaemia, as shown by long-term epidemiological evidence. Identification of insulin-resistant people with a significantly increased risk of overt dysglycaemia could provide significant clinical and economic benefits [20]. Generally, reference intervals are used in the diagnostic process as reference points for the interpretation of the laboratory parameters values obtained for patients who are suspected to have abnormalities [21, 22, 23]. Nonetheless, there is still a lack of adequate procedures for insulin resistance diagnosis, especially in young people, on the basis of appropriate reference intervals. From this aspect, the presented study is important and of
Table 2. Percentile values for the analyzed indices.

<table>
<thead>
<tr>
<th>Indices</th>
<th>5th percentile</th>
<th>25th percentile</th>
<th>75th percentile</th>
<th>95th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=6</td>
<td>n=32</td>
<td>n=97</td>
<td>n=123</td>
</tr>
<tr>
<td>HOMA1-IR</td>
<td>1.14</td>
<td>1.84</td>
<td>2.78</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>0.80–1.35</td>
<td>1.77–2.00</td>
<td>2.61–3.10</td>
<td>3.45–4.21</td>
</tr>
<tr>
<td>HOMA2</td>
<td>0.78</td>
<td>1.14</td>
<td>1.72</td>
<td>2.27</td>
</tr>
<tr>
<td></td>
<td>0.59–0.84</td>
<td>1.08–1.21</td>
<td>1.56–1.79</td>
<td>2.03–2.45</td>
</tr>
<tr>
<td>HOMA2 C-pep.</td>
<td>1.16</td>
<td>1.53</td>
<td>2.63</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td>0.89–1.22</td>
<td>1.37–1.66</td>
<td>2.35–3.08</td>
<td>3.64–5.24</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.31</td>
<td>0.33</td>
<td>0.35</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>0.31–0.32</td>
<td>0.32–0.33</td>
<td>0.34–0.37</td>
<td>0.36–0.4</td>
</tr>
<tr>
<td>Matsuda Index</td>
<td>3.19</td>
<td>4.31</td>
<td>7.48</td>
<td>11.33</td>
</tr>
<tr>
<td>ISSI-2</td>
<td>206</td>
<td>261</td>
<td>370</td>
<td>502</td>
</tr>
<tr>
<td></td>
<td>181–221</td>
<td>247–269</td>
<td>344–394</td>
<td>441–647</td>
</tr>
</tbody>
</table>

ISSI-2 – Insulin Secretion-Sensitive Index 2, HOMA – Homeostasis Model Assessment; QUICKI – Quantitative Insulin Sensitivity Check Index; n – number of participants in 5th, 25th, 75th and 95th percentile, respectively.
daily clinical practice, due to the lack of clear criteria for the diagnosis of these conditions based on indirect indices of insulin resistance. The use of different criteria for the setting of reference and/or cut-off values determines the achievement of different IR recognition frequencies. It should be emphasized that the CLSI protocol used in the current study for establishing reference intervals is still not widely used in laboratory medicine. Therefore, the authors decided to discuss the results of their study as results observed for the 6th and 123rd ranked patients’ values, which correspond to the 5th and 95th percentiles according to CLSI protocol, as well as the results observed in the 32th and 97th ranked patients, which correspond to the 25th and 75th of all 130 participants included in the analysis for the purposes of comparison with data in the literature. This is indicated by the importance of the use of unified, recommended criteria to determine these values. As provided by the data from the literature, the values of IR indices might also differ between nations, because heterogeneity in ethnicity contributes to discrepancies in the degree of insulin resistance. However, it is unreasonable to directly compare these studies unless HOMA1-IR reference limits are determined by a standardized procedure [9]. The interchangeable use of cut-offs (established as the 75th or 90th percentile) and reference intervals, causes discrepancies in the interpretation and adoption of appropriate index values, and it is therefore invariably important to use the same procedures, e.g. according to the CLSI. Establishing reference intervals in a similar way to their use in this study will facilitate the use of insulin resistance indices in routine clinical practice. This approach will enable the accurate identification of individuals at risk of metabolic diseases and the development of personalized therapeutic interventions.

**Study limitations.** The authors are aware of the basic limitations of the presented study with regard to the laboratory methods used. While glucose methodology is almost perfectly standardized, the methods of insulin determination used in laboratories are very diverse, which is very much due to the lack of an international standard. For this reason, the trimmed-out reference limit has an application in laboratories using the same methodology. Another significant disadvantage is the limited number of participants in the study, particularly in relation to the male group, which was due to the much lower reporting of males during the study. The narrow age range of study participants also limited the use of the reference intervals of the analyzed indices established. However, the young age of participants in this study could also be counted as an advantage, because to the best of the knowledge of the authors, no such extensive publications on insulin resistance indices concerning this age group exist.

**CONCLUSIONS**

The reference intervals obtained, when applying the same laboratory methods for HOMA1-IR ≤4.00, HOMA2 ≤2.27, HOMA2 C-pep≤4.10, QUICKI ≥0.31, Matsuda Index ≥3.19 and ISSI-2 ≥2.06, can be used for recognition of insulin resistance, reduced insulin sensitivity and decreased ability of the pancreas beta cell, respectively, in a Polish population aged 18–31. Establishing cut-off values for these indices is significant regarding the possibility of comparing results with those of other authors, due to the scant information about reference values in epidemiological studies. To the best of the authors’ knowledge, this is the first reference interval study of indirect insulin resistance indices for a young Polish population, which follows the stringent CLSI document. This is especially important for clinical practice, due to the lack of appropriate guidelines dedicated to this age group. For this reason, it is crucial to determine the indicator RI’s relative to populations and particular medical laboratories. In order to improve the diagnostic process of insulin resistance recognition, population studies should be performed and the reference intervals and decision limits for insulin resistance, insulin sensitivity and pancreatic beta cell function for different age groups should be determined.

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