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Age- and sex-specific reference values for fasting serum insulin levels and insulin resistance/sensitivity indices in healthy Iranian adults: Tehran Lipid and Glucose Study

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ABSTRACT

Objectives: Increased insulin concentration is a surrogate for insulin resistance and early assessment of fasting insulin may help in identifying those who are potentially at high risk of type 2 diabetes, hypertension, and cardiovascular disease. The aim of this study was to determine age- and sex-related reference values for serum insulin and insulin resistance/sensitivity indices in Iranian subjects.

Design and methods: Serum insulin levels were measured by electrochemiluminescence immunoassay in 5786 participants of the Tehran Lipid and Glucose Study. After application of exclusion criteria, 309 non-obese healthy subjects (124 men and 185 women), aged 24–83 y, were included. The International Federation of Clinical Chemistry guidelines (non-parametric method) and the robust method were used for determining reference values.

Results: Overall 95% reference values for fasting insulin were 1.61–11.37, 2.34–11.98, and 2.11–12.49 μU/mL in men, women, and total population respectively. Mean fasting insulin concentration showed a decreasing trend with age in both genders (p for trend ≤ 0.001). Age, waist circumference, and systolic blood pressures were biological determinants of fasting insulin in both genders; in addition, insulin was modulated by triglycerides in men and fasting glucose in women. Reference intervals for HOMA1-IR, HOMA2-IR, and QUICKI were 0.63–2.68, 0.40–1.80, and 0.33–0.42, respectively.

Conclusion: This study presents the first set of reference values for fasting serum insulin to be 2–12 μU/mL for both genders in a healthy sample of Iranian adults along with the reference values for insulin resistance/sensitivity indices. These values could be used for identifying subjects with insulin resistance in epidemiological and clinical research.

Introduction

At present, investigating the pathogenesis of hypoglycemia is almost the only clinical indication of serum insulin measurement [1,2]. The diagnosis of insulinoma, as a cause of hypoglycemia, requires the presence of Whipple's triad (symptoms, signs, or both of hypoglycemia, a low measured plasma glucose concentration, and resolution of symptoms and signs after increasing the plasma glucose), and plasma insulin ≥3 μU/mL, plasma C-peptide ≥0.6 ng/mL, and plasma pro-insulin ≥5 pmol/L in the presence of plasma glucose <3 mmol/L [3]. Regardless of limited clinical utility, serum insulin is frequently measured for research purposes [1,2]; increased insulin concentration is a surrogate marker to estimate insulin resistance [1]. Recently, Xun et al. in a meta-analysis of prospective cohort studies found that an elevated fasting insulin concentration was significantly associated with an increased risk of hypertension and coronary heart disease [4]. Although, serum insulin has no clinical value in the diagnosis of diabetes [2], many studies showed that insulin level per se is an independent risk factor of type 2 diabetes [5–8]. Hence, early assessment of fasting insulin may help clinicians to identify those who are potentially at high risk of type 2 diabetes, hypertension, and cardiovascular disease (CVD). In

Abbreviations: BMI, body mass index; CLSI, Clinical and Laboratory Standards Institute; CV, coefficients of variation; CVD, cardiovascular disease; ECLIA, electrochemiluminescence immunoassay; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; IFCC, International Federation of Clinical Chemistry; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; TG, triglycerides; TLGS, Tehran Lipid and Glucose Study; WC, waist circumference.

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addition, other endocrine disorders such as hyperthyroidism and Cushing's syndrome are also related to higher serum insulin levels [9].

Hyperinsulinemic euglycemic clamp is the most reliable direct method for estimating insulin resistance [10,11]. However, this method is invasive, expensive, time-consuming, and needs experienced personnel, which limit its clinical utility [11,12]. On the other hand, surrogate indices of insulin resistance/sensitivity such as the homeostasis model assessment-insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) are simple, inexpensive, and validated alternative tools, which are calculated from simultaneous fasting glucose and insulin concentrations [10,11]. Both high levels of HOMA-IR and low levels of QUICKI have been suggested to be related with incident diabetes in many studies [13–15].

Few studies have reported reference values for serum insulin concentrations and insulin resistance/sensitivity indices. In addition, serum insulin reference values supplied by kit producers are based on small numbers and most often are difficult to interpret [2,16]. The aim of this study was to determine age- and sex-specific reference values for serum insulin concentrations and insulin resistance/sensitivity indices in healthy adult Iranian.

Subjects and methods

Subjects

This analysis has been conducted on data of participants of the Tehran Lipid and Glucose Study (TLGS), which is a prospective study being conducted with the aim of determining the prevalence of non-communicable disease risk factors. The baseline survey was performed between 1999 and 2001 (phase 1), when a multistage stratified cluster random sampling technique was used to select 15,005 persons, aged over 3 y. All participants were followed-up at three-year intervals [17,18]. In the fourth phase of the TLGS, July 2008 to October 2010, insulin was measured for 5786 participants, aged 23–94 y. Participants with any components of metabolic syndrome defined by the Joint Interim Statement [19] were excluded; however, waist circumference (WC) cut-off points for Iranian men and women according to the first report of the Iranian National Committee of Obesity were used [20]. Participants with diabetes mellitus, history of CVD, renal dysfunction (creatinine >123.8 μmol/L), chronic diarrhea, cancer, significant weight loss during past 6 months, and hospitalization during the past 3 months were excluded; in addition, smokers (smoked ≥1 cigarette per day or used the waterpipe), pregnant and lactating women, and subjects using diuretics, calcium channel blockers, angiotensin converting enzyme inhibitors, beta-blockers and other cardiac-related drugs, steroids, female and male hormones, thyroid medications, or aspirin, and finally subjects with missing data were excluded. After application of exclusion criteria, 309 subjects (124 men and 185 women, aged 24–83 y) were included in the study. Before estimating the reference values, outliers were removed from the data set; outliers may partly be due to subjects with unknown medical problems leading to widening of the reference intervals [21]. Separate analysis was done for 17 healthy menopausal women. This study was conducted in accordance with the principles of the Declaration of Helsinki and the proposal of the study was approved by the local ethics committee of the Research Institute for Endocrine Sciences of Shahid Beheshti University of Medical Sciences and informed written consent was obtained from all participants.

Anthropometric, clinical, and laboratory assessments

Details of data collection in TLGS have been published previously [18]; in brief, weight and height were measured according to standard protocols. Body mass index (BMI) was calculated as weight (kg) / [height (m)]^2. Two measurements of systolic and diastolic blood pressure were performed using a standardized mercury sphygmomanometer on the right arm after a 15 min rest in a sitting position; the mean of the two measurements was considered the subject's blood pressure.

Blood samples were collected between 7:00 and 9:00 AM after 12–14 hour overnight fasting using anticoagulant-free tubes and centrifuged at 3000 rpm for 10 min within 30–45 min of collection; all blood analyses were done at the TLGS research laboratory on the day of blood collection. For the oral glucose tolerance test, a solution containing 75 g anhydrous glucose was administered orally to subjects and a blood sample was taken 2 h later.

Serum glucose, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using the enzymatic colorimetric method. For glucose measurement, glucose was oxidized to gluconic acid and H_2O_2 by glucose oxidase. For TC assay, cholesteryl ester was converted to cholesterol by cholesteryl ester hydrolase; cholesterol was oxidized by cholesterol oxidase to cholesterol-4-en-3-one and H_2O_2. For TG measurement, glycerol was released from TG by lipoprotein lipase followed by phosphorylation of glycerol to glycerol phosphate by glycerokinase; glycerol phosphate oxidase converts glycerol phosphate to dihydroxyacetone phosphate and H_2O_2. Measurement of HDL-C was done after precipitation of the apolipoprotein B containing lipoproteins with phosphotungstic acid and magnesium ions. In all the above-mentioned assays, the colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by H_2O_2 and measured at 546 nm. Low-density lipoprotein cholesterol (LDL-C) concentrations in samples with TG <4.52 mmol/L were calculated with the Friedewald equation; LDL-C = TC − HDL-C − TG / 5 [22]. Serum creatinine was measured using the photometric Jaffe method in which creatinine reacts with picrate in an alkaline medium to yield an orange–red color, read at 505 nm. The analyses were performed using commercial kits (Pars Azmoon Inc., Tehran, Iran) and a Selectra Pro M auto-analyzer (Vital Scientific, Spankeren, Netherlands). The quality of assays was monitored using assayed serum controls in two different concentrations. Intra- and inter-assay coefficients of variation (CV) were both less than 2.0% for glucose, 1.8% for TC and TG, 2.9% for HDL-C, and 2.9% for creatinine.

Fasting serum insulin was determined by the electrochemiluminescence immunoassay (ECLIA) method, using Roche Diagnostics kits and the Roche/Hitachi Cobas e-411 analyzer (GmbH, Mannheim, Germany). Lyophilized quality control material (Lyphochek Immunoassay Plus Control, Bio-Rad Laboratories) was used to monitor accuracy of assays; intra- and inter-assay CVs were 1.2% and 3.5%, respectively. A pooled serum was prepared, aliquoted and stored at −80° and assayed in all runs for calculating inter-assay CVs; intra-assay CVs were calculated every 10 runs using 8 repeated measurements of pooled serum and the largest one was considered as intra-assay CV.

Determining outliers

The Dixon outlier range statistic was used for determining outliers as it has been recommended by the Clinical and Laboratory Standards Institute (CLSI) [23]. In the Dixon test, if the D/R ratio exceeds 1/3, the extreme value is considered as outlier and should be deleted, where D is the absolute difference between the most extreme value and the next most extreme value, and R is the range of the values.

Determining serum insulin reference values

For determining reference values, the CLSI/International Federation of Clinical Chemistry (IFCC) guidelines (non-parametric method) and the robust method were used for sample sizes ≥120 and <120 respectively [24,25]. The retrospective (posteriori) selection of individuals from a population-based study was used as it is considered ideal for the study of exclusion and partitioning criteria according to IFCC [26]. For the IFCC non-parametric method, values were sorted in ascending order and rank numbers were assigned to values. Rank numbers of