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A prospective guide for clinical implementation of selected OGTT-derived surrogate indices for the evaluation of β-cell function and insulin sensitivity in patients with transfusion-dependent β-thalassaemia

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Abstract. The gold standard for the measurement of insulin secretion is the hyperglycemic clamp and for insulin sensitivity the hyperinsulinemic euglycemic clamp, respectively. Their disadvantages are the non-physiological route, the complexity of protocols requiring an experienced operator and the high costs; thus, they are mainly used for research purposes. A number of surrogate indices, derived from plasma glucose and insulin levels at a fasting state or after oral glucose load, have been proposed to estimate β-cell response, and the ability of β-cells to compensate for changes of insulin sensitivity by modulating insulin secretion (disposition index). Starting from the current recommendations for the annual screening of glucose dysregulation in patients with transfusion dependent β-thalassemia (β-TDT), this article summarizes the most frequently used indirect indices of insulin secretion and resistance derived from the oral glucose tolerance test (OGTT) and discusses the strengths and weaknesses of selected indices and the basic concepts underlying each method for the appropriate evaluation of glucose regulation. Basal indices for β-cell function and insulin sensitivity, albeit simple and cheap, have limited usefulness due to a high coefficient variation and the lack of data about response to glucose load. Therefore, measurement of indices during an OGTT, despite being costly and time-consuming, is suggested since it can detect, even subtle, dynamic changes in insulin secretion and glucose handling. In patients with β-TDT, the indices derived from OGTT may offer an additional factor to evaluate the efficiency of iron chelation therapy and detect patients who may need intensification of iron chelation therapy and/or pharmacological intervention.(www. actabiomedica.it)

Key words: Transfusion dependent β-thalassemia (β-TDT), oral glucose tolerance test (OGTT), insulin sensitivity, insulin secretion, disposition index, surrogate indices, recommendations

Background

Glucose dysregulation (GD) is a common complication in patients with transfusion dependent β-thalassemia (β-TDT). GD usually occurs during adolescence and the incidence increases with age (1). The severity and type of glucose disturbances vary greatly in different studies (2). The highest prevalence of impaired

fasting glucose (IFG) reported in a meta-analysis, including a total of 35 studies from 1994 to 2018, was found to be in the Middle East (27.8%) and the highest prevalence of impaired glucose tolerance (IGT) on the Mediterranean coast (15.1%). The overall prevalence of diabetes mellitus (DM) was 6.5% (2). β-TM patients with IGT and IFG plus IGT are at higher risk for developing a type of DM which has many similarities with type 2 diabetes mellitus (T2DM) and a few with type 1 diabetes mellitus (T1DM) (1).

The pathogenesis of GD in β-TDT is complex, multifactorial and not completely understood. The primary defect of GD appears to be a low degree insulin resistance (IR), as estimated by HOMA-IR, followed by an insulin secretion defect (3). However, it should be noted that the cohort of these patients was very heterogenous group with some patients exhibiting mainly insulin deficiency and others predominantly IR (3-8). The defect in β-cell insulin secretion is mainly due to the toxic effects of iron deposition in the pancreas (1,3,4) and IR has been attributed to iron deposition in both liver (where iron deposits may interfere with insulin's ability to suppress hepatic glucose production) and muscle (where iron deposits may decrease glucose uptake because of muscle damage) (3,6-8).

In addition to iron overload, other risk factors for developing GD in β-TM patients include: chronic hypoxia due to anemia (1,3), chronic liver disease (1,9), overweight/obesity (10), associated endocrine complications (1), zinc deficiency (11), genetic factors (12,13), pancreatic fatty replacement (14), splenectomy (15), low insulin growth factor -1 (IGF-1) (16) and reduced physical activity (17).

Taking into consideration that pancreatic iron loading starts in early childhood, mainly in patients receiving large quantities of red blood cells and suboptimal iron chelation (18), early identification of GD and a precise characterization of insulin secretion, sensitivity and β-cell function (19-22), provide useful information of susceptible patients prior to the onset of GD. This is essential, facilitating measures to prevent or, at least, delay the deterioration of glucose homeostasis .

Based on our recent preliminary study and analysis of 11 patients with β-TDT (mean age 25.1 ± 5.7 years), the risk of progression from prediabetes to incipient DM in patients with β-TM is secondary to a defect of pancreatic early β-cell response to glucose load, reduced insulin sensitivity and reduced ability of β-cells to compensate for changes of insulin sensitivity (IS) by modulating insulin secretion (disposition index) (23).

The gold standard for the measurement of insulin secretion and insulin sensitivity (IS) are the hyperglycemic clamp (HC) and the hyperinsulinemic euglycemic clamp (HEC), respectively (24-26). Their disadvantages include the non-physiological route of tests, the complexity of protocols requiring an experienced operator and the high costs (26). The minimal model analysis based on frequent samples after intravenous glucose tolerance test (fs-IVGTT) is easier than the glucose clamp method, but it still involves intravenous infusions with multiple blood sampling as in the glucose clamp (26). These disadvantages have not favoured the extensive clinical application of these tests and have promoted the development of different approaches to the evaluation of insulin secretion and IS, in both clinical and research areas.

Compared to glucose clamp or IVGTT, oral glucose tolerance test (OGTT) is a simpler and less expensive alternative method that provides an estimation of β-cell response, IS and the ability of β-cells to compensate for changes of IS by modulating insulin secretion from blood insulin and glucose concentrations, both under fasting conditions (steady state) and during the test (dynamic) using simple equations (28- 30).

Starting from the current recommendations for the screening of GD in β-TM patients, this article summarizes the most useful indirect indices of insulin secretion and IR derived from OGTT with the aim of understanding the strengths and weaknesses of selected indices and the basic concepts underlying to each method.

Standard OGTT screening for GD in thalassemia

The assessment of pancreatic β-cell function in vivo is complex because of its interplay with other key variables of glucose homeostasis, i.e. blood glucose levels, IS and hepatic insulin extraction. Following glucose ingestion, the liver is the first major organ that plays a critical role in insulin metabolism; the increase

of plasma glucose (PG) stimulates insulin secretion into the portal circulation. Insulin that is not metabolized by the liver reaches the rest of the body where it restores normoglycemia. Substantially, 80% of endogenously secreted insulin is cleared by the liver (~50% at first pass), ~15% is cleared by the kidney and ~5% by muscle and adipose tissue (31,32) .

According to the "Standards of Medical Care in Diabetes", published by the American Diabetes Association (ADA) (33) and the World Health Organization (WHO) guidelines (available from: https://www.who.int/publications/i/item/9789241565257), diabetes may be diagnosed based on the concentration of PG, either with fasting plasma glucose (FPG) or 2-h PG during OGTT (1.75 g/Kg body weight, max. 75 g) or based on glycated hemoglobin A1c (HbA1c) concentration.

Prediabetes is an intermediate hyperglycemic state in which glycemic markers, such as PG and HbA1c, are above the threshold considered normal, but below the diagnostic criteria for DM. In addition, age, race, ethnicity, and any clinical condition that alters the lifetime of erythrocytes or hemoglobin levels can alter HbA1c independent of PG concentration (34).

In mild to moderately iron overloaded β-TM patients, a routine OGTT screening (at 0, 30, 60, and 120 minutes for plasma glucose and insulin measurements, after an overnight fast for 10-12 hours) is recommend at 10, 12, 14, and 16 years and annually thereafter (35,36), following the ADA criteria (33). According to these criteria, the cut-off value for the diagnosis of IFG is between 100-125 mg/dL (5.6–6.9 mmol/l) compared to 110-125 mg/dL [6.1-6.9 mmol/l]) of WHO.

An extended duration of 3-h OGTT may be requested when some particular mathematical modelling is utilized to calculate derived indices of insulin secretion and sensitivity.

In the early stages of GD, fasting and 2- h PG levels during an OGTT may be normal or slightly elevated. The use of continuous glucose monitoring system (CGMS) is a useful and valid tool in detecting hyperglycaemia (37,38). Nevertheless, studies with a larger sample size are needed in order to collect additional information to establish a consensus on screening parameters/thresholds, e.g. the number of glucose elevations required to indicate prediabetes or DM and its correlation with clinical outcomes (39).

There is a myriad of variables that affect the reproducibility and accuracy of the OGTT in terms of the total testing process (pre-analytical, analytical and post-analytical phases). To minimize the influence of these factors, a good laboratory test should conform to the specific analytical regulatory criteria, as recommended by the National Academy of Clinical Biochemistry (NACB). In particular, for glucose measurement, the recommended targets are imprecision < 2.9%, bias < 2.2%, and total maximum allowable error <6.9%. Nevertheless, even within these targets, there is no precise absolute estimate of the OGTT glucose levels, and this theoretically impacts GD prevalence (40).

Alongside this, age, gender, adiposity, ethnicity, and variability in the rate of gastric emptying and glucose absorption from the gastrointestinal tract, affect glucose tolerance and insulin levels (41,42). Additionally, insulin concentration after OGTT is influenced by the gastrointestinal incretin hormones [glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)] that are released from the gut into the bloodstream, where they bind to their specific receptors on the pancreas to regulate the amount of insulin (from 50% to 70%) and glucagon secreted for regulating blood glucose levels after meal intake (43,44). In T2DM patients, the incretin effect is severely reduced and the impairment is thought to explain an important part of the impaired insulin secretion seen in T2DM (45).

Use and interpretation of selected OGTT-derived surrogate indices of insulin response and insulin sensitivity

Numerous indices derived from fasting glucose or OGTT have been proposed to evaluate insulin response and IS, ranging from complex to simple indices (27-30). Each of these methods has distinct advantages and limitations. Some methods rely on steady-state analysis of glucose and insulin, whereas others rely on dynamic testing. Surrogate indices based on changes in insulin and glucose during the OGTT incorporate both hepatic and peripheral IS; hepatic glucose production changes most during the

first hour of the OGTT and peripheral glucose uptake is best measured during the second hour (46). The usefulness of surrogate indices is influenced by the degree of correlation with gold standard indicators, by their reproducibility and their ability to predict incidence of diabetes similarly to more complex methods. Thus, optimal choice and employment of a specific method depend on the nature of the study (47,48).

a. Assessment of early β-cell response

Insulin release in response to OGTT occurs in two phases: the early phase peak within the first 15-30 min that is responsible for limiting the initial rise of glucose, and the late phase of insulin response occurring later than 30 min after oral glucose intake, that may persist for several hours. This delayed burst of insulin response is responsible for returning glucose to baseline fasting levels. Of note, when glucose is administered intravenously, plasma insulin concentrations increase rapidly with a peak from 2 to 4 min, decrease within 10 to 15 min (first-phase insulin response), and gradually increase over 120 min (second-phase insulin response) (24-26).

The 1st phase of insulin response is primarily mediated at the level of the liver, allowing prompt inhibition of endogenous glucose production (EGP) and the 2nd phase of insulin response reduces EGP as in the 1st phase, but to a lesser extent (49,50). It is well known that the first phase of insulin response plays an important role in priming the liver and inhibiting endogenous glucose production during OGTT (38). Thus, it is well accepted that a reduction in first-phase insulin response is the earliest detectable and one of the most sensitive indicators of a defect in β-cell function in subject destined to develop T2DM (51).

The most common surrogate indices of β-cell response, reported in the literature, include: *the homeostatic model assessment of β-cell function* (HOMA-β: fasting plasma insulin [(μIU/mL) × 360/ (fasting plasma glucose (mg/dL) −63)] (52,53); the early insulin response also known as the *insulinogenic index* (GI: 30-min insulin – fasting insulin/(30-min glucose – fasting glucose in mg/dL) (54*); the corrected insulin response* (CIR: 100 × 30-min insulin)/(30-min glucose × [30-min glucose – 70 mg/dl]) (55); *the Stumvoll first-phase* $(1283 + 1.829 \times Ins_{30} 138.7 \times Glu_{30} + 3.772 \times Ins_0$ and *second-phase Stumvoll* $(286 + 0.416 \times Ins_{30} - 25.94 \times Glu_{30} + 0.926 \times Ins_0)$. For these indices, insulin is expressed in pmol/L and the PG in mmol/L (56.57), *modified β-cell function index* (MBCI: fasting insulin [mIU/L] × fasting glucose [mmol/L])/ (2–h plasma glucose [mmol/L] + 1–h plasma glucose [mmol/L] –7) (58), and the *ratio of insulin/glucose area under the curves* (AUC_{ins}/AUC_{gluc}) analysed by the trapezoidal method (59).

With the exception of HOMA-β and AUC_{ins}/AUC_{gluc} ratio, these indices were not specifically designed to emulate first-phase β- cell response as measured by IVGTT or clamp, but rather to capture signs of pancreatic β-cell dysfunction in response to glucose load, which is compromised in subjects with diabetes .

In our experience, a small number of β-TM patients exhibited a negative IGI index due to either a decrement in insulin or PG value at 30 min after glucose load (VDS, personal observation). Due to the small number of occurrences, we were unable to ascertain if this result was due to a physiological early insulin response or if it was secondary to a slow/delayed glucose absorption (60,61). Nevertheless, its role, especially in β-TM patients with diabetes, may represent an element requiring further exploration.

C-peptide to glucose ratio (CPRI) is considered a marker of β cell function. Postprandial PCPRI (timing of sampling:1 or 2 h after a meal) appears to reflect the maximal β cell functional capacity more accurately compared with fasting C-peptide to glucose ratio and may have, in conjunction with other clinical parameters, the potential role for improving the glycemic control in patients with T2DM. A reported cut-off value of postprandial C-peptide (ng/mL) to glucose (mg/dL) ratio (×100) less than 2.02 may represent a prediction index for starting insulin therapy with sensitivity of 81% and specificity of 63% (62).

b. Assessment of insulin sensitivity (IS)

IS is defined as the ability of insulin to exert its physiological effects of glucose uptake in the muscles and adipose tissue and to suppress hepatic gluconeogenesis while insulin resistance (IR) is a pathological

situation characterized by a lack of physiological response of peripheral tissues to insulin action, leading to the metabolic disturbances (63).

To further improve the ability to identify subjects at high risk of T2DM early, many indices to assess IS have been developed using formulae derived from fasting PG and insulin concentrations or from OGTT. The most commonly used in clinical practice are: *the homeostasis model of IR* (HOMA 1-IR:[fasting insulin (μU/mL)]×[fasting glucose (mg/dL)]/405] and the secretion HOMA-β% (360 × fasting insulin (μU/L))/(fasting glucose (mg/dL) – 63)], and the *quantitative insulin sensitivity check index* (QUICKI: 1/[log (fasting insulin, μIU/mL)+log (fasting glucose, mg/dL)].

The log-transformation included in the formula of QUICKI results in greater accuracy compared to HOMA1-IR in calculations over a broad range of insulin sensitivity. The *Stumvoll insulin sensitivity index (ISI Stumvoll)* is calculated as follows: 0.226 – (0.0032 × BMI) – (0.0000645 × I_{120}) – (0.00375 × G_{90}) (57).

HOMA1-IR model describes the glucose-insulin homeostasis derived by an empirical nonlinear equation. It uses fasting values for estimation and mostly describes hepatic IR and steady state insulin secretion. The constant 405 (22.5 if glucose is expressed in mmol/L) is a normalising factor, obtained from an "ideal" and "normal" individual, calculated as a product of fasting insulin equal to 5 μU/mL and a FPG level of 81 mg/dL (4.5 mmol/L).

The major advantage of the HOMA1-IR and QUICKI models is that they both require one blood sample from a fasting patient. The coefficient of variation for HOMA-IR varies considerably depending upon the number of fasting samples obtained and the insulin assay used. Therefore, since fasting insulin levels have a non-normal skewed distribution, log transformation may improve the linear correlation with SI_{clamp} (27-29,64-66). Moreover, HOMA1-IR does not take into account whether the secretion of insulin by the pancreatic β cells is altered or if the alteration is secondary to insulin removal (clearance) (64). Therefore, the PG measurement alone will not accurately reflect insulin sensitivity in subjects with β-cell dysfunction (53, 64, 65).

In most cases, to define the cut-off point for HOMA-IR, the determination is made in percentiles (80th or 90th percentile value of non-diabetic and non-obese subjects), using the values in the general population (66,67). The 90th percentile in different countries is 2.7 in Brazil (68), 2.77 in Italy (69), 2.33 in Portugal (70), 2.48 in Iran (71), 1.55 in Thailand (72), 3.8 in France (73) and Spain (74),17% in Denmark (75), and 2.7 in North Americans (68). Age and gender should be taken also into account in the estimation of their values in different populations (76-78).

HOMA1-IR was updated in 1998 with a computer version (HOMA2-IR) to provide a more accurate physiological adjustment of the previous index version (67). Compared to HOMA1- IR, HOMA2-IR considers variations in hepatic and peripheral glucose resistance, increases in the insulin secretion curve for PG concentrations above 180 mg/dL, and contribution of circulating proinsulin (67).

HOMA2-% β is a marker of insulin secretion and HOMA2-% S is a marker of IS. Both provide a fasting-based measure of the β-cells' function relative to the prevailing FPG concentration (acceptable range:54-450 mg/dL = 3–25 mmol/l) (66). HOMA2-%S and HOMA2-% β may be calculated using the version 2.2 of HOMA2 calculator. The associated estimation of HOMA2-% S is advisable because simple variability between insulin assays can provide different estimates of β-cell function on the same sample or may reflect proportionate differences in insulin sensitivity (78).

The log-transformation included in the formula of QUICKI results in greater accuracy compared to HOMA1-IR in calculations over a broad range of IS. The reported references for normal values and IR are >0.339 and ≤ 0.339, respectively (79).

The Matsuda-Defronzo index or WIBSI (*whole body insulin sensitive index)* is a model that uses dynamic PG and insulin values obtained during OGTT for an accurate evaluation of IS. This index is calculated from the glucose and insulin values during an OGTT using the formula $104/(I_0 \times G_0 \times I_m \times G_m)1/2$, where G_0 and G_m are the fasting and mean glucose and I_0 and I_m are the fasting and mean insulin (80). Despite its wide use, a universal cutoff value or reference range has not been established for clinical classifications of normal, IR, prediabetes, and/or T2DM (25). However, some agreement exists for the individual models. Alternatively, cutoff points and reference ranges provided

by ADA are normal values: > 2.5, IR values ≤ 2.5) (79). In youth, WBISI correlates significantly with the euglycemic-hyperinsulinemic clamp (r~0.8) (83).

Mari et al. (82) proposed a method based on a physiological glucose-insulin model for measuring IS from OGTT. They demonstrated that *oral glucose insulin sensitivity index (OGIS$_{120}$*: = f (G_0, G_{90}, G_{120}, I_0, I_{90}, I_{120}, DO; http://www.isib.cnr.it/bioing/ogis/home.html*)* was tightly correlated with glucose clamp and is considered mainly an index of glucose clearance than IS in the liver, muscle and adipocytes (83). The reported $OGIS_{180}$ value in subjects with normal OGTT was 440 ± 16 mL/min/m^2 (82).

Other specific indices, including the Gutt index, Avignon index and Belfiore index use particular sampling protocols during the OGTT. These indices derived from PG and insulin concentrations reflect both muscle and liver sensitivity (29).

c. Disposition index

The product of insulin secretory capacity and insulin sensitivity during OGTT is commonly expressed as oral *"disposition index"* (oDI; early-phase insulin response x IS). The IS is calculated by the *composite whole body insulin IS index*, called WBISI or Matsuda-Defronzo index [$1000/\sqrt{(G_0 \times I_0)} \times (G_{mean} \times I_{mean})$] (81). The index includes both hepatic and peripheral tissue and have been developed from combined of PG and insulin excursions during the OGTT.

DI is an expression of the acute insulin response adjusted for prevailing insulin sensitivity (IS) and is utilized as a measure of β-cell function (84). In healthy subjects, the product of IS and insulin secretion is constant. Substantially, any environmentally or physiological reduction of IS (e.g., increased body weight, puberty, pregnancy or aging) would be compensated by an appropriate enhancement of insulin release in proportion to the increase of IR and represents a measure of β-cell health status. Thus, β-cell function and insulin action can change, but the DI would remain constant.

In subjects with reduced IS, insulin secretion increases but in cases of associated β-cell failure the capacity to compensate decreases resulting in lower DI. Efficient and accurate methods to estimate IS and β-cell function are of great importance for studying the pathogenesis and treatment effectiveness in T2DM (29, 84,85).

Insulin Secretion-Sensitivity Index-2 (ISSI-2) is defined as the ratio of the area under the insulin curve to the area under the PG curve (as a measure of the β-cell response) multiplied by the Matsuda-Defronzo index to estimate β- cell function (86). It constitutes a surrogate measure of insulin secretion relative to IS and emphasizes the pivotal role of impaired insulin secretion in the development of dysregulation of glucose homeostasis. Substantially, it refers to the relationship between IS and insulin secretion.

In 29 β-TDT patients with normal OGTT and 17 with 1-h post-load PG levels ≥ 155 mg/dl (8.6 mmol/L) we found an inverse correlation between 1-h PG value during OGTT and ISSI-2 (r: -0.3298; P: 0.025). Moreover, a significant negative correlation was detected between ISSI-2 and ALT (r: -0.3262; P: 0.027) (87). In support of these finding, Karadas et al. (88) suggested that ISSI-2 index in combination with pancreas R2* MRI were valuable parameters to identify β-TDT patients at the highest risk for developing GD.

A pragmatic clinical approach to GD in patients with β-TDT: Strengths and weaknesses

The use and interpretation of selected OGTT-derived surrogate indices can be challenging since patients with β-TDT may exhibit a combination of decreased insulin secretion and impaired peripheral utilization of glucose (23) in the face of elevated oxidative stress that can damage DNA, phospholipids and proteins of pancreatic β-cells (89). The strong susceptibility to oxidative stress of these cells is a consequence of their high metabolic activity (90), and high rate of ROS production, coupled with their weak defence mechanisms against oxidative insults (90,91).

Therefore, the main problem for an investigator is to understand the role that each index provides and to choose the most appropriate indices for clinical purpose.

In the authors' experience, indices based on a repeated PG and insulin measurements, obtained during the OGTT, give a more precise estimation of the β-cell response, insulin sensitivity and β-cell function than those calculated only in fasting state or in a single occasion because the physiology of insulin secretion and IR is multidimensional (particularly in β-TDT patients) and therefore it is unlikely to be covered adequately by

a single index (30, 92-94). It is, therefore, wise to use more than one index in each study to avoid erroneous conclusions (94,95).

Fasting insulin determinations strongly depend upon the precision of the assay and small errors greatly affect these indices, especially when are calculated on a single determination (96). The insulin coefficient of variation of four manual enzyme-linked immunosorbent assay (ELISA) kits and three automated chemiluminescent (CLIA) assay kits varied between 1.7% and 23.2% (92). In view of these results, it is highly recommended that, in any given study, one source of insulin assay is used throughout and possibly two baseline samples are obtained (commonly at -15 and 0 minutes) for PG and insulin measurements.

In clinical practice, due to the lack of appropriate guidelines and scanty information about reference of surrogate derived OGTT indices in β-TDT patients, establishing their cut-off values is crucial for the better validity of results. In general, their usefulness is considered to be influenced by the degree of correlation with gold standard indicators, by their reproducibility and by their ability to predict diabetes incidence to more complex methods (90,97,98).

The most common way for evaluating surrogate indices consists in using correlation coefficients with the HC and/or HEC. Correlation coefficients describe the strength and direction of an association between variables. A number of different measures of correlation exist, the two most common being the Pearson's correlation coefficient and the Spearman's rank correlation coefficient. Noteworthy, Pearson correlation is a measure of a linear association between 2 normally distributed random variables and Spearman's rank correlation coefficient is a nonparametric (distribution-free) rank statistic proposed as a measure of the strength of the association between two variables. It is a measure of a monotone association that is used when the distribution of data makes Pearson's correlation coefficient undesirable or misleading (99).

Conclusions

The OGTT is used both in clinical practice and research to assess glucose tolerance. In addition, blood samples from the OGTT can be utilized for surrogate measures of β-cell response, insulin sensitivity and β-cell function. Several homeostatic approaches have been developed in recent years, each with its merits and deficiencies. Nevertheless, data in literature on β-TDT patients are still scarce because investigators have mainly focused their attention on basal indices of IR and have neglected the assessment of early β-cell response and the product of insulin secretory capacity and insulin sensitivity during OGTT (disposition index). These indices can detect subtle disturbances of glucose metabolism and may help in understanding the sequences of pathophysiology underlying glucose dysregulation in β-TDT patients. Therefore, a complete panel of surrogate indices should be measured, when feasible, because it may improve the patient's risk prediction with the vision of enabling more targeted interventions in the era of personalized medicine. Finally, if the implementation of conventional OGTT is problematic for some β-TDT patients and/or centers, preliminary personal observations have shown that 1-h PG during OGTT ≥ 155 mg/dL could facilitate greater access to screening in clinical practice and may serve as a simple biomarker to detect high-risk patients (87). The potential additional use of other parameters, like repeated trajectories of post-load 2-h PG linear increase and progressive decline of HOMA2-%β and HOMA2-%S indices (100,101) have not yet tested in β-TDT patients.

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References

1. De Sanctis V, Soliman A, Tzoulis P, Daar S, Fiscina B, Kattamis C. The Pancreatic changes affecting glucose homeostasis in transfusion dependent β-thalassemia (TDT): a short review. Acta Biomed. 2021; 92(3):e2021232. doi:10.23750/abm.v92i3.11685.
2. He LN, Chen W, Yang Y, et al. Elevated prevalence of abnormal glucose metabolism and other endocrine disorders in patients with β-thalassemia major: A meta-analysis. Biomed Res Int. 2019; 2019:6573497. doi:10.1155/2019/6573497.
3. De Sanctis V, Daar S, Soliman AT, Tzoulis P, Karimi M, Kattamis C. Evolution of glucose-insulin homeostasis in children with β- thalassemia major (β-TM): A twenty-year retrospective ICET- A observational analysis from early childhood to young adulthood. Acta Biomed. 2022; 93(3): e2022243. doi: 10.23750/abm. v93i3.12643.
4. Soliman AT, el Banna N, alSalmi I, Asfour M. Insulin and glucagon responses to provocation with glucose and arginine in prepubertal children with thalassemia major before and after long-term blood transfusion. J Trop Pediatr. 1996;42:291–6. doi:101093/tropej/42.5.291.
5. Angelopoulos NG, Zervas A, Livadas S, et al. Reduced insulin secretion in normoglycaemic patients with beta-thalassaemia major. Diabet Med. 2006;23:1327–31. doi:10.1111/j.1464-5491.
6. Dmochowski K, Finegood DT, Francombe W, Tyler B, Zinman B. Factors determining glucose tolerance in patients with thalassemia major. J Clin Endocrinol Metab. 1993;77:478– 83.doi:10.1210/jcem. 77.2. 8345055.
7. Pappas S, Donohue SM, Denver AE, Mohamed-Ali V, Goubet S, Yudkin JS. Glucose intolerance in thalassemia major is related to insulin resistance and hepatic dysfunction. Metabolism. 1996;45:652–7.doi: 10.1016/s0026 -0495(96)90038-4.
8. Cario H, Holl RW, Debatin KM, Kohne E. Insulin sensitivity and beta-cell secretion in thalassaemia major with secondary haemochromatosis: assessment by oral glucose tolerance test. Eur J Pediatr. 2003;162:139–46.doi:10.1007 /s00431-002-1121-7.
9. Patel S, Jinjuvadia R, Patel R, Liangpunsakul S. Insulin Resistance is associated with significant liver fibrosis in chronic hepatitis C patients: A systemic review and meta-analysis. J Clin Gastroenterol. 2016; 50(1):80-4. doi:10.1097 /MCG.0000000000000400.
10. De Sanctis V, Daar S, Soliman AT, Tzoulis P, Yassin M, Kattamis C. The effects of excess weight on glucose homeostasis in young adult females with β-thalassemia major (β-TM): a preliminary retrospective study. Acta Biomed 2023; 94 (5): e2023225. doi: 10.23750/abm.v94i5.14909.
11. Fung EB, Gildengorin G, Talwar S, Hagar L, Lal A. Zinc status affects glucose homeostasis and insulin secretion in patients with thalassemia. Nutrients. 2015;7(6):4296-307. doi:10.3390/nu7064296.
12. Saudek CD, Hemm RM, Peterson CM. Abnormal glucose tolerance in β-thalassemia major. Metabolism. 1977; 26 (1):43-52. doi.org/10.1016/0026-0495(77)90126-3.
13. Farmakis D, Porter J, Taher A, Cappellini MD, Angastiniotis M, Eleftheriou A. 2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia Hemasphere. 2022; 6(8):e732. doi: 10.1097/HS9.0000000000000732.
14. Meloni A, Nobile M, Keilberg P, et al. Pancreatic fatty replacement as risk marker for altered glucose metabolism and cardiac iron and complications in thalassemia majorEur Radiol. 2023; 33(10): 7215–25. doi: 10.1007/s00330-023-09630-z.
15. Matter RM, Allam KE, Sadony AM. Gradient-echo magnetic resonance imaging study of pancreatic iron overload in young Egyptian beta-thalassemia major patients and effect of splenectomy. Diabetol Metab Syndr. 2010;2:23. doi: 10.1186/1758-5996-2-23.
16. De Sanctis V, Soliman A, Daar S, et al. Insulin-Like Growth Factor-1 (IGF-1) and Glucose Dysregulation in Young Adult Patients with β-Thalassemia Major: Causality or Potential Link? Acta Biomed. 2022;93 (6): e2022331. doi: 10.23750/abm.v93i6.13288.
17. Fung EB, Pinal J, Leason M. Reduced physical activity patterns in patients with thalassemia compared to healthy controls. J Hematol Oncol Research. 2015; 2(1):7-21. doi. org/10.14302/issn.2372-6601.jhor-15-776.
18. Berdoukas V, Nord A, Carson S, et al. Tissue iron evaluation in chronically transfused children shows significant levels of iron loading at a very young age. Am J Hematol. 2013;88(E):283–5.doi:10.1002/ajh. 23545.
19. Bergman RN, Piccinini F, Kabir M, Kolka CM, Ader M. Hypothesis: role of reduced hepatic insulin clearance in the pathogenesis of type 2 diabetes. Diabetes. 2019;68 (9): 1709-16. PIMD:31431441.23545.
20. Bonadonna RC, Boselli L, Dei Cas A, Trombetta M. Methods to assess in vivo insulin sensitivity and insulin secretion. In: Bonora E, DeFronzo R eds. Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment. Endocrinology. Springer; 2018:pp.317-68.
21. Rijkelijkhuizen JM, Girman CJ, Mari A, et al. Classical and model-based estimates of beta-cell function during a mixed meal vs. an OGTT in a population-based cohort. Diabetes Res Clin Pract.2009; 83:280-8. doi:10.1016/j .diabres2008.11.017.
22. Defronzo RA. Lilly lecture 1987. The triumvirate: beta cell, muscle, liver. A collusion responsible for NIDDM. Diabetes.1988; 37(6): 667–87.doi:10.2337/diab.37.6.667.
23. De Sanctis V, Soliman AT, Daar S, Tzoulis P, Kattamis C. Can we predict incipient diabetes mellitus in patients with β-transfusion dependent thalassemia (β-TDT) referred with a history of prediabetes? Submitted for publication, 2023.
24. Pacini G, Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. Best Pract Res Clin Endocrinol Metab. 2003;17:305–22. doi:10.1016 /S1521-690X(03)00042-3.
25. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab. 2008; 294(1):E15–E26. doi .org/10.1152/ajpendo.00645.2007.

26. Muniyappa R, Ritu Madan R, Varghese RT. Assessing insulin sensitivity and resistance in humans. Endotext [Internet]. Last Update: August 9, 2021.
27. Otten J, Ahrén B, Olsson T. Surrogate measures of insulin sensitivity vs the hyperinsulinaemic–euglycaemic clamp: a meta-analysis. Diabetologia.2014;57:1781–8. doi:10.10.1007/s00125-014-3285-x.
28. Kahn SE, Chen YC, Esser N, et al. The β Cell in Diabetes: Integrating Biomarkers With Functional Measures. Endocrine Rev. 2021; 42 (5): 528–83. doi.org/10.1210/endrev /bnab021.
29. Singh B, Saxena A. Surrogate markers of insulin resistance: A review. World J Diabetes. 2010;1(2): 36–47. doi: 10.4239 /wjd.v1.i2.36.
30. Placzkowska S, Pawlik-Sobecka L, Kokot I, Piwowar A. Indirect insulin resistance detection: Current clinical trends and laboratory limitations. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2019; 163(3):187-99. doi:10.5507/bp.2019.021.
31. Ferrannini E, Wahren J, Faber OK, et al. Splanchnic and renal metabolism of insulin in human subjects: A dose-response study. Am J Physiol Metab. 1983;244: E517–E27. doi.10.1152/ajpendo.1983.244.6.E517.
32. Wood AC, Jensen ET, Bertoni AG, et al. Defining the relative role of insulin clearance in early dysglycemia in relation to insulin sensitivity and insulin secretion: The microbiome and insulin longitudinal evaluation Study (MILES). Metabolites. 2021;11(7):420; doi.org/10.3390/metabo 11070420.
33. American Diabetes Association. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes - 2020. Diabetes Care. 2020; 43(Suppl.1): S14-S31.doi.org/10.2337 /dc20-S002.
34. Klonoff DC. Hemoglobinopathies and hemoglobin A1c in diabetes mellitus. J Diabetes Sci Technol. 2020;14:3–7. doi:10.1177/1932296819841698.
35. De Sanctis V, Daar S, Soliman AT, Tzoulis P, Karimi M, Di Maio S, Kattamis C. Screening for glucose dysregulation in β-thalassemia major (β-TM): An update of current evidences and personal experience. Acta Biomed. 2022;93(1):e2022158. doi:10.23750/abm.v93i1.12802./
36. De Sanctis V, Soliman AT, Daar S, Tzoulis P,Di Maio S, Kattamis C. Oral glucose tolerance test: how to maximize its diagnostic value in children and adolescents. Acta Biomed. 2022; 93(5): e2022318. doi: 10. 23750/abm.v93i5.13615.
37. Soliman AT, Yasin M, El-Awwa A, De SanctisV. Detection of glycemic abnormalities in adolescents with beta thalassemia using continuous glucose monitoring and oral glucose tolerance in adolescents and young adults with β-thalassemia major: Pilot study. Indian J Endocrinol Metab. 2013; 17(3): 490–5. doi: 10. 4103/2230-8210.111647.
38. El-Samahy MH, Tantawy AA, Adly AA, Abdelmaksoud AA, Ismail EA, Salah NY. Evaluation of continuous glucose monitoring system for detection of alterations in glucose homeostasis in pediatric patients with β-thalassemia major. Pediatr Diabetes .2019;20:65-72. doi: 10.1111/pedi.12793.
39. Tzoulis P, Yavropoulou MP, Banchev A, Modeva I, Daar S, De Sanctis V. Recent advancements in glucose dysregulation and pharmacological management of osteoporosis in transfusion-dependent thalassemia (TDT): an update of ICET-A (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine): Glucose disorders and osteoporosis in thalassemia . Acta Biomed. 2023;94(3):e2023178. doi:10.23750/abm.v94i3.14805.
40. Sacks DB. Carbohydrates. Tietz textbook of clinical chemistry and molecular diagnostics. In: Burtis CA, Ash-wood ER, Bruns DE, editors. 4th ed. St. Louis: Elsevier Saunders; 2006. pp. 837–902.
41. Libman IM , Barinas-Mitchell E , Bartucci A , Robertson R, Arslanian S. Reproducibility of the oral glucose tolerance test in overweight children. J Clin Endocrinol Metab. 2008; 93: 4231–7.doi:10.1210/jc. 2008-0801.
42. Utzschneider KM, Prigeon RL, Tong J, et al. Within-subject variability of measures of beta cell function derived from a 2 h OGTT: implications for research studies. Diabetologia. 2007;50: 2516–25.doi.10.1007/s00125-007-0819-5.
43. Kim W, Egan JM. The role of incretins in glucose homeostasis and diabetes treatment. Pharmacol Rev. 2008;60(4): 470–512. doi: 10.1124/pr.108.000604.
44. Nauck MA, Homberger E, Siegel EG, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab. 1986;63(2):492–8. doi: 10.1210/jcem-63-2-492.
45. Faerch K, Vaag A, Holst JJ, Glumer C, Pedersen O, Borch-Johnsen K. Impaired fasting glycaemia vs impaired glucose tolerance: similar impairment of pancreatic alpha and beta cell function but differential roles of incretin hormones and insulin action. Diabetologia. 2008;51(5):853–61. doi: 10.1007/s00125-008-0951-x.
46. Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. Diabetes Care.2007; 30:89–94. doi.10.2337/dc06-1519.
47. Prystupa K, Renklint R, Chninou Y, et al. Comprehensive validation of fasting-based and oral glucose tolerance test–based indices of insulin secretion against gold standard measures. BMJ Open Diabetes Res Care. 2022; 10(5): e002909. doi: 10.1136/bmjdrc-2022-002909.
48. Hudak S, Huber P, Lamprinou A, et al. Reproducibility and discrimination of different indices of insulin sensitivity and insulin secretion. PLoS One. 2021; 16(10): e0258476. doi: 10.1371/journal.pone.0258476.
49. Chon S, Gautier JF. An update on the effect of incretin-based therapies on β-cell function and mass. Diabetes Metab J. 2016; 40(2):99-114.doi:10.4093/dmj.2016.40.2.99.
50. Luzi L, DeFronzo RA. Effect of loss of first-phase insulin secretion on hepatic glucose production and tissue glucose disposal in humans. Am J Physiol. 1989;257(2 Pt 1): E241-6.doi:10.1152/ajpendo.1989. 257. 2.E241.
51. Gerich JE. Is reduced first-phase insulin release the earliest detectable abnormality in individuals destined to develop type 2 diabetes? Diabetes. 2002;51(Supplement 1):S117–S21. doi: 10.2337/diabetes. 51.2007.S117.
52. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with

the euglycemic insulin clamp. Diabetes Care. 1999; 22(9): 1462–70, doi:10.2337/diacare.22.9.1462.
53. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7): 412–9, doi: 10.1007/bf00280883.
54. Seltzer HS, Allen EW, Herron AL Jr, Brennan MT. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest. 1967; 46(3):323-35. doi: 10.1172/JCI105534.
55. Sluiter WJ, Erkelens DW, Reitsma WD, Doorenbos H. Glucose tolerance and insulin release, a mathematical approach I. Assay of the beta-cell response after oral glucose loading. Diabetes.1976; 25 (4):241-4.doi.10.2337/diab.25.4.245.
56. Stumvoll M, Mitrakou A, Pimenta W, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care. 2000;23(3):295-301.doi: 10.2337/diacare.23.3.295.
57. Stumvoll M, Haeften T V, Fritsche A, Gerich J. Oral glucose tolerance test indexes for insulin sensitivity and secretion based on various availabilities of sampling times. Diabetes Care. 2001;24(4):796-7. doi:10. 2337/diacare.24.4.796.
58. Zheng S, Zhou H, Han T, Li Y, Zhang Y, Liu W, Hu Y. Clinical characteristics and beta cell function in Chinese patients with newly diagnosed type 2 diabetes mellitus with different levels of serum triglyceride. BMC Endocr Disord. 2015;15:21. doi: 10.1186/s12902-015-0018-1.
59. Powe CE, Locascio JJ, Huston Gordesky L, Florez JC, Catalano PM. Oral Glucose Tolerance Test-based measures of insulin secretory response in pregnancy. J Clin Endocrinol Metab. 2022; 107(5): e1871–e8. doi:10.1210/clinem/dgac041.
60. Faulenbach MV, Wright LA, Lorenzo C, et al. Impact of differences in glucose tolerance on the prevalence of a negative insulinogenic index. J Diabetes Complications. 2013;27(2):158–61.doi: 10.1016/j.jdiacomp.2012.09.011.
61. Faulenbach MV, Wright LA, Lorenzo C, et al. and American Diabetes Association GENNID Study Group. Impact of Differences in glucose tolerance on the prevalence of a negative insulinogenic index. J Diabetes Complications. 2013; 27(2):158–61. doi: 10.1016/j.jdiacomp. 2012.09.011.
62. Saisho Y, Kou K, Tanaka K, et al. Postprandial serum C-peptide to plasma glucose ratio as a predictor of subsequent insulin treatment in patients with type 2 diabetes. Endocr J. 2011;58:315–22. doi: 10.1507/endocrj.K10E-399.
63. Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, Carmena R. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. Diabetes Care. 2003; 26 (12):3320-5. doi. org/10.2337/diacare.26.12.3320.
64. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27(6): 1487–95. doi:102337/diacare.27.6.1487.
65. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85:2402–10.doi: 10. 1210/jcem.85.7.6661.
66. Lee CH, Shih AZL, Woo YC, et al. Optimal cut-offs of homeostasis model assessment of insulin resistance (HOMA-IR) to identify dysglycemia and type 2 diabetes mellitus: A 15-year prospective study in Chinese. PLoS ONE 11(9): e0163424. doi:10.1371/journal. pone.0163424.
67. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care.1998;21(12):2191–2.doi:10.2337/diacare.21.12.2191.
68. Geloneze B, Vasques AC, Stabe CF, et al. HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS) Arq Bras Endocrinol Metabol. 2009;53(2):281–7. doi:10.1590/s0004-273020090002000,20.
69. Bonora E, Kiechl S, Willeit J, et al. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. Diabetes. 1998;47(10):1643–9.doi: doi:10.2337/diabetes.47.10.1643.
70. Timoteo AT, Miranda F, Carmo MM, Ferreira RC. Optimal cut-off value for homeostasis model assessment (HOMA) index of insulin-resistance in a population of patients admitted electively in a Portuguese cardiology ward. Acta Med Port. 2014;27(4):473–9.doi:10.20344/amp.5180.
71. Ziaee A, Esmailzadehha N, Oveisi S, Ghorbani A, Ghanei L. The threshold value of homeostasis model assessment for insulin resistance in Qazvin Metabolic Diseases Study (QMDS): assessment of metabolic syndrome. J Res Health Sci. 2015;15(2):94–100. PMID: 26175291.
72. Do HD, Lohsoonthorn V, Jiamjarasrangsi W, Lertmaharit S, Williams MA. Prevalence of insulin resistance and its relationship with cardiovascular disease risk factors among Thai adults over 35 years old. Diabetes Res Clin Pract. 2010;89(3):303–8. doi: 10.1016/j.diabres.2010.04.013.
73. Marques-Vidal P, Mazoyer E, Bongard V, et al. Prevalence of insulin resistance syndrome in southwestern France and its relationship with inflammatory and hemostatic markers. Diabetes Care. 2002;25(8):1371–7. doi.org/10.2337/diacare.25.8.1371.
74. Ascaso JF, Romero P, Real JT, Lorente RI, Martinez-Valls J, Carmena R. Abdominal obesity, insulin resistance, and metabolic syndrome in a southern European population. Eur J Intern Med. 2003;14(2):101–6. doi.org/10.1016/S0953-6205(03)00022-0.
75. Friedrich N, Thuesen B, Jorgensen T, et al. The association between IGF-I and insulin resistance: a general population study in Danish adults. Diabetes Care. 2012; 35(4):768–73. doi:10.2337/dc11-1833.
76. Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. BMC Endocr Disord.2013;13: 47.doi.org/10.1186/1472-6823-13-47.
77. Bergman RN, Hucking K, Watanabe RM. International Textbook of Diabetes Mellitus. Measuring insulin ac78. Kahn SE, Chen YC, Esser N, et al. The β Cell in Diabetes: Integrating biomarkers with functional measures. Endocr Rev. 2021;42 (5):528–83.doi.org/10.1210/endrev/bnab021.

78. Kahn SE, Chen YC, Esser N, et al. The β Cell in Diabetes: Integrating biomarkers with functional measures. Endocr Rev. 2021;42 (5):528–83.doi.org/10.1210/endrev/bnab021.
79. Farkas GJ, Gordon PS, Trewick N, et al. Comparison of various indices in identifying insulin resistance and diabetes in chronic spinal cord injury. J Clin Med. 2021;10(23): 5591. doi:10.3390/jcm10235591.
80. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing. Diabetes Care.1999;22:1462-70.doi:10.2337/diacare.22.9.1462.
81. Utzschneider KM, Prigeon RL, Faulenbach MV, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. Diabetes Care.2009:32:335–41.doi:10. 233/dc09-0729.
82. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care. 2001;24:539-48. doi:10.2337/diacare.24.3.539.Erratum in: Diabetes Care. 214;37(7)2063.PIMD.11289482.
83. Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: Results from the veterans administration genetic epidemiology study. Diabetes. 2006;55:1430-5.doi:10.2337/db05-1200.
84. Park SY, Gautier JF, Suk Chon S. Assessment of insulin secretion and insulin resistance in human. Diabetes Metab J. 202;45(5): 641–54. doi:10.4093/dmj.2021.0220.
85. Bergman RN, Ader M, Heucking K,Van Citters G. Accurate assessment of beta-cell function. The hyperbolic correction. Diabetes. 2002; 51 S212–S20.doi:10.2337 /diabetes.512007.s212.
86. Retnakaran R, Shen S, Hanley AJ, Vuksan V, Hamilton JK, Zinman B. Hyperbolic relationship between insulin secretion and sensitivity on oral glucose tolerance test. Obesity (Silver Spring). 2008;16:1901–7. doi:10.1038/oby.2008.307.
87. De Sanctis V, Soliman A, Tzoulis P, Daar S, Pozzobon GC, Kattamis C. A study of isolated hyperglycemia (blood glucose ≥155 mg/dL) at 1-hour of oral glucose tolerance test (OGTT) in patients with β-transfusion dependent thalassemia (β-TDT) followed for 12 years. Acta Biomed. 2021; 92(4): e2021322. doi:10.23750/abm.v92i4.11105.
88. Karadas N, Yurekli B, Bayraktaroglu S, Aydinok Y. Insulin secretion-sensitivity index-2 could be a novel marker in the identification of the role of pancreatic iron deposition on beta-cell function in thalassemia major. Endocr J. 2019;66:1093–9.doi:10.1707/endocej.EJ19-0191.
89. Marku A, Galli A, Marciani P, Dule N, Perego C, Castagna M. Iron metabolism in pancreatic beta-cell function and dysfunction. Cells. 2021:10:2841.doi.org/10.3390/cells10112841.
90. Lenzen S. Oxidative Stress: The vulnerable beta-cell. Biochem Soc Trans. 2008;36: 343–7. doi.org/10.1042/BST0360343.
91. Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PIH. Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J. 2016;473:4527–50. doi:10.1042/BCJ2016050503C.
92. Rosli N, Kwon HJ, Lim J, Yoon YA. Measurement comparability of insulin assays using conventional immunoassay kits. J Clin Lab Anal.2022;36:e24521.doi.org/10.1002 /jcla.24521.
93. Borai A, Livingstone C, Shafi S, Zarif H, Ferns G. Insulin sensitivity (Si) assessment in lean and overweight subjects using two different protocols and updated software. Scand J Clin Lab Invest. 2010; 70(2):98- 103. doi: 10.3109/00365510903544585.
94. Magkos F, Lee MH, Lim M, et al. Dynamic assessment of insulin secretion and insulin resistance in Asians with prediabetes. Metabolism.2022;128:154957. doi.org/10.1016 /j.metabol.2021.154957.
95. Gastaldelli, A. Measuring and estimating insulin resistance in clinical and research settings.Obesity (Siver Spring). 2022;20(8): 1549-63. doi.org/10.1002/oby.23503.
96. Mather KJ, Hunt AE, Steinberg HO, et al. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. J Clin Endocrinol Metab. 2001;86:5457–64.doi:10. 1210/jcem .86.11.78.80.
97. Hudak S, Huber P, Lamprinou A, et al. Reproducibility and discrimination of different indices of insulin sensitivity and insulin secretion. PLoS One. 2021; 16(10): e0258476. doi: 10.1371/journal.pone.0258476.
98. Prystupa K, Renklint R, Chninou Y, et al. Comprehensive validation of fasting- and oral glucose tolerance test-based indices of insulin secretion against gold-standard measures. medRxiv. 2022. 04. 14. 22273491;doi:10.1101/2022.04.14 .22273491.
99. Hauke J, Kossowski T, Comparison of values of Pearson's and Spearman's correlation coeficient on the same sets of data. Quaestiones Geographicae.2011; 30(2):87–93.doi: 10.2478/v10117-011-0021-1.
100. Tabák AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR. Trajectories of glycemia, insulin sensitivity and insulin secretion preceding the diagnosis of type 2 diabetes: The Whitehall II study Lancet. 2009; 373(9682): 2215–21.doi: 10.1016/S0140-6736(09)60619-X.
101. Khalili D, Khayamzadeh M, Kohansal K, et al. Are HOMA-IR and HOMA-B good predictors for diabetes and pre-diabetes subtypes? BMC Endocr Disord. 2023; 23: 39. doi: 10.1186/s12902-023-01291-9.

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