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Oral glucose tolerance test: how to maximize its diagnostic value in children and adolescents

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Abstract. *Background:* Recently, the validity of the oral glucose tolerance test (OGTT) as a gold-standard test for the diagnosis of glucose dysregulation (GD) has been questioned due to the pre-analytical, analytical, and post-analytical variables which can potentially affect its reproducibility and accuracy. *Aims:* In this short update, the many variables that affect the reproducibility and accuracy of the OGTT are described and discussed aiming to enhance its diagnostic value in clinical practice. *Search strategy:* A systematic search was implemented in June 2022, using Scopus, PubMed, Embase and Google Scholar focusing on OGTT relevant papers published in the last 10 years. Moreover, the reference lists of these articles were checked for additional pertinent studies. The research and selection of articles was also supported by the long-term authors' experience in the use of OGTT for the diagnosis of GD in children and adolescents. *Conclusion:* The complexity of diagnosing GD presupposes that clinicians have specific knowledge and experience to perform rigorous assessment of glucose metabolism. It is worth mentioning that during OGTT, subjects with glucose levels close to the cut-off values proposed by WHO (World Health Organization)/ ADA (American Diabetes Association) require careful evaluation in order to avoid misclassification and unnecessary interventions. For this reason, ADA recommends a second test to confirm the diagnosis of diabetes. (www.actabiomedica.it)

Key words: Oral glucose tolerance test, glucose dysregulation, ADA recommendation, analytical variables, children, adolescents

Introduction

Diabetes mellitus is the most common endocrine disorder of carbohydrate metabolism. Diagnostic criteria for all types of glucose dysregulation (GD) in children and adolescents are based on laboratory measurement of plasma glucose levels and the presence or absence of symptoms of GD.

Type 1 diabetes mellitus (T1-DM) is characterized primarily by deficiency of insulin secretion and the presence of autoantibodies against insulin (IAA), glutamic acid decarboxylase (GADA), protein tyrosine phosphatase (IA2) and zinc transporter 8 (Znt8A) while type 2 diabetes mellitus (T2-DM) is a combination of resistance to insulin action, and an inadequate compensatory insulin secretory response for the insulin resistance. A third category of DM is secondary to other specific types of diabetes (monogenic defects of β-cell function, genetic defects of insulin action, exocrine pancreatic disease, certain endocrinopathies,

induced by drugs, chemicals and infections, uncommon immune-mediated disease and genetic syndromes associated with diabetes) (1).

In conjunction with a worldwide increased prevalence of obesity in childhood and teenage, prevalence of diabetes in youth has also increased (2). Given the fact that many children and adolescents with T2-DM have minimal symptoms (3), the American Diabetes Association (ADA) guidelines (4) recommend that obese children with BMI ≥ 95th percentile for age and sex and with additional risk factors should be screened for diabetes every two years, starting at age of 10 years or at onset of puberty. Additional risk factors include: maternal history of diabetes or gestational diabetes during the child's gestation; family history of type 2 diabetes in a first-or second-degree relative; native American, Black, Latino, Asian American, Pacific Islander; signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome, or small for gestational age birth weight).

Besides the intrinsic and extrinsic factors that modify response to the OGTT, other diseases may produce GD. Among these are: chronic diseases (transfusion dependent hemoglobinopathies, cystic fibrosis, history of hematopoietic stem cell transplantation (5-8) or renal transplantation (9), a number of endocrinopathies (Cushing's syndrome, hyperthyroidism, congenital adrenal hyperplasia) (1,4), genetic syndromes (e.g., Down's syndrome, Klinefelter's syndrome, Friedreich's ataxia, Prader-Willi syndrome, Russel-Silver syndrome and Turner's syndrome) (4), exposure to drugs known to be toxic to β-cells or causing insulin resistance (e.g., immunosuppressive drugs; glucocorticoids or some antidepressants) (1, 4) may be of potential risk to develop abnormalities of glucose homeostasis.

Tissues most vulnerable to the effects of prolonged elevated plasma glucose (PG) levels include pancreatic β-cells and vascular endothelial cells. The ensuing β-cell dysfunction reduces insulin synthesis and secretion, perpetuating the associated hyperglycemia (10). Therefore, screening for early diagnosis of GD js an advantage for patients at high risk, formulating a regular follow-up and an appropriate therapeutic intervention.

PG has a normal diurnal variation; it also varies with seasons and aging. Hyperglycemia can be assessed in at least three ways: by measuring fasting PG, post-challenge (or postprandial) glucose, and glycated hemoglobin (HbA1c) (1). The OGTT has been used in clinical practice for over 100 years and is the most widely used test in clinical practice to diagnose glucose intolerance and diabetes mellitus (DM). For OGTT, at least two blood samples (0 min and 120 min) are needed to characterize carbohydrate tolerance (12). The OGTT results are used to classify subjects as having normal glucose tolerance (NGT), impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) or both, and DM. In addition to the determination of glucose tolerance, repeated measurements made during the OGTT are frequently used to derive indices of β-cell function (11).

Recently, the validity of the OGTT as the "gold-standard" for the diagnosis of GD has been questioned, mainly due to the pre-analytical variables that potentially affect its reproducibility and accuracy (11).

Glycated hemoglobin (HbA1c) provides a reliable index of chronic glycemia. HbA1c and is used as a diagnostic criterion for diabetes and prediabetes; the cut-off values generally recommended for prediabetes and diabetes are 5.7% (39 mmol/moL) and 6.5% (48 mmol/moL), respectively (2).

HbA1c in blood provides evidence of an individual's average blood glucose levels during the previous two to three months.

The mean HbA1c value in 2,455 healthy German children and adolescents, aged between 0.5 and 18 years, was 5.06% (31.79 ± 3.3 mmol/moL) (12). Positive relation with HbA1c values were identified for age, gender, and body mass index-standard deviation score (BMI-SDS). Compared to prepuberty, the pubertal and post pubertal stages were associated with higher HbA1c levels (12).

In general, despite some advantages, the use of HbA1c has some limitations depending on the assay and spuriously low values may occur in patients with certain hemoglobinopathies or those who have

increased red-cell turnover (e.g., hemolytic anemia and spherocytosis). In contrast, falsely high glycated hemoglobin levels have been reported in subjects with iron deficiency (4,5,8). Moreover, the performance of HbA1c for diagnosing diabetes and prediabetes is poor.

Nam et al. (13) evaluated the diagnostic performance of HbA1c and determined optimal cut-off points for detecting prediabetes and diabetes in 389 children (48 overweight and 341 obese). Their mean age was 13.0 ± 2.5 years. About half of the children (203, 52.2%) had a family history of DM in first- and second-degree relatives. Based on the results of the OGTT, 197 (50.6%) subjects had normoglycemia, 121 (31.1%) had prediabetes, and 71 (18.3%) had T2 -DM. The kappa coefficients for agreement between the OGTT, FPG, 2-hr PG, and HbA1c results were 0.464, 0.396 and 0.476, respectively. In that study, the diagnosis of prediabetes would have been missed in nearly half of children without OGTT results. Therefore, the authors recommended the combination of fasting and 2-h PG levels, in addition to HbA1c, in the diagnosis of childhood prediabetes and diabetes. As stated by the same authors, several other studies have reported low correlation between FPG, 2-hr PG from OGTT results, and HbA1c levels (13).

In June 2022 a systematic search was implemented that included Web of Science (ISI), Scopus, PubMed, Embase, and Scholar for papers on OGTT published in the last 20 years. Moreover, we checked the reference lists of the relevant articles and previous reviews for additional pertinent studies. The research and selection of articles was also enriched by the long-term authors' experience in the use of OGTT for the diagnosis of GD in children and adolescents.

Oral glucose tolerance test (OGTT)

The OGTT is the most frequently used method for assessing glucose tolerance, insulin sensitivity and secretion. It has been widely used both in clinical practice and in research settings for a long time.

Following ADA guidelines, the test should be performed in the morning after at least 3 days of unrestricted diet and normal physical activity (14). To avoid false-positive results, decades-old literature recommends carbohydrate loading prior to OGTT, ensuring adequate carbohydrate intake (> 150 g/day and > 50 g the evening meal prior to the overnight fast). Low carbohydrate intake can contribute to impaired glucose metabolism via loss of first-phase insulin secretion phase and reduced insulin sensitivity. In addition, smoking, caffeine consumption and exercise should be avoided immediately prior to OGTT, because they also may impact the results (14).

An overnight fast for 10-12 hours, during which only administration of water is allowed, should precede the test. The presence of factors that may influence test results (medication, infection etc.) should be avoided or recorded. After collection of the fasting blood sample, the patient should drink 75 g of glucose (anhydrous and not monohydrate) dissolved in 250-300 mL of cold water.

For children, the test load is 1.75 g per kg of ideal body weight (max, dose 75 grams) in the form of a chilled, 25-30% solution. The timing of the test starts with the beginning of the drink, and the glucose should be consumed within 5 minutes (15). During the test, carbohydrates should not be consumed and the patient should remain seated or lying down throughout the 2 hours of the test.

An extended glucose tolerance test may be conducted to detect other abnormalities of glucose metabolism and insulin secretion with samples taken at 0, 30, 60, 90, 120 and 180 minutes. After the test is completed, the subject can resume usual life activities.

The analytical factors that could influence the OGTT results are its reproducibility (usually expressed as coefficient of variation) and bias (i.e., the difference from the true value, usually expressed as the percentage of the true value). 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 impairs GD prevalence (16).

Current criteria for the diagnosis of prediabetes and diabetes mellitus (DM)

Prediabetes is an intermediate state of hyperglycemia with glycemic parameters between normal and diabetes threshold. Prediabetes remains a state of high risk for developing T2-DM in adults with an annual conversion rate of 5% -10% (17).

The World Health Organization (WHO) has defined prediabetes as a state of intermediate hyperglycemia using two specific parameters: IFG, defined as fasting plasma glucose (FPG) of 110 to 125 mg/dL (6.1-6.9 mmol/L) and IGT defined as 2- h plasma glucose of 140-199 mg/dL (7.8-11.0 mmol/L) after OGTT (18) (Figure 1).

The ADA, on the other hand, has a lower cut-off value for IFG (100-125 mg/dL; 5.6 -6.9 mmol/L) but the same cut-off value for IGT (140-199 mg/dL), and, in addition, has a HbA1c level of 5.7% to 6.4% for the definition of prediabetes (19) (Figure 1). Because HbA1c is a measure of chronic hyperglycemia, it may reflect impairment in both fasting and 2-h glucose.

Both organizations define DM as: (a) a fasting glucose of ≥126 mg/dL (≥ 7.0 mmol/L), or (b) a 2-hour glucose on an OGTT of ≥ 200 mg/dL (≥ 11.1 mmol/L). In the absence of unequivocal hyperglycemia, the ADA recommends that the result should be confirmed with repeat testing or (c) a random glucose of ≥ 200 mg/dL (11.1 mmol/L) with classic diabetes symptoms (19,20).

The mechanism behind IFG is still not fully understood, but IFG seems to be a result of impaired insulin secretion, indicating β-cell dysfunction, and the result of hepatic IR (21) while IGT is primarily the result of pancreatic β-cell dysfunction and IR in skeletal muscle (22).

In adults, IFG is a predictor of T2-DM (23) and is associated with fatal and non-fatal cardiovascular disease (24) and increased risk for cancers (25). IFG in obese children is associated with increased intima-media thickness which is considered a predictive factor for atherosclerosis (26) .

In a large number of obese children and adolescents (aged 2–18 years) in Germany (32,907 subjects) and in Sweden (2,726 subjects), the total prevalence of IFG in Germany according to ADA criteria was 5.7% and according to the WHO was 1.1%. In Sweden, the corresponding prevalence was 17.1% and 3.9%, respectively. IFG risk correlated positively with increasing age, male sex and degree of obesity (27).

In brief, the differences in the diagnostic cut-off points for the identification of IFG between ADA

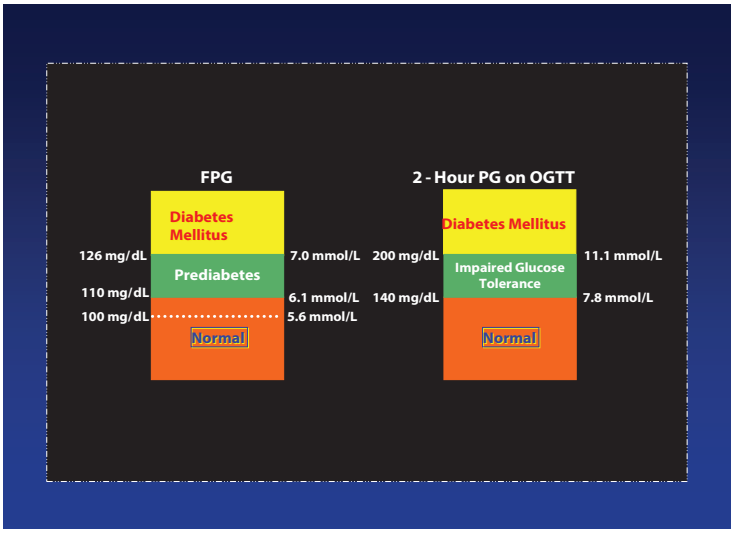

Figure 1. Current diagnostic criteria for the diagnosis of diabetes mellitus and other categories of hyperglycaemia based on FPG and 2-hour PG on OGTT.

and WHO have left clinicians in a conundrum as to which diagnostic criteria should be used for a specific population. The lower threshold for IFG in the ADA guidelines compared with the WHO was established as the result of an inter-subjective consensus among numerous actors from different institutions and professional groups.

Reproducibility of fasting plasma glucose and OGTT

Limited data are available on the reproducibility of the OGTT in children and adolescents.

A study in overweight children 4–7 yrs old showed that screening with FPG alone would have missed 64% of children with GD (28).Youth with discordant OGTTs, compared with those with concordant results, were more insulin resistant, had a lower oral disposition index (oDI) and had higher low-density lipoprotein cholesterol without differences in physical characteristics (29). However, these studies were hampered by small number of enrolled children and adolescents.

Kostopoulou et al. (30) reported in 81 children and adolescents (55 obese, 17 overweight and 9 with normal weight but positive family history of T2-DM), with mild or moderate disorders of glucose metabolism (such as IFG and IGT); in these patients a second test was needed to make an accurate diagnosis. However, when glucose metabolism was profoundly impaired, as in T2-DM, a single OGTT was more reliable and adequate for establishing the diagnosis

Although, these studies were hampered by the small number of enrolled children and adolescents, they substantially support the ADA recommendation that a second OGTT should be performed to confirm the diagnosis of GD (4).

Very little is known in young patients with chronic diseases. Some information is available in patients with cystic fibrosis and β-thalassemia major. A high variability in glucose tolerance was observed over time in 4,643 standardized OGTTs of 1,128 cystic fibrosis (CF) patients [median age at first test: 15.5 (range: 11.5 - 21.5) years, 48.8% females] (31). Compared to the general population, the overall variability in CF patients, at 2-h after OGTT, was from 1.5 to 1.8-fold higher (32).

In another study, only 4 of 25 (16%) children and adults with newly diagnosed cystic fibrosis-related diabetes mellitus (CFRD) had elevated FPG (33). In any case prevalence of GD was positively related to age.

During an ongoing ICET-A retrospective study on GD in β-TM patients, we collected data on 397 patients (aged 5-40 years; 56.3% males) followed from January 1988 to June 2021 (34). Both ADA and WHO criteria for IFG missed the diagnosis of thalassemia related diabetes (Th-RD) in 4 of 91 patients (4.3%) and 11 of 59 patients (18.6%), respectively. Moreover, ADA criteria used for the diagnosis of IFG identified an additional group of patients with IGT. Although the identification of the optimal method for identifying patients at risk for deterioration of glucose homeostasis is still challenging, the recent retrospective personal observations confirm the utility of OGTT screening using ADA criteria for the detection of IGT and Th-RD in β-TM patients with normal fasting plasma glucose (FPG), preferably combined with assessment of insulin secretion, at 10, 12, 14, and 16 years and annually thereafter. Venous blood samples for PG and insulin measurements should be collected at 0, 30, 60, and 120 min. (34).

Additional markers OGTT-derived to identify subjects at high-risk for GD

Recent studies have provided additional information that can be obtained from the OGTT, which renders this test even more useful, especially in at-risk youth for whom prolonged and/or costly diagnostic testing may be challenging in the outpatient setting.

a. ***Delayed timing of post-load glucose:*** A peak >30 minutes is associated with decline of β-cell function, blunted incretin secretion, lower insulin sensitivity to glucose and FFA metabolism (35-37). It remains to be determined if late-peak glucose predicts the future development of type 2 diabetes in these high-risk youth (38).

   Thirty healthy adults underwent three replicate OGTTs to assess the reproducibility

of the following parameters: time to insulin peak, shape of the glucose curve, glucose nadir below baseline, 1-h post-challenge glucose, and time to glucose peak. Of the five analyzed parameters, only time to glucose peak displayed reliable reproducibility on repeated testing (κ = 0.76) (39).

b. ***1-h PG ≥ 155 mg/dL (≥ 8.6 mmol/L):*** This is considered an early and sensitive marker of dysglycemia (40) and is associated with a worse clinical and metabolic phenotype, characterized by alterations in insulin sensitivity, β-cell function, and insulin clearance. It may prospectively predict progression to prediabetes in obese youths with NGT (41). However, the 1-hr glucose threshold, used alone, had low diagnostic sensitivity (40%), increasing the risk of false negative diagnosis (42).

c. ***The shape of the glucose response curve during OGTT:*** This identifies physiologically distinct groups of individuals with abnormalities in insulin secretion and insulin sensitivity (43)

Subjects with a monophasic OGTT glucose response curve (i.e., a gradual increase in glucose concentrations between 30' and 90' min until a peak is reached, followed by a subsequent decrease in glucose of ≥ 4.5 mg/dL (≥ 0.25 mmol/L), have lower insulin sensitivity and decreased β-cell function compared with subjects with a biphasic OGTT glucose response curve [i.e., a second rise of glucose concentration of ≥ 4.5 mg/dL (≥ 0.25 mmol/L)] after the first decline in glucose) (44,45). A small percentage of individuals with an OGTT glucose response curve that does not fit either are designated as "unclassified" (Figure 2). This latter category is rare in children, ranging from 1% to 12% depending on the study (44,45). The greater risk of type 1 diabetes in the monophasic group could be explained by a lower early C-peptide response, which has been shown to decline with progression to type 1 diabetes (46).

Data from 287 obese adolescents without diabetes demonstrate that 56.8% had a monophasic OGTT glucose response curve, 39.7% had a biphasic glucose response curve, and 3.5% had a gradual continuous rise or an incessant increase (47). The monophasic and unclassified curves, compared to the biphasic curve, are associated with lower insulin sensitivity and decreased β-cell function (48). However, the application of simple shape changes to diagnosing prediabetes and/or diabetes is challenging, as described recently (49).

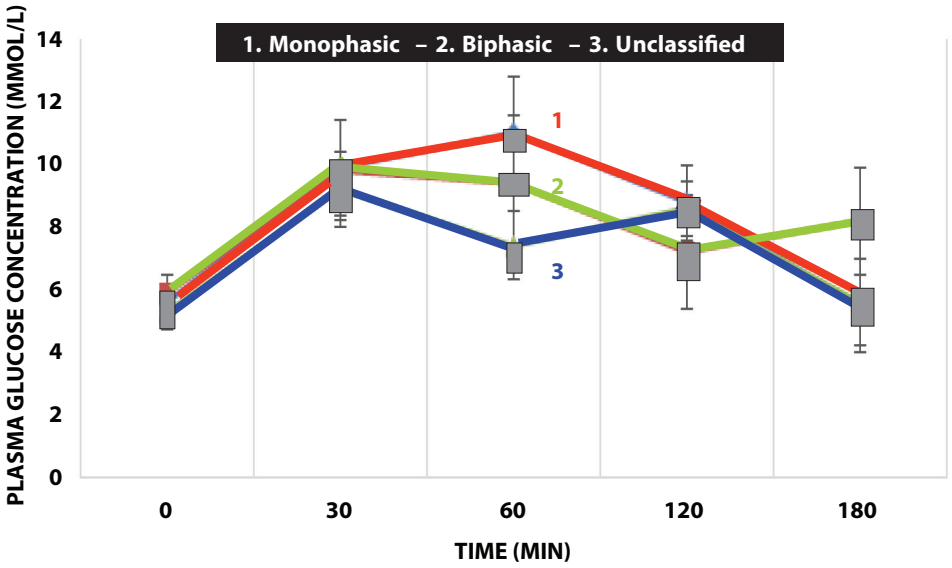

Figure 2. Shapes of the glucose curve: monophasic, biphasic and unclassified.

d. ***The glucose area under the curve (AUC):*** This has been applied in scientific reports to show the variations in increased blood glucose during the OGTT. It is an index of whole glucose excursion after glucose loading and has been widely used for calculating the glycemic index and for evaluating the efficacy of medications for postprandial hyperglycemia (50,51). PG-AUC can be calculated by trapezoidal approximation with the following formula. PG- AUC (mgh/dL) =PG (0) + PG (30) × 2+ PG (60) ×3 + PG (120) × 2/4. The cutoff value of the glucose AUC for glucose intolerance screening in adults has been set at 290 mg-h/dL based on the analysis of ROC curves (52).

In conclusion, the OGTT is highly sensitive and specific for detecting glucose intolerance because it can demonstrate post-challenge glucose excursion. However, the 2-h PG levels, a criterion for glucose intolerance during OGTT, may not provide complete information regarding the processing of PG after glucose loading. The combined use of three main morphological features of the glucose curve (time to glucose peak > 30 mins, 1- hr glucose concentration ≥155 mg/dL (≥ 8.6 mmol/L), and the monophasic curve shape) can be used as biomarkers for prediabetes risk stratification. However, before these indices can be adopted as a mainstream prognostic tool, longitudinal analyses are needed.

Insulin response during OGTT and surrogates measures of β-cell function

During the OGTT, insulin action and secretion modulate the rate in increase and decrease of PG and the time required for PG to peak and to return to the fasting levels (2). First-phase insulin secretion and hepatic insulin resistance index (HIRI) are important determinants of the initial rise of PG following glucose ingestion. The rate of decline in PG concentration back towards the fasting level seems to depend on late phase of insulin secretion and muscle insulin sensitivity (53). When insulin sensitivity declines, the appropriate physiologic response is for insulin secretion to increase in a compensatory manner. The calculated line linking these factors, which exhibit a square hyperbolic relationship, is commonly expressed as the 'disposition index' (DI, insulin sensitivity).

In early stages of GD, fasting and 2 h PG levels during an OGTT may be normal or slightly elevated, but the amount of insulin necessary to maintain this equilibrium is supra-physiological. Insulin resistance (IR) is an important risk factor for diabetes and other diseases (54). Because both insulin sensitivity and insulin response have varying influences on the patterns of insulin concentration during an OGTT, these patterns provide important and valuable information for predicting the subsequent development of T2-DM.

A multiplicity of mathematical representations calculating insulin sensitivity/resistance from glucose and insulin levels obtained during OGTT have been reported (55). The HOMA-IR and Matsuda insulin sensitivity indices are widely utilized to quantify whole-body insulin resistance from fasting and average postprandial glucose/insulin levels respectively. However, in recent years, the increased recognition of tissue-specific insulin resistance leading to metabolically distinct phenotypes, has resulted in the development of the hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI) to quantify hepatic and skeletal muscle insulin resistance from OGTT responses (55).

In conclusion, since IR occurs in multiple organs and in varying degrees, and since the interventions that improve IR are organ dependent (physical activity for muscle insulin resistance, metformin for hepatic insulin resistance, and weight loss and thiazolidinediones for muscle and hepatic insulin resistance), it is important to have simple method(s) that can assess the contribution of each organ to whole-body insulin resistance.

How to improve the reliability of OGTT

1. Pre-analytical phase of OGTT:

Pre-analytical variables begin with the request by the clinician, patient's preparation, collection of primary samples, transportation to and within the laboratory and end when the analytical procedures start.

In order to minimize pre-analytical errors, subjects are advised to limit exercise the day before, to follow a 3-day diet with a minimum of 150 g of carbohydrate per day and maintain an overnight fast prior to the OGTT. A very low carbohydrate meal (6.7% carbohydrate, <10 grams) immediately prior to OGTT has been shown to alter OGTT results. The mechanisms of how low-carbohydrate diets impact glucose metabolism are complex and incompletely understood. Some proposed that the mechanism is in part due to loss of first-phase insulin release resulting in decreased peripheral and hepatic glucose uptake and incomplete suppression of hepatic glucose production (15).

Previous studies have shown that the time of day for the glucose measurement and fasting duration influence the glucose level in adults (56,57). For a random blood glucose sample a fasting duration of 5-6 hours seems to be sufficient for a reliable blood glucose measurement (58) and an overnight fast > 8 h for assessing GD after OGTT (59).

Very little is known about increasing palatability of oral glucose solutions (OGS) and thus improving compliance to testing. Polycal® liquid is flavoured and may be more palatable. When calculating the dose for children, Polycal® (113 mL) should be followed by 150 mL water (total volume should be 250- 300mL). Polycal® contains 0.66 g anhydrous glucose per mL (or 1.51 mL contains 1g anhydrous glucose). Rapilose® OGTT solution comes in liquid form and is available in a ready-to-use 300 mL pouch containing 75 g anhydrous glucose. Rapilose® has be customised for patients with a body weight ≥ 43 kg where they should consume the entire contents of one pouch, but patients who weigh under 43 kg should have the volume adjusted accordingly. Lucozade® solution contains caffeine which may affect glucose metabolism. However, it has been suggested that the composition of OGS, including the excipients added to improve taste and smell, can have a potential impact on blood glucose level and endogenous insulin secretion after OGTT (60).

Body size has a negative inverse association with 2h PG concentration in an OGTT even within a physiological plasma glucose range. This may cause underestimation of glucose disorders in individuals with larger body surface area (BSA) and overestimation in individuals with smaller BSA when using an OGTT (61). Therefore, it is a possibility that the diagnosis of T2-DM made by an OGTT gives a false positive result in a relatively small individual, and a false negative result in a relatively larger individual (62). Therefore, adjustment for height and BMI is needed for accurate interpretation of OGTT (63).

Glucose can be measured in whole blood, serum, or plasma, but plasma is recommended for diagnosis (64). Plasma equivalent glucose (mmol/L or mg/dL) is equal to whole blood glucose (mmol/L or mg/dL) x 1.11. A decrease in hematocrit causes increase in plasma equivalent glucose concentration and vice versa. Moreover, once the blood is drawn, the concentration of glucose will continue to decrease because of glycolysis, occurring in erythrocytes, white blood cells (WBCs), and platelets (65).

The loss of glucose in blood samples has been studied for many years (66). Glucose is lost through glycolysis at a rate of 5% −7%/h at concentrations near the reference interval. In absolute terms, a loss in glucose of about 12 mg/dL (0.67 mmol/L) occurs at a concentration of 100 mg/dL (5.55 mmol/L) after 2 h at room temperature (67). Higher rates of loss occur commonly, such as with increased ambient temperature and in samples with high white blood cell counts.

The original recommendations of the WHO and ADA for the stabilization of blood glucose, indicates the following: (a) immediate centrifugation and separation of plasma from blood cells, (b) immediate cooling of the sample tube in an ice-water slurry, and plasma separation within 30 min from blood draw (68,69). Guidelines recommend blood samples be immediately immersed in an ice slurry and analyzed within 30 minutes of collection, but this is difficult to achieve in patient care settings (69).

When fast separation of the cells is not possible, blood should be collected into a tube containing a glucose preservative. Sodium fluoride (NaF) is commonly used to inhibit glycolysis in samples collected during OGTT but is inadequate as it does not stop glycolysis for two or more hours (69). NaF can be used alone or with anticoagulants such as potassium oxalate, ethylenediaminetetraacetic (EDTA), citrate, or lithium heparin. Another important disadvantage of NaF is its impact on erythrocyte integrity, as shown by data on cell-free hemoglobin. Overall, the percentage of significant hemolysis using this additive can be as high as 94% of all samples (70).

There have been many studies to see if the glycolytic process can be minimized and several types of additives have been considered (71-74). The use of citrate buffer, a strong inhibitor of hexokinase, an enzyme located upstream in the glycolytic pathway, seems to produce more reliable measurements of PG in samples left uncentrifuged for over 30 min (70). A recent systemic review, made by Hal-Hinai et al.(75), reported that the mixture tubes (venosafe, gluco EXACT and Glucomedic) gave the best results for glucose stability. If the mixture tubes are not available, the use of a sample tube containing rapid glycolysis inhibitor (such as citrate buffer)is suggested.

In summary, careful attention to the pre-analytical phase variables is essential to ensure accurate glucose measurements. Blood samples should be drawn in the morning after an overnight fast. When fast separation of the cells is not possible, blood should be collected into a tube containing a glucose preservative. The recent recommendation for stabilization of blood glucose is the use of tubes containing a rapid glycolysis inhibitor, i.e. citrate/EDTA buffer, which inhibits the upstream enzymes involved in glycolysis unlike NaF which inhibits the downstream enzyme and allows glycolysis to continue for a considerable time.

2. Analytical phase

Glucose is measured almost exclusively by enzymatic methods. Specific and sensitive enzymatic assays, routinely used for the plasma glucose measurement, have considerably improved the quality parameters, in both accuracy and reproducibility terms. The principal used analytical methods for glucose determination are enzymatic assays, based on the hexokinase (recommended) or on the glucose oxidase reaction. These methods are highly standardized with an inter-laboratory imprecision (CV) < 2.6%,which is the gold standard enzymatic method for glucose estimation (76).

3. Post-analytical phase

Analytical factors that could influence the OGTT results are its reproducibility (usually expressed as coefficient of variation) and bias (i.e., the difference from the true value, usually expressed as the percentage of the true value). To minimize these factors, a good laboratory test should conform with specific analytical regulatory criteria, as recommended by the National Academy of Clinical Biochemistry (NACB) (66,69).

Conclusion

The complexity of diagnosis and treatment of GD require that healthcare professionals have specific and expertise knowledge to perform rigorous assessment of glucose metabolism. According to Walshaw (77), "a screening test should have a high sensitivity and a high specificity. Furthermore, it needs to be relevant to the condition in question, be efficient to carry out, and, if possible, be the 'gold standard' for that particular condition". The measurement of PG plays a central role in recognizing disturbances in carbohydrate metabolism, with established decision limits that are globally accepted. This requires that PG results be reliable and unequivocally valid no matter where they are obtained. Although FPG is more practical and less expensive compared to OGTT, the latter might be of greater utility in the detection of prediabetes and diabetes as reported by Chan et al. (78) but not confirmed by Færch et al. (79). On the other hand, during an OGTT, morphological features of the glucose curve (monophasic curve, glucose peak >30 minutes and 1-hour glucose ≥ 155 mg/dL) have been associated with higher prediabetes risk.

In general, the OGTT remains the preferred screening method because it is more sensitive for diabetes than the FPG although its reproducibility is poor.

The recommended preparation for, and administration of, the OGTT should be carefully followed to ensure precise results. A number of preanalytical factors like sample type, transport conditions, time to analysis, temperature and type of test tube that can influence glucose concentration should be considered. Each factor introduces a certain degree of variability. To minimize influence of these factors, a good laboratory test should conform with specific analytical regulatory criteria, as recommended by the National Academy of Clinical Biochemistry (NACB) (69). It is important to keep in mind that during OGTT, subjects with glucose values closer to the cut-off values of

criteria reported by WHO or ADA require a pedantic close attention in order to avoid GD misclassification and unnecessary intervention. That is why the ADA recommends a second test to confirm the diagnosis of diabetes.

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Ethics: The updated article did not require Ethical Committee approval.

Author Contributions: VDS reviewed the literature and wrote the first draft. ATS, SD, PT, SDM and CK contributed actively to the text editing and improvement of content. All the authors approved the final version of manuscript.

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