PANCREATIC ISLET FUNCTION TESTS

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Updated March 8, 2021

ABSTRACT

Objective: To describe testing indications and protocols for the evaluation of pancreatic islet function. **Methods:** A review of the literature and consensus guidelines concerning testing of pancreatic islet function was performed. **Results:** Indications for screening for diabetes mellitus are reviewed. Diagnostic criteria for diagnosis are fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/l) or random glucose ≥200 mg/dl (11.1 mmol/l) with hyperglycemic symptoms, hemoglobin A1c (HbA1c) ≥6.5%, and oral glucose tolerance testing (OGTT) 2-h glucose ≥200 mg/dl (11.1 mmol/l) after 75 g of glucose. One-step and two-step strategies for diagnosing gestational diabetes using pregnancy-specific criteria as well as use of the 2-h 75-g OGTT for the postpartum testing of women with gestational diabetes (4-12 weeks after delivery) are described. Testing for other forms of diabetes with unique features are reviewed, including the recommendation to use the 2-h 75 g OGTT to screen for cystic fibrosis-related diabetes and post-transplantation diabetes, fasting glucose test for HIV positive individuals, and genetic testing for monogenic diabetes syndromes including neonatal diabetes and maturity-onset diabetes of the young (MODY). Elevated measurements of pancreatic islet autoantibodies (e.g., to the 65-KDa isoform of glutamic acid decarboxylase (GAD65), tyrosine phosphatase related islet antigen 2 (IA-2), insulin (IAA), and zinc transporter (ZnT8)) suggest autoimmune type 1 diabetes (vs type 2 diabetes). IAA is primarily measured in youth. The use of autoantibody testing in diabetes screening programs are recommended only in first degree relatives of an individual with type 1 diabetes or in research protocols. C-peptide measurements >3 years after clinician diagnosis of type 1 diabetes in adults can be helpful in identifying those who have type 1 diabetes (low or undetectable c-peptide) from those who may have type 2 or monogenic diabetes. Use of OGTTs to examine insulin secretory reserve and intravenous glucose tolerance testing are also reviewed. These tests are primarily used in research studies. Evaluation of glycemic control is discussed, with special attention to hemoglobin A1c (HbA1c) and its correlation with mean blood glucose levels as well as assays of other glycated serum proteins. Finally, protocols used to evaluate hypoglycemia (glucose < 55 mg/dl (3.1 mmol/l)) are described, such as the supervised prolonged fast, during which measurements of glucose, insulin, c-peptide, oral insulin secretagogues, proinsulin, and beta-hydroxybutyrate are obtained. Insulinoma is suggested by elevated insulin, proinsulin and c-peptide levels, beta-hydroxybutyrate < 2.7 mmol/l, and undetectable insulin secretagogues. Use of a modified OGTT in the evaluation of the dumping syndrome is also described, as are the mixed meal test, glucagon tolerance test, c-peptide suppression test and evaluation of autoimmune hypoglycemia.

SCREENING FOR DIABETES MELLITUS AND PREDIABETES

Early detection and treatment of diabetes mellitus is important in preventing the chronic and acute complications of this disease. Individuals with symptoms suggestive of hyperglycemia, such as polyuria, polyphagia, polydipsia, unexplained weight loss, blurred vision,

excessive fatigue, or infections or wounds that heal poorly should be promptly tested.

The American Diabetes Association (ADA) recommends routinely screening for type 2 diabetes in adults every three years beginning at age 45. In asymptomatic people, testing for type 2 diabetes should be considered in adults of any age if they are overweight or obese (BMI ≥ 25 kg/m^2, or ≥ 23 kg/m^2 if Asian), planning pregnancy, and/or if they have additional risk factors as listed below in Table 1. Repeat screening should be performed at least every three years. Patients with prediabetes should be screened yearly (1). The US Preventive Services Task Force recommends glucose screening for all asymptomatic overweight or obese adults ages 40-70 (2); the American Association of Clinical Endocrinologists recommends screening at risk individuals at any age (3).

Table 1. Risk Factors for the Development of Type 2 Diabetes (1)
Physical inactivity
First-degree relative with diabetes
High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
Women who delivered a baby weighing >9 lb. or were diagnosed with Gestational Diabetes
Hypertension (≥140/90 mm Hg or on therapy for hypertension)
HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
Women with polycystic ovary syndrome
HbA1C ≥5.7%, Impaired Glucose Tolerance (IGT), or Impaired Fasting Glucose (IFG) on previous testing
Other clinical signs or conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans)
History of cardiovascular disease
HIV

Type 2 diabetes is becoming a growing problem in children and adolescents in high-risk populations. To address this issue, the ADA recommends screening overweight [body mass index (BMI) ≥85th percentile] or obese (BMI ≥95th percentile) youth at least every 3 years, beginning at age 10 or at the onset of puberty, if they have 1 or more additional risk factors listed below in Table 2. Repeat testing should be done more frequently if BMI is increasing (1).

Table 2. Risk Factors for Type 2 Diabetes in Children and Adolescents
Family history of type 2 diabetes (first and second-degree relatives)
High risk ethnicity (Native Americans, African-Americans, Latino, Asian/Pacific Islanders)
Signs of or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, small-for-gestational-age birth weight, or polycystic ovary syndrome)
Maternal history of diabetes or gestational diabetes during child's gestation

DIAGNOSING DIABETES AND PREDIABETES

The diagnosis of diabetes can be made using the fasting plasma glucose, random plasma glucose, oral glucose tolerance test, or hemoglobin A1c (HbA1c) (1). Testing should be performed on 2 separate days using one or more of the above tests, unless unequivocal hyperglycemia is present. Alternatively, in the absence of symptoms of hyperglycemia, diabetes can be diagnosed if there are two different abnormal test results from the same sample (1).

HbA1c

The use of the HbA1c assay was recommended for the diagnosis of diabetes in 2009 by an International Expert Committee (4). HbA1c levels reflect overall glycemic control and correlate with the development of microvascular complications. An HbA1c ≥ 6.5% on two separate occasions can be used to diagnose diabetes. An HbA1c level of 6.0% to less than 6.5% identifies high risk of developing diabetes. The ADA considers individuals with a HbA1c of 5.7% to 6.4% at increased risk for developing diabetes (1). HbA1c should not be used to diagnose gestational diabetes, diabetes in HIV positive individuals, post-organ transplantation, or in people with cystic fibrosis.

Table 3. ADA Criteria for the Diagnosis of Diabetes (1)
HbA1C ≥6.5%. The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay.
FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.
2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during an Oral Glucose Tolerance Test (OGTT). The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L) without repeat testing for confirmation.

Fasting and Random Plasma Glucose

Fasting plasma glucose is one method recommended by the ADA for the diagnosis of diabetes in children and non-pregnant adults (1). The test should be performed after an 8 hour fast. For routine clinical practice, fasting plasma glucose may be preferred over the oral glucose tolerance test because it is rapid, easier to administer, is more convenient for patients and providers, and has a lower cost (1). A random plasma glucose level, which is obtained at any time of the day regardless of the time of the last meal, can also be used in the diagnosis of diabetes in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.

Table 4. Fasting Plasma Glucose Criteria	
	Fasting Plasma Glucose
Normal glucose tolerance	<100 mg/dl (5.6 mmol/l)
Impaired fasting glucose (pre-diabetes)	100-125 mg/dl (5.6-6.9 mmol/l)
Diabetes mellitus	≥126 mg/dl (7.0 mmol/l)

For the diagnosis of diabetes, standard venous plasma glucose specimens should be obtained. Specimens should be processed promptly, since glucose is metabolized at room temperature. This process is influenced by storage temperature, storage time as well as other factors, and is accelerated in the presence of bacteria or leukocytosis.

Whole blood glucose specimens obtained with point-of-care devices should not be used for the diagnosis of diabetes because of the inaccuracies associated with these methods. Capillary and venous whole blood glucose concentrations are approximately 15% lower than plasma glucose levels in fasting specimens.

Oral Glucose Tolerance Test (OGTT)

OGTTS FOR THE DIAGNOSIS OF DIABETES AND IMPAIRED GLUCOSE TOLERANCE IN NON-PREGNANT INDIVIDUALS

Formal oral glucose tolerance tests can be used to establish the diagnosis of diabetes mellitus. They are more cumbersome and costlier than the fasting plasma glucose test, however, the use of only the fasting plasma glucose may not identify a proportion of individuals with impaired glucose tolerance or diabetes (5). A plasma glucose level

2-hours after a glucose challenge may identify additional individuals with abnormal glucose tolerance who are at risk for microvascular and macrovascular complications, particularly in high-risk populations in which postprandial (versus fasting) hyperglycemia is evident early in the disease (6,7).

When using an OGTT, the criteria for the diagnosis of diabetes is a 2 h glucose >200 mg/dl (11.1 mmol/l) after a 75-gram oral glucose load (ADA and WHO criteria). The 75-gram glucose load should be administered when the patient has ingested at least 150 grams of carbohydrate for the 3 days preceding the test and after an overnight fast. Dilution of the 75-gram oral glucose load (300-900 ml) may improve acceptability and palatability without compromising reproducibility (8). The patient should not be acutely ill or be taking drugs that affect glucose tolerance at the time of testing, and should abstain from tobacco, coffee, tea, food, alcohol and vigorous exercise during the test.

Table 5. Oral Glucose Tolerance Test Glucose Criteria	
	2-h Plasma Glucose (after 75-gram Glucose Load)
Normal glucose tolerance	<140 mg/dl (7.8 mmol/l)
Impaired glucose tolerance(pre-diabetes)	140-199 mg/dl (7.8-11.1 mmol/l)
Diabetes mellitus	≥200 mg/dl (11.1 mmol/l)

OGTTS FOR THE DIAGNOSIS OF GESTATIONAL DIABETES

The prevalence of gestational diabetes (GDM) varies among racial and ethnic groups and between screening practices, testing methods, and diagnostic criteria. The overall frequency of GDM in the 15 centers participating in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study was 17.8% (9), and regional estimates may vary from 10% to 25 % depending on the population studied (10). The prevalence increases with increased number of risk factors, such that 33% of women with 4 or more risk factors have gestational diabetes (11). This condition is important to diagnose early because of the increased perinatal morbidity associated with poor glycemic control.

The US Preventive Task Force recommends screening for gestational diabetes in asymptomatic women after 24 weeks (12); the ADA recommends screening all pregnant women routinely between 24- and 28-weeks' gestation. If the woman has risk factors, however, screening should be performed at the initial prenatal visit using standard criteria (1).

Table 6. Risk Factors for the Development of Gestational Diabetes
Overweight or obese
Previous history of impaired glucose tolerance, gestational diabetes, or delivery of a baby weighing >9 lb.
Glycosuria or history of abnormal glucose tolerance
Family history of diabetes (especially first degree relative)
Polycystic ovarian syndrome, hypertension, glucocorticoid use
History of poor obstetric outcome
Age (>25 years)
High risk ethnicity
Multiple gestation

Table 7. Low Risk for the Development of Gestational Diabetes
Age (< 25 years)

Normal weight pre-pregnancy
Low risk ethnicity
No first-degree relatives with diabetes
No history of abnormal glucose tolerance
No history of poor obstetric outcome

Table 8. Time of Initial Testing for Gestational Diabetes

Risk of Development of Gestational Diabetes	Time of Initial Testing for Gestational Diabetes
Low risk	24-28 weeks gestation
Average risk	24-28 weeks gestation
High risk	As soon as feasible; repeat at 24-28 weeks if earlier testing normal

More than one method has been recommended for the screening and diagnosis of gestational diabetes. The criteria for the diagnosis of this condition remain controversial because the glucose thresholds for the development of complications in pregnancies with diabetes remain poorly defined. Currently, the ADA suggests screening for GDM with either the “one-step” or “two-step” approach (1). Long term outcome studies evaluating pregnancies complicated by GDM are currently underway and hopefully a uniform approach will be adopted.

One-Step Strategy

The International Association of Diabetes and Pregnancy Study Group (IADPSG), an international consensus group with representatives from multiple obstetrical and diabetes organizations including the ADA recommend that all women not previously known to have diabetes undergo a 75-gram 2-hour OGTT at 24-28 weeks of gestation. This approach, which has been adopted internationally, is expected to increase the prevalence of GDM as only one abnormal value is sufficient to make the diagnosis (1,13). In 2017, the American College of Obstetricians and Gynecologists (ACOG) stated that clinicians may make the diagnosis of gestational diabetes based on only one elevated blood glucose value if warranted, based on their population, although this organization still supports the “two step” approach for diagnosis of GDM (14).

Table 9. Oral Glucose Tolerance Test Glucose Criteria for the Diagnosis of GDM

75-gram 2- hour OGTT: Performed at 24-28 weeks gestation in the morning after an overnight fast of at least 8 hours

GDM is diagnosed when any of the following values are exceeded:

Fasting	≥ 92 mg/dL (5.1 mmol/L)
One Hour	≥ 180 mg/dL (10.0 mmol/L)
Two Hour	≥ 153 mg/dL (8.5 mmol/L)

These glucose thresholds were based on outcome data of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study that conveyed an odds ratio for adverse maternal, fetal and neonatal outcomes of at least 1.75 based on fully adjusted logistic regression models (15).

Two-Step Strategy

The American College of Obstetricians and Gynecologists (ACOG) as well as the National Institutes of Health (NIH) have been in support of the "two step" approach which consists of universal screening of all pregnant women at 24-28 weeks gestation with a 50-gram glucose challenge regardless of timing of previous meals, followed by a 100-gram three-hour OGTT in screen positive patients (14, 16).

In the two-step approach, first a 50-gram oral glucose load is administered regardless of the timing of previous meals. The following thresholds have been defined as a positive screen: ≥130 mg/dL, ≥135 mg/dL, or ≥140 mg/dL (7.2 mmol/L, 7.5 mmol/L, or 7.8 mmol/L); the lower threshold has an estimated sensitivity and specificity of 88-99% and 66-77% compared to 70-88% and 69-89% respectively for the higher cutoff values of ≥135 mg/dL or ≥140 mg/dL (1).

Table 10. Abnormal Glucose Level on Screening Test	
50-gram Glucose Load	
1-h Plasma Glucose	≥130 mg/dl (7.8 mmol/l)

If the screening test is abnormal, the diagnosis of gestational diabetes should be confirmed using a formal 100-gram OGTT. This test should be performed after an overnight (8-14 h) fast. It is generally recommended that the woman ingest at least 150 grams of carbohydrate/day for the 3 days prior to testing to prevent false positive results; however, the necessity of this preparatory diet in normally nourished women has been challenged (17). The ADA recommends using the Carpenter/Coustan criteria (1). At least 2 of the following 4 venous plasma glucose levels must be attained or exceeded to make the diagnosis of GDM (1).

Table 11. Oral Glucose Tolerance Test Glucose Criteria for the Diagnosis of GDM		
	Carpenter/Coustan	**National Diabetes Data Group**
Fasting	≥95 mg/dl (5.3 mmol/l)	≥105 mg/dl (5.8 mmol/l)
One Hour	≥180 mg/dl (10.0 mmol/l)	≥190 mg/dl (10.6 mmol/l)
Two Hours	≥155 mg/dl (8.6 mmol/l)	≥165 mg/dl (9.2 mmol/l)
Three Hours	≥140 mg/dl (7.8 mmol/l)	≥145 mg/dl (8.1 mmol/l)

OGTTS FOR POSTPARTUM TESTING OF WOMEN WITH GESTATIONAL DIABETES

Women with a history of GDM are at a higher risk of developing type 2 diabetes than women without GDM (18,19). Women at the highest risk are those with multiple risk factors, those who had more severe gestational diabetes, and those with poorer beta cell function (11). The ADA recommends testing women 4-12 weeks after delivery using a two-hour 75-gram OGTT. Women with normal results should be retested at least every 3 years. It is recommended that women with impaired fasting glucose or impaired glucose tolerance be retested on a yearly basis (1).

Special Populations

OGTTS FOR DIAGNOSIS OF CYSTIC FIBROSIS-RELATED DIABETES

Diabetes is common in patients with cystic fibrosis and is associated with adverse effects on nutritional status as well as pulmonary function. Annual screening for diabetes is recommended for individuals over age 10 with cystic fibrosis (1). HbA1c and fructosamine can be inaccurate in this population. In a retrospective analysis of the Toronto cystic fibrosis database, screening for diabetes using a HbA1c cutoff of 5.5% had a sensitivity of 91.8% and specificity of only 34.1% (20) but more studies need to be performed before the use of HbA1c is generally recommended for the diagnosis of diabetes in these individuals.

The use of the 2-hour 75 gm OGTT is recommended for the screening of healthy outpatients with cystic fibrosis. For patients receiving continuous drip feedings, laboratory glucose levels at the midpoint or immediately after feedings should be obtained. The diagnosis of diabetes is based on glucose levels ≥200 mg/dL on 2 separate occasions. If the patient is acutely ill or ingesting glucocorticoids, a FPG ≥126 mg/dL or 2-hour postprandial glucose ≥200 mg/dL that persists for >48 hours is sufficient to diagnose diabetes (21, 22).

FASTING GLUCOSE FOR DIAGNOSIS OF PREDIABETES AND DIABETES IN PEOPLE LIVING WITH HIV

Screening for prediabetes and diabetes by measuring fasting glucose before and 3-6 months after starting or changing antiretroviral therapy is recommended for everyone living with HIV (1). If normal, a fasting glucose test should be performed yearly. Screening using a HbA1c test is not recommended for diagnosis due to risk of inaccuracies (1, 23).

OGTTS FOR DIAGNOSIS OF POST-TRANSPLANTATION DIABETES

After an individual has had an organ transplant and is on stable immunosuppressive therapy, routine screening for diabetes is recommended. The recommended screening test is an OGTT post- transplantation (1).

ESTIMATING INSULIN SENSITIVITY AND SECRETION

The hyperinsulinemic euglycemic insulin clamp procedure is the gold standard for measuring insulin resistance, and the hyperglycemic clamp is the gold standard for measuring insulin secretion. These are only used in research studies. Fasting data and data from OGTTs are more often used due to ease of performance and lower cost. A simple widely used research method, the Homeostasis Model (HOMA), uses fasting glucose (G) and insulin (I) levels (or c-peptide (C) instead of insulin) to estimate beta cell function and insulin sensitivity. The HOMA calculator as well as additional information concerning this method can be found at: http://www.dtu.ox.ac.uk/homacalculator/. Insulin secretion has also been estimated using the Insulinogenic Index [IGI; $\Delta I_{30}/\Delta G_{30}$] and the C-peptide Index ($\Delta C_{30}/\Delta G_{30}$). Additional estimates of insulin sensitivity include the Quantitative Insulin Sensitivity Check Index [QUICKI; 1/log(FI) + log (FG)] and the Whole-Body Insulin Sensitivity Index [WBISI]. The Oral Disposition Index is a measure of insulin secretion relative to insulin sensitivity [$1/I_F$x ($\Delta C_{30}/\Delta G_{30}$)]. These measures are not used in routine clinical care. A surrogate marker of insulin resistance is the lipid accumulation product (LAP) index, which uses information that can be obtained in routine clinical practice (24). It is calculated as follows: females (waist circumference-58) x (triglyceride [mmol/L]); males (waist circumference-68) x (triglyceride [mmol/L]). The LAP cannot be used if triglycerides are >15 mmol/L.

Intravenous Glucose Tolerance Test

The short intravenous glucose tolerance test (IVGTT) is used in research studies to assess first phase insulin release. This acute insulin secretory response is typically lost early in the development of both type 1 and type 2 diabetes due to reduction of beta cells and islet cell dysfunction. Abnormal IVGTT results can occur prior to the onset of the diabetes. The test is performed after an overnight 10 h fast, and the patients are instructed to ingest at least 150 grams of carbohydrate for the 3 days preceding the test. A 25-gram glucose bolus (of a 25% glucose solution) is given intravenously, and the acute insulin response calculated from the third to fifth minute after the glucose bolus. The short intravenous glucose tolerance test is sometimes used to assess pancreatic function after pancreatic transplantation.

In the Diabetes Prevention Trial Type 1, a glucose load was given intravenously (0.5 g/kg body weight up to a maximum of 35 grams) over 3 minutes, and insulin levels at 1- and 3-minutes post-load were used to estimate acute insulin production (25). Individuals with low insulin response (<100 uU/ml) and positive autoantibodies were at high risk of developing type 1 diabetes. Until effective interventions are established, however, the routine use of this test for the detection of early type 1 diabetes is not recommended.

The standard intravenous glucose tolerance test is used in research studies to estimate insulin sensitivity (SI) and glucose effectiveness (SG) using minimal model methodology. The procedure for the standard intravenous glucose tolerance test is to intravenously inject glucose (0.33 g/kg body weight) over 2 minutes and to frequently sample for glucose and insulin over 3-4 hours. Modifications include the addition of a tolbutamide (125 mg/m^2) or insulin (20-30 mU/kg) infusion 20-25 minutes after the glucose load. These tests are not used in clinical practice.

Additional information can be found in the chapter entitled “Assessing Insulin Sensitivity and Resistance in Humans” in the Diabetes or Endocrine Testing Protocol sections of Endotext.

C-Peptide Testing

During the processing of proinsulin to insulin in the beta cell of the pancreas, the 31 amino acid connecting peptide which connects the A and B chains, called c-peptide, is enzymatically removed and secreted into the portal vein. C-peptide circulates independently from insulin and is mainly excreted by the kidneys. Levels are elevated in renal failure. Standardization of different c-peptide assays is still suboptimal. C-peptide testing is used to examine insulin secretory reserve in people with diabetes. Another important use of c-peptide measurements is in the evaluation of hypoglycemia, described below (see Section "Evaluation of Hypoglycemia").

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At the time of type 1 diabetes diagnosis, c-peptide levels commonly overlap with those observed in type 2 diabetes, and cannot reliably distinguish between these diabetes types. With longer duration, there is progressive loss of c-peptide, and although c-peptide levels in many individuals with long-standing type 1 diabetes are extremely low or undetectable, there is heterogeneity in residual beta cell function with detectable c-peptide being more common in adult-onset type 1 diabetes (26). In type 1 diabetes, detectable c-peptide is associated with better glycemic control, less hypoglycemia, and decreased microvascular disease (27-28).

Type 2 diabetes is heterogeneous, with many individuals having progressive loss of beta cell function over many years evidenced by decreasing c-peptide levels. Fasting and glucose-stimulated c-peptide levels have been used in the past to distinguish type 1 (severe insulin deficiency) from type 2 diabetes with limited success. However, targeted testing may be more discriminatory. When random c-peptide testing was performed >3 years after clinical diagnosis of type 1 diabetes, 13% had a c-peptide ≥200 pmol/L, and after islet autoantibody and genetic testing, 6.8% of these were reclassified: 5.1% as having type 2 diabetes and 1.6% as having monogenic diabetes (29).

C-peptide stimulation using glucagon or a mixed meal such as Sustacal, has also been used to help differentiate between type 1 and type 2 diabetes, and to determine the need for insulin therapy in type 2 diabetes. In the glucagon stimulation test, glucose, insulin and c-peptide levels are measured 6 and 10 min after the intravenous injection of 1 mg of glucagon. Normal stimulation of c-peptide is a 150-300% elevation over basal levels. In the mixed meal tolerance test, Sustacal (6 mg/kg up to a maximum or 360 ml) is ingested over 5 minutes, and glucose and c-peptide are measured 90 min after oral ingestion.

These tests have had limited general clinical utility since they do not reliably discriminate between patients who require insulin therapy. They have been used in research studies and in the evaluation of patients after pancreatectomy and pancreatic transplantation. In the Diabetes Control and Complications Trial, a basal c-peptide value of <0.2 pmol/ml and stimulated level of <0.5 pmol/ml were used to confirm the presence of type 1 diabetes at entry (30).

PANCREATIC AUTOANTIBODIES

Islet autoantibodies can be detected early in the development of type 1 diabetes and are considered markers of autoimmune beta cell destruction. They predict progressive beta cell destruction and ultimately beta cell failure. The autoantibodies for which specific immunoassays are available include the 65-KDa isoform of glutamic acid decarboxylase (GAD65), insulin autoantibodies (IAA), zinc transporter antibodies (ZnT8), islet cell antigen 512 autoantibodies (ICA512), and autoantibodies to the tyrosine phosphatase related antigens islet antigen 2 (IA-2) and IA-2b. Measurements of ICA512, which are autoantibodies to parts of the IA-2 antigen, are no longer recommended. The presence of high levels of 2 or more antibodies is strongly predictive of type 1 diabetes mellitus. These antibodies may be detected before the onset of type 1 diabetes, at the time of diagnosis and for variable amounts of time after diagnosis. They have been used in screening for type 1 diabetes in first-degree relatives of an individual with type 1 diabetes or in research studies related to the early detection, treatment and prevention of type 1 diabetes (www.diabetestrialnet.org). These measurements are not recommended for use in general screening programs in low-risk individuals.

Commercially available assays for autoantibodies are sometimes useful in distinguishing type 1 diabetes from type 2 diabetes. The absence of detection of these antibodies, however, does not exclude the diagnosis of

type 1 diabetes. Since IAA can form in response to insulin therapy, detection can be the result of insulin injections or autoimmune insulin antibody formation. GAD65 antibodies are frequently observed early in the course of type 1 diabetes. They are also present in the rare neurological disorder, stiff-man syndrome, and in some patients with polyendocrine autoimmune disease.

In adults with newly diagnosed diabetes for whom type 1 diabetes is a possible diagnosis, GAD65 is commonly measured first, along with or followed by IA2 and ZnT8. IAA are more commonly detected in young children who develop type 1 diabetes and are generally not measured in adults.

Lynam and coworkers (31) developed a clinical multivariable model to help differentiate between type 1 and type 2 diabetes in adults ages 18-50 years. The model includes age at diagnosis, BMI, islet autoantibodies (GAD, IA-2), and a type 1 diabetes genetic risk score. The authors define type 1 diabetes by a non-fasting c-peptide <200 pmol/L and rapid insulin requirement within the first 3 years of diagnosis. The definition of type 2 diabetes was not requiring insulin treatment within the first 3 years after diagnosis or, if insulin was used, having a c-peptide measurement of >600 pmol/L at ≥5 years post-diagnosis. Since the measures of the genetic variants in the type 1 diabetes genetic risk score are not widely available, this model is not used clinically in the United States.

GENETIC TESTING FOR MONOGENIC DIABETES SYNDROMES

Monogenic diabetes syndromes account for 1%-5% of all individuals with diabetes and have been primarily classified as neonatal diabetes or Maturity-Onset Diabetes of the Young (MODY) based on clinical characteristics. More than 50 affected genes have been described. A *Diabetes Care* Expert Forum assembled in 2019 to re-consider the classification of monogenic diabetes syndromes. They recommend a classification system based upon molecular genetics, listing the affected gene, inheritance/phenotype, disease mechanism/special features, and the treatment implications (32).

When genetic testing is considered, involvement of centers with expertise in the diagnosis and treatment of monogenic diabetes syndromes is recommended (1). Laboratories performing genetic testing should participate in quality assurance programs (32). Proper diagnosis is critical since treatment approaches will differ depending upon the gene affected.

Table 12. When to Consider Genetic Testing for Monogenic Diabetes Syndromes
Diabetes diagnosed younger than 6 months of age
Diabetes in children and young adults not characteristic of type 1 or type 2 (negative pancreatic auto-antibodies, non-obese, no features of metabolic syndrome) and with a strong family history (diabetes in successive generations suggesting dominant inheritance)
Fasting glucose 100-150 mg/dL, stable A1c (5.6-7.6%), especially if in a non-obese child or young adult

The ADA recommends immediate genetic testing for all infants diagnosed with diabetes within the first 6 months of life (1). Common causes of neonatal diabetes include mutations in the following genes: KCNJ11 (potassium inward-rectifying channel, subfamily J, member 11), ABCC8 (ATP-binding cassette, sub-family C, member 8 of the potassium channel), INS (preproinsulin), 6q24 (PLAGL1, HYMA1), GATA6, EIF2AK3, EIF2B1 and FOXP3.

MODY most commonly manifests before age 25 years but can be diagnosed in older individuals. The inheritance is typically autosomal dominant. Individuals who have positive islet autoantibody test results and/or low c-peptide concentrations should not be tested for monogenic diabetes syndromes (33). The number of genetic mutations responsible has been increasing each year. Most common forms include: HNF4A-MODY (hepatocyte nuclear factor-4 alpha; MODY 1), GCK-MODY (glucokinase; MODY 2), HNF1A-MODY (hepatocyte nuclear factor 1 homeobox A;MODY 3), PDX1-MODY (MODY 4), HNF1B-MODY (hepatocyte nuclear factor-1 beta; MODY 5), NEUROD1-MODY (MODY 6), and INS-MODY (MODY 10). A MODY risk calculator is available at: https://www.diabetesgenes.org/mody-probability-calculator.

EVALUATION OF GLYCEMIC CONTROL IN DIABETES MELLITUS

Hemoglobin A1c

Glycosylated hemoglobin, or the hemoglobin A1c (HbA1c) assay, is the most widely accepted laboratory test for the measurement of glycemic control and is recommended for routine use in the management of patients with diabetes mellitus. HbA1c levels reflect average blood glucose levels over the preceding 2-3 months. Although the life span of erythrocytes is approximately 120 days, HbA1c levels represent a weighted average of blood glucose levels, with youngest red blood cells, reflecting mean glucose levels over the past month, contributing to a greater extent than older ones.

The International Federation of Clinical Chemistry Working Group on HbA1c defines the HbA1c as the hemoglobin A that is irreversibly non-enzymatically glycosylated at one or both N-terminal valines of the beta-chains of the hemoglobin. Multiple methods have been certified to measure HbA1c. The National Glycohemoglobin Standardization Program, which was started in 1996, has been largely successful in its goal to standardize HbA1c assays throughout the United States to the HPLC method used in the Diabetes Control and Complications Trial. The process has involved certification and proficiency testing, and long-term monitoring of quality control data. Providers should only use laboratories that are certified by the National Glycohemoglobin Standardization Program. Information concerning certified methods and laboratories can be found on their website http://www.ngsp.org/.

A consensus statement on the international standardization of HbA1c assays was issued by the American Diabetes Association (ADA), the European Association for the Study of Diabetes, the International Diabetes Federation, the International Federation of Clinical Chemistry and Laboratory Medicine, the International Society for Pediatric and Adolescent Diabetes, the Japanese Diabetes Society and the National Glycohemoglobin Standardization Program (34). HbA1c assays should be calibrated to this reference method and results reported in a standardized manner (A1c (%); A1c (mmol/mol), and estimated average glucose).

The ADA recommends determining HbA1c levels every 3 to 6 months to monitor glycemic control (1). Reducing the HbA1c level to as close to normal as possible is directly related to the reduction in the development and progression of the chronic complications of diabetes (31, 35-37). The ADA goal HbA1c is <7% (if this can be accomplished safely) but states that lower goals may be appropriate in individual patients. Higher HbA1c goals may be appropriate for patients with a history of severe hypoglycemia, limited life expectancy, advanced complications, and/or comorbid conditions and those in whom a lower goal is difficult to attain (1). The American Association of Clinical Endocrinologists target HbA1c is 6.5% for otherwise healthy patients at low risk for hypoglycemia. HbA1c targets should be individualized in patients with concurrent illness or those at risk for hypoglycemia (38).

The international A1c-Derived Average Glucose Study (ADAG) utilized frequent self-monitoring of blood glucose in adults with type 1 diabetes, type 2 diabetes, and no diabetes. The study described a linear relationship between HbA1c and average glucose level (39). In the ADAG study, there was no significant difference in the regression lines between HbA1c and mean glucose levels among ethnic and racial groups, although there was a trend toward a difference in regression lines between African/African-American and Caucasian adults. Other studies have shown differences in HbA1c by race and ethnicity, but the reasons for this remain unknown and the individual differences within racial groups are greater than the variation between races (40-42). At this time the recommended HbA1c target does not differ based on race or ethnicity.

Table 13. Correlation of HbA1c with Mean Blood Glucose Concentrations (39, 42-43)

Hemoglobin A1c (%)	Approximate Mean Plasma Glucose (mg/dL)	
	Nathan et al 2008 (ADAG study; reference 31)	Beck et al 2017 (reference 35)

6	100-152	101-163
7	123-185	128-190
8	147-217	155-218
9	170-249	182-249
10	193-282	209-273

Table 14. Relationship of HbA1c Levels with Mean Glucose Levels

Hemoglobin A1c (%)	Mean Glucose Concentrations (95% CI; reference 1)			
	Fasting (mg/dL)	Premeal (mg/dL)	Post meal (mg/dL)	Bedtime (mg/dL)
5.5-6.49	122 (117-127)	118 (115-121)	144 (139-148)	136 (131-141)
6.5-6.99	142 (135-150)	139 (134-144)	164 (159-169)	153 (145-161)
7.0-7.49	152 (143-162)	152 (147-157)	176 (170-183)	177 (166-188)
7.5-7.99	167 (157-177)	155 (148-161)	189 (180-197)	175 (163-188)
8.0-8.5	178 (164-192)	179 (167-191)	206 (195-217)	222 (197-248)

Depending upon the assay method being used, certain hemoglobinopathies may interfere with results. This problem is highly method-dependent. Inaccurate results may be obtained in the presence of salicylates, chronic alcohol or opiate use, hyperbilirubinemia, liver or renal disease, iron deficiency, vitamin C, vitamin E, hypertriglyceridemia, lead poisoning, recent blood transfusions, and when there are conditions of abnormal red blood cell turnover such as in anemia, hemolysis, pregnancy, or use of erythropoiesis-stimulating agents. See www.ngsp.org/interf/asp for a full list of interferences for different methods.

Because of the improved standardization and reference method for the HbA1c assay, both the ADA and an International Expert Committee suggest that a HbA1c > 6.5% on two occasions is diagnostic of diabetes (1,4). Benefits of the use of HbA1c for the diagnosis of diabetes are that the test is easy to perform, does not have to be performed in the fasting state, and does not require any special preparation. Potential problems include interference by factors associated with abnormal red blood cell turnover and cost (44). The HbA1c range that indicates high risk of developing diabetes is considered 6.0% to <6.5% by the International Expert Committee (4) and 5.7% to 6.4% by the ADA (1).

Fructosamine, Glycated Albumin and 1,5-Anhydroglucitol

Assays of glycated serum proteins, which mostly measure glycated serum albumin, can reflect short-term glycemic control. The fructosamine assay is most commonly used. Since albumin has a short half-life (14-20 days), this test indicates average blood glucose levels over the past few weeks, which can be helpful in certain conditions such as pregnancy or in patients with hemoglobinopathy or abnormal red blood cell turnover (1,45). These tests may be affected by hypertriglyceridemia, hyperbilirubinemia, hyperuricemia, hypothyroidism and hyperthyroidism, as well as by low serum protein and albumin levels. The relationship between these measures of glycemic control and HbA1c, fasting glucose and mean glucose have been reported in few studies shown below:

Table 15. Relationship Between Tests to Measure Glycemic Control					
Hemoglobin A1c (%)	**Mean Fasting Glucose (mg/dL)**	**Mean CGM Glucose (mg/dL)**	**Mean (Range) Fructosamine (µmol/L)**	**Mean (Range) Glycated Albumin (%)**	**Mean (Range) 1,5-anhydroglucitol (µg/mL)**
5.4[a]	102[a]		219 (89-240)	12.2 (5.6-13.5)	
	100 [b]		224.9	12.5	27.5
5.7[b]			241.4	13.6	29.1
	126 [b]		261.7	15.0	17.9
6.1[a]			238	13.3 (7.9-15.6)	
6.2[a]	126		236 (159-265)	13.6	
	126		250.5-276.4	15.5-16.9	5.9-15.7
6.5 [b]			270.2	15.6	22.7
6.5[c]			254.7-289.5	16.1-18.3	5.0-15.3
7.4[d]		143	293	19.6	3.4
7.7 [a]	179		305 (266-355)	18.9 (15.7-23.0)	
7.8[d]		170	312.5	22.8	4.2
8.2[d]		185	344	24.9	6.1
9.4[d]		218	427	30.4	11.6
10.5 [a]	269		445 (356-706)	30.3 (23.1-51.5)	

a-c References 46-48: ARIC study (adults)
d Reference 49: DirecNet study (youth)
CGM: continuous glucose monitoring

Assays of 1,5-anhydroglucitol (1,5-AG) are an alternative measure of hyperglycemia. In the kidney, 1,5-AG is filtered by the glomeruli and reabsorbed in the proximal tubules. This reabsorption is competitively inhibited by glucose. When high glucose levels are associated with glycosuria, there is increased 1,5-AG excretion in the urine and lower serum levels. Concentrations of 1,5-AG reflect hyperglycemia-induced glycosuria over the prior 1-2 weeks. This test may be affected by pregnancy, advanced renal disease (CKD stages 4-5), and by use of SGLT-2 inhibitors.

There are few of studies demonstrating the usefulness of the fructosamine, glycated albumin, and 1,5-AG assays in predicting the development of diabetes-related complications (46, 50). Racial differences have also been reported for these assays (50). Since their clinical usefulness is not well established, testing is generally recommended in situations where HbA1c testing is expected to be inaccurate (e.g., abnormal red blood cell turnover).

Continuous Glucose Monitoring

Glucose data from continuous glucose monitors (CGM) are increasingly being used to assess glycemic control. These data have the advantage of displaying glycemic patterns, glucose variability, time in target range, and time

in hypoglycemia. CGM is discussed in detail in the chapter entitled “Monitoring Technologies – Continuous Glucose Monitoring, Mobile Technology, Biomarkers of Glycemic Control” in the Diabetes section of Endotext.

EVALUATION OF HYPOGLYCEMIA

Symptomatic hypoglycemia is defined clinically using Whipple's triad, which includes the presence of symptoms (confusion, lightheadedness, loss of consciousness, seizure, aberrant behavior, sweating, palpitations, weakness, blurred vision, or hunger) at the time of a low plasma glucose level, with improvement of symptoms when plasma glucose levels return to normal (51). The physician should determine if the patient truly has hypoglycemia prior to seeking an etiology. A plasma glucose level < 55 mg/dl (3.1 mmol/l) should raise the suspicion for a hypoglycemic disorder and initiate further evaluation, but many authorities rely on a glucose <40 mg/dl (2.2 mmol/l) as being diagnostic (52). Although symptoms are commonly observed when plasma glucose falls to <55 mg/dl (3.1 mmol/l), levels of <45 mg/dl (2.5 mmol/l) are associated with cognitive dysfunction (neuroglycopenia). Capillary glucose determinations should not be used in the evaluation of hypoglycemic disorders due to their poor accuracy in these situations.

The Endocrine Society has published clinical practice guidelines for the evaluation and management of hypoglycemic disorders (53). In persons without diabetes, drugs or critical illnesses, hormone deficiencies, and non-islet cell tumors should be considered based on the clinical findings (54). If the cause of the hypoglycemia is not evident then plasma glucose, insulin, c-peptide, proinsulin, β-hydroxybutyrate, insulin antibodies, and a screen for oral hypoglycemic drugs should be obtained during an episode of spontaneous hypoglycemia. Glucagon 1 mg IV should then be administered with careful follow up of the glucose response. This will help determine if the condition is related to excessive endogenous insulin production. The diagnosis of pancreatic hyperinsulinemic hypoglycemia is supported by the demonstration that insulin secretion is not suppressed normally when the patient develops hypoglycemia. If testing cannot be conducted during an episode of spontaneous hypoglycemia, the prolonged fast or mixed meal test followed by the administration of glucagon is the most useful diagnostic study.

Some patients who have had bariatric surgery for the treatment of obesity, most commonly Roux-en-Y gastric bypass, will develop hypoglycemia. This is associated with abnormal OGTTs and mixed meal tests, abnormal transport of food to the small intestine, and, in some cases, hypersecretion of insulin and incretin hormones (55-58). Spontaneous hypoglycemia has been reported after islet auto-transplantation for chronic pancreatitis as well; a deficient glucagon response to hypoglycemia during a mixed meal test has been reported (59).

Prolonged Fast

The gold standard test in the evaluation of hypoglycemia is the 72-hour supervised fast although a 48-h fast is almost as effective in diagnosing patients with suspected insulinoma (60). The purpose of the fast is twofold. The first is to diagnose hypoglycemia as the cause of the patient's symptoms. The second is an attempt to determine the etiology of the hypoglycemia. Due to the risk of hypoglycemia, patients should be admitted to the hospital to undergo the fast in a monitored setting. The fast could be initiated in a carefully monitored outpatient facility, with the patient entering the hospital if the fast is not terminated prior to the closing of the site. Baseline bloodwork can include cortisol, growth hormone, glucagon, and catecholamines if deficient counterregulation is suspected.

During the fast, patients are allowed no food but can consume non-caloric caffeine-free beverages for up to 72 hours. The onset of the fast is the time of the last food consumption. During the fast all non-essential medications should be discontinued. Simultaneous insulin, c-peptide and glucose samples are obtained at the beginning of the fast and every 4-6 hours thereafter. Once the plasma glucose falls to <60 mg/dl, specimens should be taken every 1-2 hours under close supervision. Patients should continue activity when they are awake. The fast continues until the plasma glucose falls below 45 mg/dl (2.5 mmol/l) (plasma glucose <55 mg/dl (3.1 mmol/L) is recommended in the most recent Endocrine Society guidelines (53)) and symptoms of neuroglycopenia develop, at which time, insulin, glucose, c-peptide, oral insulin secretagogues, proinsulin and beta-hydroxybutyrate levels are obtained and the fast is terminated (52). Additional samples for

insulin antibodies, anti-insulin receptor antibodies, IGF-1/IGF-2, and plasma cortisol, glucagon or growth hormone can also be obtained if a non-islet cell tumor, autoimmune etiology, or hormone deficiency is suspected. A glucagon tolerance test is then frequently performed to aid in diagnosis (Glucagon, 1 mg intravenously, administered with careful follow up of the glucose response every 10 minutes for 30 minutes. Further details regarding the glucagon tolerance test are below). Patients are fed at the conclusion of the test.

The diagnosis of endogenous hyperinsulinism is likely if the patient has neuroglycopenic symptoms, a fall in plasma glucose to <55 mg/dl, inappropriately elevated beta-cell polypeptides (insulin, proinsulin and c-peptide levels; see below table), with undetectable oral insulin secretagogue levels. β-hydroxybutyrate <2.7 mmol/L, and an increase in plasma glucose ≥25 mg/dL (1.4 mmol/L) after intravenous glucagon (53).

Table 16. Distinguishing Causes of Symptomatic Hypoglycemia (glucose < 55 mg/dl (3.0 mmol/l)) After a Prolonged Fast

Insulin (μU/mL)	C-peptide (nmol/L)	Proinsulin (pmol/L)	Oral hypoglycemic medication	Interpretation
≥3	<0.2	<5	No	Exogenous insulin
≥3	≥0.2	≥5	No	Endogenous insulin[a]
≥3	≥0.2	≥5	Yes	Oral hypoglycemic (drug-induced)

[a] Insulinoma, non-insulinoma pancreatogenous hypoglycemia (NIPHS), post gastric bypass hypoglycemia.

Adapted from: Cryer, PE, et al. Evaluation and Management of Adult Hypoglycemic Disorders: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 94:709-728, 2009

Approximately 75% of patients with insulinomas are diagnosed after a 24 hour fast and 90-94% at 48 hours. Although some experts advocate conducting the prolonged fast for only 48 hours (60), others disagree, arguing that prolonging the fast up to 72 hours minimizes misdiagnosis and maximizes the probability of diagnosing an insulinoma (61).

Limitations of the prolonged fast:

- Normal subjects, especially young women, can occasionally have plasma glucose levels of <40 mg/dl (2.2 mmol/l)
- Rare insulinomas suppress their release of insulin in response to hypoglycemia
- Insulin levels can sometimes be artificially elevated in the presence of anti-insulin antibodies.

OGTT and Mixed Meal Test

When the diagnosis of the dumping syndrome is being considered, a modified OGTT has been recommended (62). After an overnight fast, a 75-gm glucose load is administered. Glucose levels are measured at baseline and every 30 min up to 180 min. To diagnose hypoglycemia due to the dumping syndrome, a glucose reading of <50 mg/dL is observed, typically between 60 and 180 min after receiving the glucose load.

For patients with hypoglycemic symptoms several hours after meals, an OGTT or mixed meal test may be performed. The mixed meal test has not been well standardized. This test is typically done after an overnight fast. Patients eat a meal similar to one that provokes their symptoms. If this is not possible then a commercial mixed meal may be used. Patients are then observed for several hours. Samples for plasma glucose, insulin, c-peptide, and proinsulin are collected prior to the meal and every 30 minutes thereafter for 5 hours. If symptoms occur prior to the end of the test then additional samples for the above are collected prior to administration of carbohydrates. If Whipple's triad is demonstrated, testing for oral hypoglycemic drugs and testing for insulin antibodies should be done. Interpretation of test results is the same as for the 72-hour fast or spontaneous hypoglycemia

Glucagon Tolerance Test

The glucagon tolerance test serves as a supplemental study to aid in the diagnosis of hypoglycemic disorders when results from the prolonged fast are inconclusive. Following an overnight fast (or at the conclusion of a prolonged fast), 1 mg of glucagon is injected intravenously over 2 minutes. Plasma glucose and insulin levels are measured at baseline, and either 10, 20, and 30 minutes after glucagon, or at 3, 5, 10, 15, 20, and 30 minutes after glucagon injection. In normal patients, maximum insulin response occurs rapidly and usually does not exceed 100 uU/ml (peak insulin 61+19 uU/ml at 3-15 minutes), and the serum glucose levels peak at 20-30 minutes (140 +24 mg/dl) (63).

Insulinoma patients demonstrate an exaggerated insulin response to glucagon, with values often exceeding 160 uU/ml within 15-30 minutes of the injection (peak insulin 93-343 uU/ml at 15 minutes) (54). In the hypoglycemic patient at the conclusion of the prolonged fast, an increase in plasma glucose of >25 mg/dl (1.4 mmol/l) post-glucagon suggests an insulin-mediated etiology (63).

Patients with malnutrition or hepatic disease may be unable to have a hyperglycemic response to glucagon due to depleted hepatic glycogen stores. Insulin responses in these subjects may be increased but not to the degree seen in subjects with an insulinoma. Drugs such as diazoxide, hydrochlorothiazide and diphenylhydantoin can cause false negative results (62). Patients with non-islet cell tumors such as hemangiopericytomas and meningeal sarcomas can have similar glucose elevations (30 mg/dl) as subjects with insulinomas following glucagon injection (65).

Another limitation of the glucagon stimulation test is the failure of some insulinoma patients to hypersecrete insulin following glucagon injection. This problem was reported in 8% of patients with insulinomas in one study (66). In addition, patients with cirrhosis with portocaval anastomosis can have peak insulin levels that are indistinguishable from subjects with insulinomas. Obese subjects and patients with acromegaly can also have exaggerated peak insulin responses, as can patients treated with sulfonylurea drugs and aminophylline.

An additional disadvantage of this test is the danger of causing hypoglycemia after 90-180 min (66) as well as inducing nausea and vomiting. Because of the possibility of severe hypoglycemia, a physician needs to be present during the test.

Autoimmune Hypoglycemia

The insulin autoimmune syndrome is a rare condition whereby antibodies, either directed against insulin or against the insulin receptor, are responsible for hypoglycemia. Autoimmune hypoglycemia due to insulin antibodies should be suspected when the hypoglycemia is associated with high insulin levels (usually >100 uU/mL) and incompletely suppressed C-peptide levels. Insulin levels are rarely >100 uU/mL in the presence of hypoglycemia due to an insulinoma. Although these elevated insulin levels can be observed with exogenous insulin administration, the associated c-peptide levels are usually extremely low. Autoimmune hypoglycemia is most often seen in people of Japanese descent but has been described in other populations (67). Autoimmune hypoglycemia may also be due to antibodies to the insulin receptor. These patients will have mildly elevated insulin levels (thought to be due to decreased clearance of insulin) and suppressed c-peptide levels, and may have other autoimmune conditions. Antibodies to insulin and/or proinsulin and insulin receptor antibodies can interfere with the measurements of pancreatic hormones using immunoassays (68-69). Insulin, proinsulin and/or insulin receptor antibody testing is needed to confirm the diagnosis of autoimmune hypoglycemia. This testing does not need to be done at the time of hypoglycemia.

C-Peptide Suppression Test

C-peptide and insulin are secreted in equimolar concentrations in the pancreas, making c-peptide levels a good marker of endogenous insulin secretion. The c-peptide suppression test can be used to test for an insulinoma or to provide supplemental diagnostic information, especially if the results of a supervised fast are not definitive. The c-peptide suppression test must be carefully administered, since the patient is given intravenous insulin to induce hypoglycemia. The advantage of the test is that it is of much shorter duration than the supervised fast.

The c-peptide suppression test is performed following an overnight fast. The procedure is to infuse regular insulin, 0.125 U/kg body weight, intravenously over 60 minutes. Blood samples are obtained from the contralateral arm at 0, 30, 60, 90, and 120 minutes for determination of insulin, c-peptide, and plasma glucose levels. An abnormal result is a lower percentage decrease of c-peptide at 60 minutes compared to normative data appropriately adjusted for the patient's body mass index and age (70). For example, an abnormal result for a 45-year-old with a BMI of 25-29 kg/m^2 would be <61% suppression of c-peptide at 60 minutes (70). An alternative method (Regular insulin 0.075 IU/kg/hr. infused intravenously over 2 hours) using a different classification plot has been proposed (71) but few data using it have been published.

Limitations of this test include the fact that some patients with a documented insulinoma have normal c-peptide levels including normal percent decrease in c-peptide levels. There is also the danger of inducing severe hypoglycemia. In addition, little data concerning the reliability, sensitivity and safety of this test are published.

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