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Evidence-based
Laboratory Medicine
and Test Utilization

Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus

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Background: **Multiple laboratory tests are used in the diagnosis and management of patients with diabetes mellitus. The quality of the scientific evidence supporting the use of these assays varies substantially.**
Approach: **An expert committee drafted evidence-based recommendations for the use of laboratory analysis in patients with diabetes. An external panel of experts reviewed a draft of the guidelines, which were modified in response to the reviewers' suggestions. A revised draft was posted on the Internet and was presented at the AACC Annual Meeting in July, 2000. The recommendations were modified again in response to oral and written comments. The guidelines were reviewed by the Professional Practice Committee of the American Diabetes Association.**
Content: **Measurement of plasma glucose remains the sole diagnostic criterion for diabetes. Monitoring of glycemic control is performed by the patients, who measure their own plasma or blood glucose with meters, and by laboratory analysis of glycated hemoglobin. The potential roles of noninvasive glucose monitoring, genetic testing, autoantibodies, microalbumin, proinsulin, C-peptide, and other analytes are addressed.**
Summary: **The guidelines provide specific recommendations based on published data or derived from expert consensus. Several analytes are of minimal clinical value at the present time, and measurement of them is not recommended.**

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An Approved Guideline of the National Academy of Clinical Biochemistry "Laboratory Medicine Practice Guidelines". These guidelines were reviewed by the Professional Practice Committee of the American Diabetes Association (ADA) in June 2001 and were found to be consistent in those areas where the ADA has also published Clinical Practice Recommendations. The Clinical Practice Recommendations of the ADA are updated annually, and the most recent version should be consulted.

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Executive Summary

The following guidelines provide recommendations based on the best available evidence derived from published data or expert consensus.

GLUCOSE

Accredited laboratory

Glucose should be measured in an accredited laboratory to establish the diagnosis of diabetes and to screen high-risk individuals. Analysis in an accredited laboratory is not recommended as the primary means for routine monitoring or evaluating therapy in individuals with diabetes. Blood should be drawn after the individual has fasted overnight. If plasma cannot be separated from the cells within 60 min, a tube containing a glycolytic inhibitor should be used. Glucose should be measured in plasma.

Although methods for glucose analysis exhibit low imprecision at the diagnostic decision limits of 7.0 mmol/L [(126 mg/dL), fasting] and 11.1 mmol/L [(200 mg/dL), post glucose load], the relatively large intraindividual biological variability (CVs of ~5–7%) may produce classification errors. On the basis of biological variation,

glucose analysis should have analytical imprecision ≤3.3%, bias ≤2.5%, and total error ≤7.9%.

Portable meters

Portable meters are used by healthcare workers in acute and chronic care facilities, in physicians' offices, and by patients. Because of the imprecision and variability among meters, they should not be used to diagnose diabetes and have limited value in screening.

Self-monitoring of blood glucose (SMBG) is recommended for all insulin-treated patients. It should be performed at least three times a day for patients with type 1 diabetes. The efficacy of SMBG in patients with type 2 diabetes has not been established.

Multiple performance goals for portable glucose meters have been proposed. These targets vary widely and lack consensus. Clinical studies are needed to determine these analytical goals. We recommend meters that measure and report plasma glucose concentrations.

Oral glucose tolerance test (OGTT)

We do not recommend the OGTT for the routine diagnosis of type 1 or 2 diabetes. This issue is controversial, and the WHO supports its use. The key limitation of the OGTT is its poor reproducibility. Proponents, however, argue that it has slightly higher sensitivity than fasting glucose for diagnosing diabetes.

Noninvasive or minimally invasive glucose analyses

Noninvasive glucose analyses cannot be recommended at present as replacements for SMBG or glucose measurements by an accredited laboratory. Although promising, clinical studies remain limited. Several methodologies are available, but no analytical performance goals have been established.

ketones

Ketones should be measured in urine or blood by patients with diabetes at home and in hospitals or clinics as an adjunct to the diagnosis of diabetic ketoacidosis (DKA). Methods based on the nitroprusside reaction should not be used to monitor treatment of DKA. Although specific measurement of β -hydroxybutyrate is available, further studies are needed to ascertain whether this offers clinical advantage.

glycated hemoglobin (GHb)

GHb should be measured at least biannually in all patients with diabetes to document their glycemic control. Treatment goals should be based on the results of prospective randomized clinical trials, such as the Diabetes Control and Complications Trial (DCCT), that documented the relationship between glycemic control (quantified by GHb analysis) and the risks for the development and progression of chronic complications of diabetes.

US laboratories should use GHb assays certified by the National Glycohemoglobin Standardization Program (NGSP) as traceable to the DCCT reference. GHb concentrations should be maintained at <7%, and the treatment regimen should be reevaluated if GHb is >8% as measured by NGSP-certified methods. Laboratories should participate in proficiency testing. Efforts to achieve global harmonization of GHb testing, an important goal, are underway.

genetic markers

Routine measurement of genetic markers is not recommended at this time for the diagnosis or management of patients with diabetes.

autoimmune markers

Several autoantibodies have been detected in individuals with type 1 diabetes. However, these lack specificity and are not recommended for routine diagnosis or screening of diabetes. Until type 1 diabetes can be prevented, islet cell autoantibody measurement should be essentially confined to research protocols.

microalbuminuria

Diabetes is the leading cause of end-stage renal disease. Annual microalbuminuria testing should be performed in patients without clinical proteinuria. To be useful, semiquantitative or qualitative screening tests must be shown to be positive in >95% of patients with microalbuminuria. Positive results of such tests must be confirmed by quantitative testing in an accredited laboratory.

miscellaneous potentially important analytes

Several other analytes are measured in patients with diabetes. All adults with diabetes should receive annual lipid profiles. There is no role for routine testing for insulin, C-peptide, or proinsulin in most patients with diabetes. These assays are useful primarily for research purposes. Similarly, measurement of amylin or leptin is not of value at this time in the management of patients with diabetes.

Introduction

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia. The disease is classified into several categories. The revised classification, published in 1997 *(1)* is shown in Table 1[1]. Type 1 diabetes mellitus, formerly known as insulin-dependent diabetes mellitus or juvenile-onset diabetes mellitus, is caused by autoimmune destruction of the β-cells of the pancreas, rendering the pancreas unable to synthesize and secrete insulin *(2)*. Type 2 diabetes mellitus, formerly known as non-insulin-dependent diabetes mellitus or adult-onset diabetes, results from a combination of insulin resistance and inadequate insulin secretion *(3, 4)*. Other types of diabetes are rare. Type 2 is the most common form, accounting for 90–95% of diabetes in developed countries.

In 1992, the costs of diabetes in the US were estimated

Table 1. Classification of diabetes mellitus.[a]

Type 1 diabetes
- A. Immune mediated
- B. Idiopathic

Type 2 diabetes

Other specific types
- Genetic defects of β-cell function
- Genetic defects in insulin action
- Diseases of the exocrine pancreas
- Endocrinopathies
- Drug or chemical induced
- Infections
- Uncommon forms of IMD
- Other genetic syndromes sometimes associated with diabetes

GDM

[a] From ADA *(1)*.

to be $98 billion *(5)*. The mean annual per capita healthcare costs for an individual with diabetes are approximately fourfold higher than those for individuals who do not have diabetes *(5)*. Similarly, in the United Kingdom, diabetes accounts for roughly 10% of the National Health Service budget (£49 billion).

The high costs of diabetes are attributable to care for both acute conditions (such as hypoglycemia and ketoacidosis) and debilitating complications *(6)*. The latter include both microvascular complications—predominantly retinopathy, nephropathy, and neuropathy—and macrovascular complications, particularly stroke and coronary artery disease (CAD).[8] Together these make diabetes the seventh most common cause of death in the developed world *(7)*.

The American Diabetes Association (ADA) publishes in January each year a supplement, entitled *Clinical Practice Recommendations*, to *Diabetes Care*. This is a compilation of all ADA position statements related to clinical practice and is an important resource for healthcare professionals who care for people with diabetes. The National Academy of Clinical Biochemistry has developed evidence-based guidelines for the practice of laboratory medicine. The guidelines in this document are based on the best available published evidence. An assessment was made of virtually all analytes used in the diagnosis and management of individuals with diabetes. The resulting guidelines, intended for use by laboratorians and providers of patient care, have been reviewed by the ADA Professional Practice Committee and found to be consistent in those areas where the ADA has also published *Clinical Practice Recommendations*. The guidelines in this document are not intended to supplant the ADA Recommendations. The objective is to supplement the ADA Recommendations, with an emphasis on the laboratory aspects of diabetes.

The ADA has developed a system to grade the quality of scientific evidence (Table 2). This scheme has been used in this report to describe the quality of the evidence on which each recommendation is based. The ratings range

Table 2. ADA evidence grading system for clinical practice recommendations.

Level of evidence	Description
A	Clear evidence from well-conducted, generalizable, randomized controlled trials that are adequately powered, including:
	Evidence from a well-conducted multicenter trial
	Evidence from a metaanalysis that incorporates quality ratings in the analysis
	Compelling nonexperimental evidence, i.e., "all or none" rule developed by Center for Evidence Based Medicine at Oxford[a]
	Supportive evidence from well-conducted randomized controlled trials that are adequately powered, including:
	Evidence from a well-conducted trial at one or more institutions
	Evidence from a metaanalysis that incorporates quality ratings in the analysis
B	Supportive evidence from well-conducted cohort studies
	Evidence from a well-conducted prospective cohort study or registry
	Evidence from a well-conducted prospective cohort study
	Evidence from a well-conducted metaanalysis of cohort studies
	Supportive evidence from a well-conducted case–control study
C	Supportive evidence from poorly controlled or uncontrolled studies
	Evidence from randomized clinical trials with one or more major or three or more minor methodologic flaws that could invalidate the results
	Evidence from observational studies with high potential for bias (such as case series with comparison to historical controls)
	Evidence from case series or case reports
	Conflicting evidence, with the weight of evidence supporting the recommendation
E	Expert consensus or clinical experience

[a] Either all patients died prior to therapy and at least some survived with therapy, or some patients died without therapy and none died with therapy. Example: use of insulin in the treatment of DKA.

[8] Nonstandard abbreviations: CAD, coronary artery disease; ADA, American Diabetes Association; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; GHb, glycated hemoglobin; CAP, College of American Pathologists; CI, confidence interval; DKA, diabetic ketoacidosis; SMBG, self-monitoring of blood glucose; GDM, gestational diabetes mellitus; DCCT, Diabetes Control and Complications Trial; UKPDS, United Kingdom Prospective Diabetes Study; NHANES, National Health and Nutrition Examination Survey; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; FDA, Food and Drug Administration; AcAc, acetoacetate; βHBA, β-hydroxybutyrate; Hb, hemoglobin; NGSP, National Glycohemoglobin Standardization Program; IMD, immune-mediated diabetes; MODY, maturity onset diabetes of youth; HNF, hepatocyte nuclear factor; IPF-1, insulin promoter factor-1; ICA, islet-cell cytoplasm antibody; IAA, insulin autoantibody; GAD_{65}, 65-kDa isoform of glutamic acid decarboxylase; IA, insulinoma-associated antigen; JDF, Juvenile Diabetes Foundation; and apo, apolipoprotein.

from A to C, with A exhibiting the highest quality of evidence. Category E, expert opinion, is used for recommendations for which no evidence from clinical trials is available or where conflicting evidence has been published.

To facilitate comprehension and assist the reader, each analyte is divided into several headings and subheadings. These are use (diagnosis, screening, monitoring, and prognosis), rationale (diagnosis and screening), analytical considerations [preanalytical (including reference values) and analytical (such as methods)], interpretation (including frequency of measurement and turnaround time), and where applicable, emerging considerations, which alert the reader to ongoing studies and potential future aspects relevant to that analyte.

Glucose

USE

Diagnosis/Screening

> Recommendation: Glucose should be measured in plasma in an accredited laboratory to establish the diagnosis of diabetes.
>
> Level of evidence: A
>
> Glucose should be measured in plasma in an accredited laboratory for screening of high-risk individuals.
>
> Level of evidence: E
>
> Analysis in an accredited laboratory is not necessary for routine monitoring.
>
> Level of evidence: E

The diagnosis of diabetes is established exclusively by the documentation of hyperglycemia (increased glucose concentrations in the plasma). In 1997, the diagnostic criteria *(8)* were modified *(1)* to better identify individuals at risk of retinopathy and nephropathy. The revised (current) criteria include: (*a*) symptoms of diabetes and casual (i.e., regardless of the time of the preceding meal) plasma glucose ≥11.1 mmol/L (200 mg/dL); (*b*) fasting plasma glucose (FPG) ≥7.0 mmol/L (126 mg/dL); or (*c*) 2-h postload glucose ≥11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT) *(1)*. If any one of these three criteria is met, confirmation by repeat testing on a subsequent day is necessary to establish the diagnosis. (Note that repeat testing is not necessary in patients who have unequivocal hyperglycemia with acute metabolic decompensation.) Although included as a criterion, the OGTT was not recommended for routine clinical use in nonpregnant individuals (see below).

Population screening for type 2 diabetes, previously controversial, is now recommended for those at risk of developing the disease *(1, 9)*. The ADA proposes that FPG should be measured in all asymptomatic people ≥45 years of age. If results are <6.1 mmol/L (110 mg/dL), testing should be repeated at 3-year intervals. Screening should be considered at a younger age or be carried out more frequently in individuals at increased risk of diabetes [see Ref *(1)* for conditions associated with increased risk]. Because of the increasing prevalence of type 2 diabetes in children, screening of children has been suggested recently *(10)*. Starting at age 10 years, testing should be performed every 2 years in overweight individuals who have two other risk factors, namely family history, race/ethnicity, and signs of insulin resistance *(10)*. Despite these recommendations, there is no published evidence that treatment based on screening has value. The cost-effectiveness of screening for type 2 diabetes has been estimated. The incremental cost of screening all persons 25 years or older was estimated to be $236 449 per life-year gained and $56 649 per quality-adjusted life-year gained *(11)*. Interestingly, screening was more cost-effective at ages younger than the 45 years currently recommended.

Monitoring/Prognosis

> Recommendation: Although there is evidence linking high plasma glucose concentrations to adverse outcome, substantially more data are available that directly correlate increased glycated hemoglobin (GHb) with complications of diabetes. Routine measurement of plasma glucose concentrations in an accredited laboratory is not recommended as the primary means of monitoring or evaluating therapy in individuals with diabetes.
>
> Level of evidence: E

There is a direct relationship between the degree of plasma glucose control and the risk of late renal, retinal, and neurologic complications. This correlation has been demonstrated for type 1 *(12)* and more recently for type 2 *(13)* diabetes. Persons with type 1 diabetes who maintained lower average plasma glucose concentrations exhibited a significantly lower incidence of microvascular complications, namely diabetic retinopathy, nephropathy, and neuropathy *(12)*. Although intensive insulin therapy reduced hypercholesterolemia by 34%, the risk of macrovascular disease was not significantly decreased. Similar results were obtained in patients with type 2 diabetes *(13)*. Intensive plasma glucose control in patients with type 2 diabetes significantly reduced microvascular complications, but no significant difference was detected for macrovascular disease (myocardial infarction or stroke) *(13)*. In both studies, patients in the intensive group maintained lower median plasma glucose concentrations. Analyses of the outcomes were linked to GHb, which was

used to evaluate glycemic control, rather than glucose concentration. Moreover, most clinicians use the ADA recommendations, which define a target GHb concentration as the goal for optimum glycemic control *(14)*.

There is some evidence directly linking higher glucose concentrations to a poor prognosis. For example, the 10-year survival of 6681 people in a Japanese town was reduced if FPG was ≥7.8 mmol/L (140 mg/dL) *(15)*. Similar findings were obtained in 1939 patients with type 2 diabetes followed for a mean of 15 years; multiple logistic regression revealed that the risk of death was significantly increased for patients with FPG ≥7.8 mmol/L (140 mg/dL) *(16)*. Individuals with type 2 diabetes with FPG >7.8 mmol/L (140 mg/dL) had increased cardiovascular mortality *(17)*. Furthermore, comparison of 300 patients with a first myocardial infarction and 300 matched controls revealed that a moderately increased FPG was a risk factor for infarction *(18)*. Notwithstanding these observations, neither random nor fasting glucose concentrations should be measured in an accredited laboratory as the primary means of routine monitoring of patients with diabetes. Laboratory plasma glucose testing can be used to supplement information from other testing, to test the accuracy of self-monitoring (see below), or when adjusting the dose of oral hypoglycemic agents *(9)*. In addition, individuals with well-controlled type 2 diabetes who are not on insulin therapy can be monitored with periodic measurement of FPG, although analysis need not be done in an accredited laboratory *(19, 20)*.

RATIONALE

Diagnosis

The disordered carbohydrate metabolism that underlies diabetes manifests as hyperglycemia. Therefore, measurement of plasma glucose is the sole diagnostic criterion. This strategy is indirect as hyperglycemia reflects the consequence of the metabolic derangement, not the cause. However, until the underlying molecular pathophysiology of the disease is identified, plasma glucose concentrations are likely to remain an essential diagnostic modality.

Screening

Screening is recommended for several reasons. The onset of type 2 diabetes is estimated to occur ~4–7 years before clinical diagnosis *(21)*, and epidemiologic evidence indicates that complications may begin several years before clinical diagnosis. Furthermore, at least 30% of people in the US with type 2 diabetes are undiagnosed *(22)*. Notwithstanding this recommendation, there is no evidence that population screening of plasma glucose concentrations provides any benefit. Outcome studies should be performed to justify screening.

ANALYTICAL CONSIDERATIONS

Preanalytical

> Recommendation: Blood for fasting plasma glucose analysis should be drawn after the individual has fasted overnight (at least 8 h). Plasma should be separated from the cells within 60 min; if this is not possible, a tube containing a glycolytic inhibitor such as sodium fluoride should be used for collecting the sample.
>
> Level of evidence: B

Blood should be drawn in the morning after an overnight fast [no caloric intake for at least 8 h, during which time the individual may consume water ad libitum *(1)*]. Recent evidence revealed a diurnal variation in FPG, with mean FPG higher in the morning than in the afternoon, indicating that many cases of undiagnosed diabetes would be missed in patients seen in the afternoon *(23)*. Glucose concentrations decrease ex vivo with time in whole blood because of glycolysis. The rate of glycolysis, reported to average 5–7% [~0.6 mmol/L (10 mg/dL)] per hour *(24)*, varies with the glucose concentration, temperature, white blood cell count, and other factors *(25)*. Glycolysis can be attenuated by inhibition of enolase with sodium fluoride (2.5 mg fluoride/mL of blood) or, less commonly, lithium iodoacetate (0.5 mg/mL of blood). These reagents can be used alone or, more commonly, with anticoagulants such as potassium oxalate, EDTA, citrate, or lithium heparin. Although fluoride maintains long-term glucose stability, the rates of decline of glucose in the first hour after sample collection in tubes with and without fluoride are virtually identical *(24)*. (Note that leukocytosis will increase glycolysis even in the presence of fluoride if the white cell count is very high.) After 4 h, the glucose concentration is stable in whole blood for 72 h at room temperature in the presence of fluoride *(24)*. In separated, nonhemolyzed, sterile serum without fluoride, the glucose concentration is stable for 8 h at 25 °C and 72 h at 4 °C *(26)*.

Glucose can be measured in whole blood, serum, or plasma, but plasma is recommended for diagnosis. The molality of glucose (i.e., amount of glucose per unit water mass) in whole blood and plasma is identical. Although red blood cells are essentially freely permeable to glucose (glucose is taken up by facilitated transport), the concentration of water (kg/L) in plasma is ~11% higher than that of whole blood. Therefore, glucose concentrations in plasma are ~11% higher than whole blood if the hematocrit is normal. Glucose concentrations in heparinized plasma are reported to be 5% lower than in serum *(27)*. The reasons for the latter difference are not apparent, but may be attributable to the shift in fluid from erythrocytes to plasma caused by anticoagulants. The glucose concentrations during an OGTT in capillary blood are significantly higher than those in venous blood [mean of 1.7

mmol/L (30 mg/dL), equivalent to 20–25% *(28)*], but the mean difference in fasting samples is only 0.1 mmol/L (2 mg/dL) *(28, 29)*.

Reference values. Glucose concentrations in healthy individuals vary with age. Reference intervals in children are 3.3–5.6 mmol/L (60–100 mg/dL), similar to the adult interval of 4.1–5.9 mmol/L (74–106 mg/dL) *(26)*. Note that the ADA criteria *(1)*, not the reference values, are used for the diagnosis of diabetes. Moreover, the threshold for diagnosis of hypoglycemia is variable. The reference values are not useful to diagnose these conditions. In adults, mean fasting plasma glucose increases with increasing age from the third to the sixth decade *(30)*, but does not increase significantly after age 60 *(31, 32)*. By contrast, glucose concentrations after a glucose challenge are substantially higher in older individuals *(31, 32)*. Evidence of an association of increasing insulin resistance with age is inconsistent *(33)*.

Analytical

> Recommendation: Enzymatic methods for glucose analysis are relatively well standardized. Despite the low imprecision at the diagnostic decision limits of 7.0 mmol/L (126 mg/dL) and 11.1 mmol/L (200 mg/dL), classification errors may occur. Because of the relatively large intraindividual biological variability (CVs of ~5–7%), FPG values of 5.8–6.9 mmol/L (105–125 mg/dL) should be repeated and individuals with FPG of 5.3–5.7 mmol/L (96–104 mg/dL) should be considered for follow-up at intervals shorter than the current ADA recommendation of every 3 years.
>
> Level of evidence: E

Glucose is measured almost exclusively by enzymatic methods. Analysis of proficiency surveys conducted by the College of American Pathologists (CAP) revealed that hexokinase or glucose oxidase is used in virtually all analyses performed in the US *(26)*. A few laboratories (~1%) use glucose dehydrogenase. At a plasma glucose concentration of ~8.2 mmol/L (147 mg/dL), imprecision among laboratories using the same method had a CV <4%, excluding glucose dehydrogenase *(26)*. Similar findings have been reported for glucose analysis in samples from patients. For example, comparison of plasma samples from 240 patients revealed a 5% difference in mean glucose concentrations measured by the hexokinase and glucose oxidase methods *(34)*.

No consensus has been achieved on the goals for glucose analysis. Numerous criteria have been proposed to establish analytical goals. These include expert opinion (consensus conferences), opinion of clinicians, regulation, state of the art, and biological variation *(35)*. A rational and realistic recommendation that has received some support is to use biological criteria as the basis for analytical goals. It has been suggested that imprecision should not exceed one-half of the within-subject biological CV *(36, 37)*. For plasma glucose, a CV ≤2.2% has been suggested as a target for imprecision, with 0% bias *(37)*. Although this recommendation was proposed for within-laboratory error, it would be desirable to achieve this goal for interlaboratory imprecision to minimize differences among laboratories in the diagnosis of diabetes in individuals whose glucose concentrations are close to the threshold value. Therefore, the goal for glucose analysis should be to minimize total analytical error, and methods should be without measurable bias. A national program using samples (e.g., fresh-frozen plasma) that eliminate matrix effects should be developed to assist in the achievement of this objective.

INTERPRETATION

Knowledge of intraindividual variability of FPG concentrations is essential for meaningful interpretation of patient values. An early study, which repeated the OGTT in 31 nondiabetic adults at 48-h intervals, revealed that FPG varied by <10% in 22 participants (77%) and by <20% in 30 participants (97%) *(38)*. Biological variation includes within- and between-subject variation. Careful evaluation over several consecutive days revealed that intraindividual variation of FPG in healthy individuals [mean glucose, 4.9 mmol/L (88 mg/dL)] exhibited within- and between-subject CVs of 4.8–6.1% and 7.5–7.8%, respectively *(39, 40)*. Larger studies have revealed CVs of 6.4–6.9% for FPG in 246 apparently healthy *(41)* and 193 newly diagnosed untreated patients with type 2 diabetes *(42)*. The latter study, which measured FPG by glucose oxidase (intra- and interassay CVs <2%) on 2 consecutive days, obtained 95% confidence intervals (CIs) of ± 14.8% for total variability and ± 13.7% for biological variability. If a CV (biological) of 6.9% is applied to a true glucose concentration of 7.0 mmol/L (126 mg/dL), the 95% CI would encompass glucose concentrations of 6.1–7.9 mmol/L (109–143 mg/dL). If the CV of the glucose assay (~4%) is included, the 95% CI is approximately ± 18%. Thus, the 95% CI for a fasting glucose concentration of 7.0 mmol/L (126 mg/dL) would be 7.0 mmol/L ± 18% (126 mg/dL ± 18%), namely, 5.7–8.3 mmol/L (103–149 mg/dL). Use of an assay imprecision of 4% (CV) only (excluding biological variability), would yield a 95% CI of 6.4–7.6 mmol/L (116–136 mg/dL) among laboratories for a true glucose concentration of 7.0 mmol/L (126 mg/dL). One should bear in mind that these ranges include 95% of individuals and that other individuals will be outside this range. The biological variability is substantially greater than analytical variability. Using biological variation as the basis for deriving analytical performance characteristics *(35)*, Ricos et al. *(43)* have proposed the following

desirable specifications for glucose: analytical imprecision ≤3.3%, bias ≤2.5%, and total error ≤7.9%.

A short turnaround time for glucose analysis is not usually necessary for the diagnosis of diabetes. In some clinical situations, such as acute hyper- or hypoglycemic episodes in the Emergency Department or treatment of diabetic ketoacidosis (DKA), rapid analysis is desirable. A turnaround time of 30 min has been proposed *(44)*. However, this value is based on requirements by clinicians, and no outcome data have been published that validate this value. Inpatient management of diabetic patients may on occasion require a rapid turnaround time (minutes, not hours). Bedside monitoring with glucose meters (see below) has been adopted by many as a practical solution *(45)*.

Frequency of measurement. The frequency of measurement of plasma glucose is dictated by the clinical situation. The ADA recommends that an increased FPG or abnormal OGTT must be confirmed to establish the diagnosis of diabetes *(1)*. Screening by FPG is recommended every 3 years if it is <6.1 mmol/L (<110 mg/dL), more frequently in high-risk individuals; however, frequency of analysis in the latter group is not specified. Monitoring is performed by patients themselves, who measure glucose with meters, and by assessment of GHb in an accredited laboratory (see below). Appropriate intervals between measurements of glucose in acute clinical situations (e.g., patients in hospital or patients with DKA or neonatal hypoglycemia) are highly variable and may range from 30 min to ≥24 h.

EMERGING CONSIDERATIONS

Noninvasive or minimally invasive analysis of glucose is addressed below.

Meters

Portable meters for measurement of blood glucose concentrations are used in three major settings: (*a*) in acute and chronic care facilities (at the patient's bedside and in clinics or hospitals); (*b*) in physicians' offices; and (*c*) by patients at home, work, and school. The last, self-monitoring of blood glucose (SMBG), is performed at least once a day by 40% and 26% of individuals with type 1 and 2 diabetes, respectively, in the US *(46)*. The worldwide market for SMBG is $2.7 billion per year, with annual growth estimated at 10–12% *(47)*. The ADA lists the following indications for SMBG: (*a*) achieving and maintaining glycemic control; (*b*) preventing and detecting hypoglycemia; (*c*) avoiding severe hyperglycemia; (*d*) adjusting to changes in lifestyle; and (*e*) determining the need for initiating insulin therapy in gestational diabetes mellitus (GDM) *(48)*. It is recommended that most individuals with diabetes attempt to achieve and maintain blood glucose concentrations as close to those found in nondiabetic individuals as is safely possible *(14)*.

USE

Diagnosis/Screening

> Recommendation: There are no published data to support a role for portable meters in the diagnosis of diabetes or for population screening. The imprecision of the meters, coupled with the substantial differences among meters, precludes their use in the diagnosis of diabetes and limits their usefulness in screening for diabetes.
>
> Level of evidence: E

The criteria for the diagnosis of diabetes are based on outcome data (the risk of micro- and macrovascular disease) correlated with plasma glucose concentrations, both fasting and 2 h after a glucose load, assayed in an accredited laboratory *(1)*. Whole blood is used in portable meters. Although many portable meters have been programmed to report a plasma glucose concentration, the imprecision of the current meters (see below) precludes their use in the diagnosis of diabetes. Similarly, screening by portable meters, although attractive because of convenience, ease, and accessibility, would generate many false positives and false negatives.

Monitoring/Prognosis

> Recommendation: SMBG is recommended for all insulin-treated patients with diabetes. For type 1 patients, SMBG is recommended three or more times a day. SMBG may be desirable in patients treated with sulfonylureas or other insulin secretagogues and in all patients not achieving goals.
>
> Level of evidence: B
>
> In patients with type 2 diabetes, SMBG may help achieve better control, particularly when therapy is initiated or changed. However, there are no data to support this concept. The role of SMBG in patients with stable type 2 diabetes controlled by diet alone is not known.
>
> Level of evidence: C

SMBG is recommended for all patients with diabetes who are receiving insulin. Tight glycemic control can decrease microvascular complications in individuals with type 1 *(12)* or type 2 *(13)* diabetes. Intensive plasma glucose control in patients with type 1 diabetes was achieved in the Diabetes Control and Complications Trial (DCCT) by participants performing SMBG at least four times per day *(12)*. Therapy in patients with type 2 diabetes in the United Kingdom Prospective Diabetes Study (UKPDS) *(13)* was adjusted according to FPG concentrations; SMBG was not evaluated.

Faas et al. *(49)* reviewed 11 studies, published between

1976 and 1996, that evaluated SMBG in patients with type 2 diabetes. Only one of the published studies reported that SMBG produced a significantly positive improvement, namely lower GHb. The authors of the review concluded that the efficacy of SMBG in type 2 diabetes is questionable *(49)*. Similar conclusions were drawn in a recent metaanalysis *(50)* and in a sample of patients with type 2 diabetes in the National Health and Nutrition Examination Survey (NHANES) *(51)*. Although SMBG may be useful in initiating or changing therapy in patients with type 2 diabetes, clinical studies are needed to define its role in outcome in patients with type 2 diabetes.

RATIONALE

SMBG allows patients with diabetes to achieve and maintain specific glycemic goals. Knowledge of plasma or blood glucose concentrations is necessary for insulin-requiring patients, particularly those with type 1 diabetes, to determine appropriate insulin doses at different times of the day *(48)*. Patients adjust the amount of insulin according to their plasma or blood glucose concentration. Frequent SMBG is particularly important for tight glycemic control in type 1 diabetes.

Hypoglycemia is a major, potentially life-threatening complication of the treatment of diabetes. The risk of hypoglycemia increases significantly with pharmacologic therapy directed toward maintaining the glycemic range as close to those found in nondiabetic individuals as possible *(12, 13)*. The incidence of major hypoglycemic episodes, requiring third-party help or medical intervention, was two- to threefold higher in the intensive group than in the conventional group in clinical trials of patients with type 1 and type 2 diabetes *(12, 13)*. Furthermore, many diabetic patients, particularly those with type 1 diabetes, lose the autonomic warning symptoms that usually precede neuroglycopenia ("hypoglycemic unawareness") *(52)*, increasing the risk of hypoglycemia. SMBG can be useful for detecting asymptomatic hypoglycemia and allowing patients to avoid major hypoglycemic episodes.

ANALYTICAL CONSIDERATIONS

Preanalytical

> Recommendation: Patients should be instructed in the correct use of glucose meters, including quality control. Comparison between SMBG and concurrent laboratory glucose analysis should be performed at regular intervals to evaluate the accuracy of patient results.
>
> Level of evidence: B

Multiple factors can interfere with glucose analysis with portable meters. Several of these, such as improper application, timing, and removal of excess blood *(26)*, have been eliminated by advances in technology. Important variables that may influence the results of bedside glucose monitoring include changes in hematocrit *(53)*, altitude, environmental temperature or humidity, hypotension, hypoxia, and high triglyceride concentrations *(54)*. Furthermore, most meters are inaccurate at very high or very low glucose concentrations. Another important factor is variability of results among different glucose meters. Different assay methods and architecture lead to lack of correlation among meters, even from a single manufacturer. In fact, two meters of the same brand have been observed to differ substantially in accuracy *(55, 56)*. Patient factors are also important, particularly adequate training. Recurrent education at clinic visits and comparison of SMBG with concurrent laboratory glucose analysis improved the accuracy of patients' blood glucose readings *(57)*. In addition, it is important to evaluate the patient's technique at regular intervals *(9)*.

Analytical

> Recommendation: Multiple performance goals for portable glucose meters have been proposed. These targets vary widely and are highly controversial. No published study has achieved the goals proposed by the ADA. Manufacturers should work to improve the imprecision of current meters.
>
> Level of evidence: E
>
> We recommend meters that measure and report plasma glucose concentrations to facilitate comparison with assays performed in accredited laboratories.
>
> Level of evidence: E

At least 25 different meters are commercially available and are reviewed annually in the ADA's Buyer's Guide to Diabetes Products *(58)*. Virtually all the meters use strips that contain glucose oxidase or hexokinase. A drop of whole blood is applied to a strip that contains all the reagents necessary for the assay. Some meters have a porous membrane that separates erythrocytes, and analysis is performed on the resulting plasma. Meters can be calibrated to report plasma glucose values, even when glucose is measured in whole blood. An IFCC working group recently recommended that glucose meters be harmonized to the concentration of glucose in plasma, irrespective of the sample type or technology *(59)*. The meters use reflectance photometry or electrochemistry to measure the rate of the reaction or the final concentration of the products. The meter provides a digital readout of glucose concentration. Most meters claim a reportable range of 1.7–33.3 mmol/L (30–600 mg/dL).

Several important technologic advances that decrease operator error have been made in the last few years. These include "no wipe" strips, automatic commencement of timing when both the sample and the strip are in the meter, smaller sample volume requirements, an error

signal if sample volume is inadequate, "lock out" if controls are not assayed, barcode readers, and the ability to store up to several hundred results that can subsequently be downloaded for analysis. Together these improvements have produced superior performance by new meters *(60)*.

Multiple analytical goals have been proposed for the performance of glucose meters. The rationale for these is not always clear. In 1987, the ADA recommended a goal for total error (user plus analytical) of <10% at glucose concentrations of 1.7–22.2 mmol/L (30–400 mg/dL) 100% of the time *(61)*. In addition, it was proposed that values should differ by ≤15% from those obtained by a laboratory reference method. The recommendation was modified in response to the significant reduction in complications by tight glucose control in the DCCT. The revised performance goal, published in 1996 *(48)*, is for analytical error <5%. To our knowledge, there are no published studies of glucose meters that have achieved the ADA goal of analytical error of <5%.

The CLIA '88 goal is less stringent than that of the ADA; results with meters should be within 10% of target values or ± 0.3 mmol/L (6 mg/dL), whichever is larger. NCCLS recommendations *(62)* are ± 20% of laboratory glucose at >5.5 mmol/L (100 mg/dL) and ± 0.83 mmol/L (15 mg/dL) of laboratory glucose if the glucose concentration is ≤5.5 mmol/L (100 mg/dL). These are undergoing revisions. New NCCLS guidelines, anticipated to be published in 2002, propose that for test readings >4.2 mmol/L (75 mg/dL), the discrepancy between meters and the central laboratory should be <20%; for a glucose concentrations ≤4.2 mmol/L (75 mg/dL), the discrepancy should not exceed 0.83 mmol/L (15 mg/dL; NCCLS, in preparation).

A different approach was proposed by Clarke et al. *(63)*, who developed an error grid that attempts to define clinically important errors by identifying fairly broad target ranges. In addition, two novel approaches were suggested very recently. In the first, 201 patients with longstanding type 1 diabetes were questioned to estimate quality expectations for glucose meters *(64)*. On the basis of patients' perceptions of their needs and of their reported actions in response to changes in measured glucose concentrations, a goal for analytical quality at hypoglycemic concentrations was a CV of 3.1%. Excluding hypoglycemia, the analytical CV to meet the expectations of 75% of the patients was 6.4–9.7%. The authors recommended an analytical CV of 5%, with a bias ≤5% *(64)*. The second method used simulation modeling of errors in insulin dose *(65)*. The results revealed that meters that achieve both a CV and a bias <5% rarely lead to major errors in insulin dose. However, to provide the intended insulin dosage 95% of the time, the bias and CV needed to be <1–2%, depending on the dosing schedule for insulin and the ranges of glucose concentrations for individual patients *(65)*. No meters have been shown to achieve CVs of 1–2% in routine use. Given the bias and imprecision of meters, no studies have evaluated this target, which is based on simulation modeling. The lack of consensus on quality goals for glucose meters reflects the absence of agreed objective criteria. Using the same biological variation criteria described in the "Interpretation" section above for glucose analysis in accredited laboratories, we suggest a goal for total error (including both bias and imprecision) of ≤7.9%. However, additional studies are necessary to accurately define this goal.

There is a very large variability in the performance of different meters. Although current meters, as predicted, exhibit performance superior to prior generations of meters *(60)*, imprecision remains high. For example, in a study conducted under carefully controlled conditions where all assays were performed by a single medical technologist, only ~50% of analyses met the ADA criterion of <5% deviation from reference values *(60)*. The performance of older meters was substantially worse: two of the four meters produced results within 5% of reference values for only 33% of analyses. Another recent study that evaluated meter performance in 226 hospitals by split samples analyzed simultaneously on meters and laboratory glucose analyzers revealed that 45.6%, 25%, and 14% differed from each other by >10%, >15%, and >20%, respectively *(66)*. Recent analysis of the clinical and analytical accuracy of portable glucose meters (all measurements done by one person) demonstrated that none of the meters met the ADA criterion and that only two meters had 100% of the estimations in the clinically acceptable zones by error grid analysis *(67)*.

Recommendation: Clinical studies are needed to determine the analytical goals for glucose meters. At a minimum, the end-points should be GHb and frequency of hypoglycemic episodes. Ideally, outcomes (e.g., long-term complications and hypoglycemia) should also be examined.

Level of evidence: E

Frequency of measurement. SMBG should be performed at least four times per day in patients with type 1 diabetes. Monitoring less frequently than four times a day can lead to deterioration of glycemic control *(48, 68, 69)*. Published studies reveal that self-monitoring is performed by patients much less frequently than recommended. Data from NHANES III collected between 1988 and 1994 revealed that SMBG was performed at least once a day by 39% of patients taking insulin and by 5–6% of those treated with oral agents or diet alone *(51)*. Moreover, 29% and 65% of patients treated with insulin and oral agents, respectively, monitored their blood glucose less than once per month. However, no evaluation has been performed to verify that four times a day is ideal or whether some other frequency or timing (e.g., postprandial testing) would improve glycemic control. For example, adjustment of insulin therapy in women with GDM

according to the results of postprandial, rather than preprandial, plasma glucose concentrations improved glycemic control and reduced the risk of neonatal complications *(70)*. The optimal frequency of SMBG for patients with type 2 diabetes is unknown.

Current ADA recommendations suggest daily SMBG for patients treated with insulin or sulfonylureas *(14)* to detect hypoglycemia. However, published evidence shows no correlation between the frequency of SMBG in type 2 diabetes and glycemic control *(49–51)*. There is no known role for SMBG in patients with type 2 diabetes who are treated with diet alone.

OGTT

Recommendation: The OGTT is not recommended for the routine diagnosis of type 1 or 2 diabetes mellitus. It is recommended for establishing the diagnosis of GDM.

Level of evidence: B

USE

The OGTT, once the gold standard for diagnosing diabetes mellitus, is now not recommended by the ADA for diagnosing either type 1 or 2 diabetes, but it continues to be recommended in a limited fashion by the WHO *(71, 72)*. The oral glucose challenge (or glucose tolerance test) continues to be recommended by both the ADA and the WHO for establishing the diagnosis of GDM. Neither group recommends use of the extended 3- to 5-h glucose tolerance test in routine practice.

RATIONALE

Inability to respond appropriately to a glucose challenge, i.e., glucose intolerance, represents the fundamental pathologic defect in diabetes mellitus. The rationale for the ADA not recommending that the glucose tolerance test be used routinely to diagnose type 1 and 2 diabetes is that appropriate use of FPG could identify approximately the same prevalence of abnormal glucose metabolism in the population as the OGTT. Furthermore, the OGTT is impractical in ordinary practice. The consensus was that a 2-h plasma glucose cutoff of ≥11.1 mmol/L (200 mg/dL) should be used because it was predictive of the occurrence of microangiopathy *(72)*. However, only approximately one-fourth of individuals with 2-h plasma glucose ≥11.1 mmol/L (200 mg/dL) have a FPG ≥7.8 mmol/L (140 mg/dL), which was the FPG previously recommended to diagnose diabetes mellitus. The currently recommended FPG value of 7.0 mmol/L (126 mg/dL) corresponds better to a 2-h value in the OGTT of >11.1 mmol/L (200 mg/dL), and thus with development of complications.

Use of the OGTT to classify individuals with impaired glucose tolerance (IGT) and diabetes remains controversial. Recent studies *(73–76)* indicate that individuals classified with IGT by the OGTT (WHO criteria) have increased risk of cardiovascular disease, but many of these individuals do not have impaired fasting glucose (IFG) by the new ADA criteria. Furthermore, the OGTT (WHO criteria) identifies diabetes in ~2% more individuals does than FPG (ADA criteria) *(77)*. Finally, diabetic patients with both abnormal FPG and 2-h OGTT have a higher risk of premature death than those with only an increased FPG concentration *(78)*.

The 2-h glucose tolerance test continues to be recommended for the diagnosis of GDM by both the ADA and WHO *(71, 72)*. Deterioration of glucose tolerance occurs frequently in pregnancy, especially in the third trimester. Diagnosing and treating GDM is essential to prevent associated perinatal morbidity and mortality.

ANALYTICAL CONSIDERATIONS

The reproducibility of the OGTT has received considerable attention. In numerous studies, the reproducibility of the OGTT in classifying patients was 50–66% *(79)*. Possible factors contributing to the lack of reproducibility include biological variation of plasma glucose concentrations, the variable effects of administration of a hyperosmolar glucose solution on gastric emptying, and effects of ambient temperature *(41, 79–81)*. The accuracy and reproducibility of glucose assays are not limiting factors in this regard.

INTERPRETATION

Diagnosing type 1 and 2 diabetes

The ADA and WHO have different recommendations:

ADA: Not recommended for routine clinical use except in pregnant women *(72)*.

WHO: When the FPG concentration is in the IFG range [6.1 mmol/L-7.0 mmol/L (110–126 mg/dL)], an OGTT is recommended *(71)*. After 3 days of unrestricted diet and an overnight fast (8–14 h), FPG is measured, followed by the oral ingestion of 75 g of anhydrous glucose (or partial hydrolysates of starch of the equivalent carbohydrate content) in 250–300 mL of water over 5 min. For children, the dose is 1.75 g glucose/kg up to 75 g of glucose. Blood samples are collected 2 h after the load, and plasma glucose is analyzed. Results are interpreted as detailed in Table 3.

Table 3. WHO criteria for interpreting 2-h OGTT.[a]

	Plasma glucose concentration, mmol/L (mg/dL)	
	0 h	2 h
IFG	≥6.1 (110) <7.0 (126)	<7.8 (140)
IGT	<7.0 (126)	≥7.8 to <11.1 (140–200)
Diabetes	≥7.0 (126)	≥11.1 (200)

[a] Any single abnormal value should be repeated on a separate day.

GDM

The ADA modified their recommendations for laboratory diagnosis of GDM in 2000 *(82)*. Their guidelines are as follows:

1. Low-risk patients require no testing. Low risk status is limited to women meeting all of the following:

- Age <25 years
- Weight normal before pregnancy
- Member of an ethnic group with a low prevalence of GDM
- No known diabetes in first-degree relatives
- No history of abnormal glucose tolerance
- No history of poor obstetric outcome

2. Average-risk patients (all patients who fall between low and high risk) should be tested at 24–28 weeks of gestation (see below for testing strategy).

3. High-risk patients should undergo immediate testing. They are defined as having any of the following:

- Marked obesity
- Personal history of GDM
- Glycosuria
- Strong family history of diabetes

The first step in laboratory testing is identical to that for diagnosing type 1 or 2 diabetes, i.e., a FPG ≥7.0 mmol/L (126 mg/dL) or a casual plasma glucose ≥11.1 mmol/L (200 mg/dL) confirmed on a subsequent day. However, if the above tests are normal, the ADA recommends that average- and high-risk patients receive a glucose challenge test following one of two methods:

1. One-step: Perform either a 100-g or 75-g OGTT. This one-step approach may be cost-effective in high-risk patients or populations (e.g., some Native-American groups).
   - The 100-g OGTT is the most commonly used, standard test supported by outcome data. Two or more of the venous plasma glucose concentrations indicated in Table 4 must be met or exceeded for a positive diagnosis.
   - Alternatively, a 75-g OGTT can be performed, but it is not as well validated as the 100-g test. In the 75-g test, diagnostic criteria for plasma glucose values are the same as for the 100-g test, except that there is no 3-h measurement. Two or more of the venous plasma glucose values must equal or exceed the cutoffs to diagnose GDM.
2. Two-step: The first step is a 50-g oral glucose load (the patient does not need to be fasting), followed by a plasma glucose determination at 1 h. A plasma glucose value ≥7.8 mmol/L (140 mg/dL) indicates the need for definitive testing. A value ≥7.2 mmol/L (130 mg/dL) may be used because it will detect ~10% more diabetic patients. The second and definitive test is one of the two OGTTs described above.

Table 4. Criteria for intepreting 100-g OGTT.[a]

	Plasma glucose concentration	
	mmol/L	mg/dL
Fasting	5.3	95
1 h	10.0	180
2 h	8.6	155
3 h	7.8	140

[a] The test should be done in the morning after an overnight fast of between 8 and 14 h and after a unrestricted diet (≥150 g carbohydrate per day) and unlimited physical activity. The subject should be seated and should not smoke throughout the test.

EMERGING CONSIDERATIONS

The main issues of controversy are: (*a*) the lower sensitivity of FPG compared with the OGTT in diagnosing diabetes mellitus (2% of cases missed with FPG); (*b*) the value of classifying individuals as having IGT (recommended by WHO, but not the ADA); and (*c*) the appropriate use in GDM.

The lower sensitivity of the FPG compared with the OGTT in diagnosing diabetes mellitus is closely linked to epidemiologic evidence that the OGTT better identifies patients at risk for developing complications of diabetes. This includes assessment of the risk of developing cardiovascular disease *(83)*, macrosomia *(84)* and of predicting increased risk of death *(85)*. The continuing use of the OGTT to diagnose diabetes mellitus has been supported by Australian and New Zealand diabetes professional organizations *(86)*.

The appropriate use of the OGTT for diagnosing GDM is particularly controversial. The recommendation at the Fourth International Workshop—Conference on Gestational Diabetes Mellitus *(87)*, that 5–10% lower glucose values be adopted for diagnosing gestational diabetes, is now adopted by the ADA.

There remains a lack of consensus regarding the use of the 100-g vs 75-g OGTT for the definitive diagnosis of GDM. It would seem practical and probably diagnostically acceptable to use primarily the 75-g OGTT. However, appropriate diagnostic thresholds continue to be in dispute *(86, 88)*. These discrepancies in recommendations reflect the state of knowledge about GDM, which continues to evolve with enhanced and expanded clinical research.

Urinary Glucose

> Recommendation: Semiquantitative urine glucose testing is not recommended for routine care of patients with diabetes mellitus.
>
> Level of evidence: C

USE

Semiquantitative urine glucose testing, once the hallmark of diabetes care in the home setting, has now been

replaced by SMBG (see above). Semiquantitative urine glucose monitoring should be considered only for patients who are unable to or refuse to perform SMBG because urine glucose concentration does not accurately reflect plasma glucose concentration *(89, 90)*.

RATIONALE

Although glucose is detectable in the urine in patients with grossly increased blood glucose concentrations, it provides no information about blood glucose concentrations below the variable renal glucose threshold [~10 mmol/L (180 mg/dL)]. This alone limits its usefulness for monitoring diabetes under modern care recommendations. Furthermore, the concentration of the urine affects urine glucose concentrations, and only average glucose values between voidings are reflected, further minimizing the value of urine glucose determinations.

ANALYTICAL CONSIDERATIONS

Semiquantitative test-strip methods using specific reactions for glucose are recommended. Most commercially available strips use the glucose oxidase reaction *(26)*. Test methods that detect reducing substances are not recommended because they are subject to numerous interferences, including numerous drugs and nonglucose sugars. When used, single voided urine samples are recommended *(90)*.

INTERPRETATION

Because of the limited use of urine glucose determinations, semiquantitative specific reaction-based test strip methods are adequate.

Noninvasive or Minimally Invasive Glucose Analyses

Recommendation: Noninvasive glucose analyses cannot be recommended as replacements for SMBG or glucose measurements by an accredited laboratory. Ongoing developments in the field, such as use of the new Gluco Watch Biographer, may influence this recommendation.

Level of evidence: E

USE

The need for a device for "continuous" in vivo monitoring of glucose concentrations in blood is a very high priority because patients are required to control their plasma glucose more closely *(12, 72, 90)*. Currently, there are only two devices that have been approved by the Food and Drug Administration (FDA) for noninvasive or minimally invasive glucose sensing: the Gluco Watch Biographer (Cygnus), and the Continuous Glucose Monitoring System (MiniMed). Although promising, routine use of these devices cannot be recommended at this time because clinical studies remain limited. Both devices require calibration and confirmation of accuracy with conventional SMBG.

RATIONALE

The first goal for developing a reliable in vivo glucose sensor is to detect unsuspected hypoglycemia. The importance of this goal has been increasingly appreciated with the recognition that strict glucose control is accompanied by a marked increase in the risk of hypoglycemia *(12, 90)*. Therefore, a sensor designed to detect severe hypoglycemia alone would be of value. In contrast, a full-range, reliable in vivo glucose monitor is a prerequisite for the development of an artificial pancreas that measures blood glucose concentrations and automatically adjusts insulin administration.

ANALYTICAL CONSIDERATIONS

The goal here is not to comprehensively review the status of research in this important area, but to make recommendations for current use. There have been several reviews recently on this topic *(91, 92)*, and it has been the subject of national conferences. For example, noninvasive testing technology was the subject of the AACC Oak Ridge Conference in 1999, with considerable attention focused on glucose-sensing technology *(93)*, and a symposium at the 1999 ADA meeting concentrated on noninvasive glucose sensing *(94)*.

Key technologic advances in minimally invasive or noninvasive glucose monitoring can be summarized as shown in Table 5.

The transcutaneous sensors and implanted sensors use multiple detection systems, including enzyme-based (usually glucose oxidase), electrode-based, and fluorescence-based techniques. Alternatives to enzymes as glucose recognition molecules are being developed, including artificial glucose "receptors" *(95, 96)*. Fluorescence technologies include the use of engineered molecules that exhibit altered fluorescence intensity or spectral characteristics upon binding glucose or the use of competitive binding assays incorporating two fluorescent molecules

Table 5. Minimally invasive and noninvasive methodologies for in vivo glucose monitoring.[a]

Transcutaneous needle-type enzyme electrodes
Totally implanted sensors
Enzyme electrodes
Near-infrared fluorescence-based
Sampling technologies
Microdialysis
Reverse iontophoresis
Noninvasive technologies
Near-infrared spectroscopy
Light scattering
Photoacoustic spectroscopy

[a] From Pickup et al. *(91)*.

in the fluorescent resonance energy transfer technique *(97–101)*.

Methods to sample tissue, often referred to as "noninvasive" but are in fact "minimally invasive", vary among test systems. The underlying fundamental concept is that the concentration of glucose in the interstitial fluid correlates with blood glucose. Most microdialysis systems are inserted subcutaneously *(102–105)*. In contrast, "reverse iontophoresis", which is the basis of the FDA-approved "Gluco Watch" (Cygnus), uses a low-level electrical current on the skin, which by convective transport (electroosmosis) moves glucose across the skin. The concentration of glucose is then measured by a glucose oxidase electrode detector *(106, 107)*.

Finally, considerable research has been focused on developing totally noninvasive technology for glucose sensing. Of these, near-infrared spectroscopy has been most intensively investigated, but unpredictable spectral variations continue to hinder progress *(108–112)*. Similar problems have impaired the successful use of light scattering *(113, 114)*. Finally, photoacoustic spectroscopy, although less studied, has yielded some encouraging preclinical results. In this technique, pulsed infrared light, when absorbed by molecules, produces detectable ultrasound waves, the intensity and patterns of which can theoretically be tuned to detect glucose *(115–117)*.

INTERPRETATION

Only the Gluco Watch Biographer and the Continuous Monitoring System have received FDA approval at the time of writing. Therefore, only they will be considered here. The two devices have vastly differing applications. The Gluco Watch is designed to analyze "glucose" approximately three times per hour for up to 12 h and appears best suited for detecting unsuspected hypoglycemia. In contrast, the Continuous Monitoring System is intended for one-time or occasional use, rather than ongoing daily use. The information derived by these devices is intended to assist physicians to guide patients to improve their diabetes control, the values being downloaded into a computer in the physicians' offices.

The Continuous Monitoring System consists of a subcutaneous glucose sensor, which is connected to a monitor worn externally. Glucose is monitored every 5 min for up to 72 h, and at the end of that period the data are transferred to another computer for analyses. Values are not displayed on the externally worn monitor.

The Gluco Watch provides frequent measurements for up to 12 h after a single calibration. Calibration with reference plasma glucose values is required, and sampling time limits the frequency of measurements to approximately three per hour. In limited but promising clinical trials, the Gluco Watch provided reasonable correlation with SMBG *(106, 107)*. For example, in 28 patients with type 1 diabetes tested in a clinical setting, the Gluco Watch values had a correlation of 0.90 (n = 1554 pairs of data) with capillary blood glucose. In 12 patients in the home setting, the correlation of Gluco Watch values with SMBG values was $r = 0.85$ (205 paired data points). The correlation between two Gluco Watches worn simultaneously was $r = 0.94$ *(107)*. Despite the recent approval of the Gluco Watch by the FDA, its use has not been rigorously tested in a clinically relevant home setting, nor has it been tested in children. However, if it is demonstrated to reliably detect unsuspected hypoglycemic episodes in such settings, we may see widespread use of the Gluco Watch and continued improvement of the technology.

Currently, there are no analytical standards for noninvasive and minimally invasive glucose analyses. Such standards will clearly need to be different for different proposed uses. For example, the reliability, precision, and accuracy requirements for a glucose sensor that is linked to a system that automatically adjusts insulin doses will be vastly different from the requirements for a sensor in a system designed to sound an alarm in cases of apparent extreme hyper- or hypoglycemia. It seems intuitively obvious that a larger imprecision can be tolerated in instruments that make frequent readings during each hour than in an instrument used only two or three times per day to adjust a major portion of a person's daily insulin dose.

EMERGING CONSIDERATIONS

With the first approvals of self-monitoring, noninvasive glucose sensors by the FDA, it is anticipated that there will be renewed efforts to bring other technologies forward into clinical studies. Ultimately, we shall see improved methods for noninvasive or minimally invasive glucose measurements that will complement current self glucose monitoring techniques.

Ketone Testing

USE

> Recommendation: Ketones should be measured in urine or blood by patients with diabetes in the home setting and in the clinic/hospital setting as an adjunct to the diagnosis of DKA.
>
> Level of evidence: E

The ketone bodies acetoacetate (AcAc), acetone, and β-hydroxybutyric acid (βHBA) are catabolic products of free fatty acids. Determinations of ketones in urine and blood are widely used in the management of patients with diabetes mellitus as adjuncts for both diagnosis and ongoing monitoring of DKA. Measurements of ketone bodies are routinely performed both in an office/hospital setting and by patients at home.

The ADA recommends that initial evaluation of patients with diabetes mellitus include determination of urine ketones and that urine ketone testing should be available in the physician's office for immediate use as

needed *(14)*. The ADA further recommends that urine ketone testing is an important part of monitoring by patients with diabetes, particularly in those with type 1 diabetes, pregnancy with preexisting diabetes, and GDM *(9)*. All patients with diabetes mellitus should test their urine for ketones during acute illness, stress, persistent hyperglycemia [plasma glucose >16.7 mmol/L (300 mg/dL)] pregnancy, or symptoms consistent with DKA, such as nausea, vomiting, or abdominal pain *(9, 14)*.

RATIONALE

Ketone bodies are usually present in urine and blood, but in very low concentrations (e.g., total serum ketones <0.5 mmol/L). Increased ketone concentrations in patients with known diabetes mellitus or in previously undiagnosed patients presenting with hyperglycemia suggest impending or established DKA, a medical emergency. The two major mechanisms for the high ketone concentrations in patients with diabetes are increased production from triglycerides and decreased utilization in the liver; both are results of absolute or relative insulin deficiency and increased counterregulatory hormones, including cortisol, epinephrine, glucagon, and growth hormone *(118)*.

The principal ketone bodies, βHBA and AcAc, are usually present in approximately equimolar amounts. Acetone, usually present in only small quantities, is derived from spontaneous decarboxylation of AcAc. The equilibrium between AcAc and βHBA is shifted toward formation of βHBA in any condition that alters the redox state of hepatic mitochondria to increase concentrations of NADH, such as hypoxia, fasting, metabolic disorders (including DKA), and alcoholic ketoacidosis *(119–121)*. Thus, assay methods for ketones that do not include measurement of βHBA may provide misleading clinical information by underestimating total ketone body concentration *(90, 122)*.

ANALYTICAL CONSIDERATIONS

Urine ketones

Preanalytical. Usually the concentrations of ketones in the urine are below the detection limits of commercially available testing materials. False-positive results have been reported with highly colored urine and in the presence of several sulfhydryl-containing drugs, including angiotensin-converting enzyme inhibitors *(123)*. Urine test reagents deteriorate with exposure to air, giving false-negative readings; testing material should be stored in tightly sealed containers and discarded after the expiration date on the manufacturer's label *(124)*. False-negative readings have also been reported with highly acidic urine specimens, such as after large intakes of ascorbic acid. Loss of ketones from urine attributable to microbial action can also cause false-negative readings. Because acetone is a highly volatile substance, specimens should be kept in a closed container. For point-of-care analyses in medical facilities and for patients in the home setting, control materials (giving both negative and positive readings) are not commercially available but would be desirable to assure accuracy of test results.

Analytical. Several assay principles have been described. Most commonly used is the colorimetric reaction that occurs between ketones and nitroprusside (sodium nitroferricyanide), which produces a purple color *(26)*. This method is widely available in the form of dipsticks and tablets and is used to measure ketones in both urine and blood (either serum or plasma). Several manufacturers offer dipsticks that measure glucose and ketones; a combination dipstick is necessary only if the patient monitors urine glucose instead of or in addition to blood glucose. The nitroprusside method measures only AcAc unless the reagent contains glycine, in which case acetone is also measured. The nitroprusside-containing reagent is much more sensitive to AcAc than acetone with respect to color generation. Importantly, this reagent does not measure βHBA *(122)*.

Blood ketones

Preanalytical. Serum/plasma ketones can be measured using tablets or dipsticks routinely used for urine ketone determinations. Although specimens can be diluted with saline to "titer" the ketone concentration (results are typically reported as "positive at a $1/x$ dilution"), as with urine ketone testing, βHBA, the predominant ketone body in DKA, is not detected.

For specific determinations of βHBA, as described below, specimen requirements differ among methods. In general, blood samples can be collected into heparin, EDTA, fluoride, citrate, or oxalate (for the BioScanner Ketone system, fluoride and oxalate have not been tested, according to the manufacturer). Ascorbic acid interferes with some assay methods. AcAc interferes with some assay methods unless specimens are highly dilute. Specimen stability differs among methods, but in general, whole blood specimens are stable at 4 °C for up to 24 h. Serum/plasma specimens are stable for up to 1 week at 4 °C and for at least several weeks at −20 °C (long-term stability data are not available for most assay methods).

Analytical. Although several different assay methods (e.g., colorimetric, gas chromatography, capillary electrophoresis, and enzymatic) have been described for blood ketones, including specific measurement of βHBA, enzymatic methods for quantification of βHBA appear to be the most widely used for routine clinical management *(125–127)*. The principle of the enzymatic methods is that βHBA in the presence of NAD^+ is converted to AcAc and NADH by β-hydroxybutyrate dehydrogenase. Under alkaline conditions (pH 8.5–9.5), the reaction favors formation of AcAc from βHBA. The NADH produced can be quantified spectrophotometrically (usually kinetically) with use of a peroxidase reagent (Analox Instruments USA). One manufacturer offers a method that uses a test card impregnated with the reagents (KetoSite; GDS Diagnostics). Most methods permit use of whole blood,

plasma, or serum specimens (required volumes are generally ≤200 μL). Some methods permit analysis of multiple analytes and are designed for point-of-care testing. Several methods are available as hand-held meters, which are FDA approved for both laboratory use and for over-the-counter use by patients [e.g., BioScanner Ketone (PolymerTechnology Systems) and MediSense Precision Xtra (Abbott Laboratories)] *(127)*. These methods use dry-chemistry test strips to which a drop of whole blood, serum, or plasma is added. Results are displayed on the instruments within ~2 min.

INTERPRETATION

Urine ketone determinations

> Recommendation: Urine ketone determinations should not be used to diagnose or monitor the course of DKA.
>
> Level of evidence: A

In a patient with known diabetes mellitus or in a patient not previously diagnosed with diabetes but who presents with typical symptoms of diabetes and hyperglycemia, the presence of positive urine ketone readings suggests the possibility of impending or established DKA. Although DKA is most commonly associated with type 1 diabetes mellitus, it may rarely occur in type 2 patients *(128)*. Patients with alcoholic ketoacidosis will have positive urine ketone readings, but hyperglycemia is not usually present. Positive urine ketone readings are found in up to 30% of first morning void urine specimens from pregnant women (with or without diabetes), during starvation, and after hypoglycemia *(90, 122, 129)*.

Blood ketone determinations

> Recommendation: Blood ketone determinations that rely on the nitroprusside reaction should be used only as an adjunct to diagnose DKA and should not be used to monitor treatment of DKA. Specific measurement of βHBA in blood can be used for diagnosis and monitoring of DKA. Further studies are needed to determine whether the test offers any clinical advantage over more traditional management approaches (e.g., measurements of serum CO_2, anion gap, or pH).
>
> Level of evidence: E

Blood ketone determinations that rely on the nitroprusside reaction should be used with caution for diagnosis of DKA because results do not quantify βHBA, the predominant ketone in DKA. The test should not be used to monitor the course of therapy because AcAc and acetone may increase as βHBA decreases during successful therapy *(90, 118–122)*. Blood ketone determinations that measure βHBA specifically are useful for both diagnosis and ongoing monitoring of DKA *(121, 130–132)*. Reference intervals for βHBA differ among assay methods, but concentrations in healthy individuals fasted overnight are generally <0.5 mmol/L. Patients with well-documented DKA [serum CO_2 <17 mmol/L; arterial pH <7.3; plasma glucose >14.9 mmol/L (250 mg/dL)] generally have βHBA concentrations >2 mmol/L.

Further studies are also needed to determine whether blood ketone determinations by patients with diabetes mellitus are preferable (e.g., better accepted by patients than urine testing, with more prompt diagnosis of DKA) to urine ketone determinations.

GHb

USE

> Recommendations: GHb should be measured routinely in all patients with diabetes mellitus to document their degree of glycemic control. Treatment goals should be based on the results of prospective randomized clinical trials such as the DCCT and UKPDS. These trials have documented the relationship between glycemic control, as quantified by serial determinations of GHb, and risks for the development and progression of chronic complications of diabetes.
>
> Laboratories should be aware of potential interferences, including hemoglobinopathies, that may affect GHb test results. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population.
>
> Level of evidence: A

Measurement of glycated proteins, primarily GHb, is widely used for routine monitoring of long-term glycemic status in patients with diabetes mellitus.[9] GHb is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications *(90, 122, 133)*. This test is also being used increasingly by quality assurance programs to assess the quality of diabetes care, e.g., requiring that healthcare providers document the frequency of GHb testing in patients with

[9] The terms glycated hemoglobin, glycohemoglobin, "glycosylated" (which should not be used) hemoglobin, Hb A_1, and Hb A_{1c} have all been used to refer to hemoglobin that has been modified by the nonenzymatic addition of glucose residues. However, these terms are not interchangeable. Glycated hemoglobins comprise Hb A_1 and other hemoglobin-glucose adducts, whereas Hb A_1 is made up of Hb A_{1a}, Hb A_{1b}, and Hb A_{1c}. Hb A_{1c} is the major component of Hb A_1, accounting for ~80% of Hb A_1. To eliminate this confusing nomenclature, the term "A1c test" has been suggested. As described in the text, most of the clinical outcome data that are available for the effects of metabolic control on complications (at least for the DCCT and UKPDS) used assay methods that quantified Hb A_{1c}. In this report, we use the abbreviation GHb to include all forms of glycated hemoglobin.

diabetes and the proportion of patients with GHb values below a specified value *(134, 135)*.

The ADA and other organizations that have addressed this issue recommend measurement of GHb in patients with both type 1 and 2 diabetes, first to document the degree of glycemic control, then as part of continuing care *(14)*. The ADA has recommended specific treatment goals for GHb based on the results of prospective randomized clinical trials, most notably the DCCT *(12, 133)*, but also the more recent UKPDS *(13)*. Because different GHb assays can give different GHb values, the ADA recommends that laboratories use only assay methods that are certified as traceable to the DCCT GHb reference *(122, 133)*; these results are reported as hemoglobin (Hb) A_{1c}.

RATIONALE

Glycated proteins are formed posttranslationally from the slow, nonenzymatic reaction between glucose and amino groups on proteins *(136)*. For hemoglobin, the rate of synthesis of GHb is principally a function of the concentration of glucose to which the erythrocytes are exposed. GHb is a clinically useful index of mean glycemia during the preceding 120 days, the average life span of erythrocytes *(90, 136–143)*. Although carefully controlled studies have documented a close relationship between the concentration of GHb and mean glycemia, routine determinations of blood glucose by patients or by their healthcare providers are not considered as reliable as GHb to quantify mean glycemia *(19, 90, 137, 138, 144–146)*. Concentrations of other blood-based glycated proteins (e.g., glycated serum/plasma proteins, "fructosamine") also reflect mean glycemia, but over a much shorter time than GHb: 15–30 days and 60–120 days, respectively *(90, 136–144, 147, 148)*. However, the clinical utility of glycated proteins other than hemoglobin has not been clearly established, and there is no convincing evidence that relates their concentration to the chronic complications of diabetes *(90, 122)*.

ANALYTICAL CONSIDERATIONS

> Recommendations: Laboratories should use only GHb assay methods that are certified by the National Glycohemoglobin Standardization Program (NGSP) as traceable to the DCCT reference. In addition, laboratories that measure GHb should participate in a proficiency-testing program, such as the CAP Glycohemoglobin Survey, that uses fresh blood samples with targets set by the NGSP Laboratory Network.
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> Level of evidence: B

There are many (>30) different GHb assay methods in current use. These range from low-throughput research laboratory component systems and manual minicolumn methods to high-throughput automated systems dedicated to GHb determinations. Most methods can be classified into one of two groups based on assay principle *(90, 139, 149)*. The first group includes methods that quantify GHb based on charge differences between glycated and nonglycated components. Examples include cation-exchange chromatography and agar gel electrophoresis. The second group includes methods that separate components based on structural differences between glycated and nonglycated components. Examples include boronate affinity chromatography and immunoassay. Most charge-based and immunoassay methods quantify Hb A_{1c}, defined as Hb A with glucose attached to the NH_2-terminal valine of one or both β chains. Other methods quantify "total glycated hemoglobin", which includes both Hb A_{1c} and other hemoglobin-glucose adducts (e.g., glucose-lysine adducts and glucose-α chain NH_2-terminal valine adducts). Generally, results of methods using different assay principles show excellent correlation, and there are no convincing data to show that any one method or analyte is clinically superior to any other. However, the reported GHb results from the same blood sample could differ considerably among methods unless they are standardized to a common reference, e.g., without standardization, the same blood sample could be read as 7% in one laboratory and 9% in another *(90, 139, 149–155)*.

In 1996, the NGSP was initiated to standardize GHb test results among laboratories to DCCT-equivalent values *(154–156)*. The rationale for standardizing GHb test results to DCCT values was that the DCCT had determined the relationship between specific GHb values and long-term outcome risks in patients with diabetes mellitus *(12, 14, 90, 122)*. The NGSP was developed under the auspices of the AACC and is endorsed by the ADA, which recommends that laboratories use only GHb methods that have passed certification testing by the NGSP. In addition, the ADA recommends that all laboratories performing GHb testing participate in the CAP proficiency-testing survey for GHb, which uses fresh whole blood specimens *(157)*.

The NGSP Laboratory Network includes a variety of assay methods, each calibrated to the DCCT reference. The DCCT reference is a HPLC cation-exchange method that quantifies Hb A_{1c} and is a NCCLS-designated comparison method *(140, 158)*. The assay method has been used since 1978 and has demonstrated good long-term imprecision (between-run CVs consistently <3%) *(157)*. The laboratories in the network interact with manufacturers of GHb methods to assist them first in calibrating their methods and then in providing comparison data for certification of traceability to the DCCT. Certification is valid for 1 year. An important adjunct to the program is the GHb proficiency-testing survey administered by CAP. Since 1996 (starting with a pilot project that included 500 laboratories and expanded to all laboratories in 1998), the survey has used fresh whole blood samples with NGSP-assigned target values. Since initiation of the NGSP in

1996, the survey has documented a steady improvement in comparability of GHb values among laboratories, both within and between methods. In general, NGSP-certified methods have demonstrated less variability and better comparability to NGSP-assigned target values than non-certified methods *(157)*. The NGSP website (http://www.missouri.edu/~diabetes/ngsp.html) provides detailed information on the certification process and maintains a listing of certified assay methods.

Preanalytical

Patient variables. There are no clinically significant effects of age, sex, ethnicity, or season on GHb or GHb test results. The effects of age on GHb are controversial *(159–161)*. Some studies have shown age-related increases in GHb, ~0.1% per decade after age 30. Other reports have shown little or no increase. Differences in results among the studies are probably attributable to differences in the selection of study participants; when studies were restricted to participants with normal glucose tolerance (i.e., fasting and postprandial plasma glucose concentrations within established reference intervals), little or no age-related increase in GHb was found. Results were also not significantly affected by acute illness.

Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia) falsely lowers GHb test results regardless of the assay method *(90)*. Vitamins C and E are reported to falsely lower test results, possibly by inhibiting glycation of hemoglobin *(162, 163)*, but vitamin C may increase values with some assays *(162)*. Iron-deficiency anemia is reported to increase test results *(164)*. There is no substantial effect of food intake on test results. Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, chronic ingestion of salicylates, and opiate addiction are reported to interfere with some assay methods, falsely increasing results *(139, 165–167)*.

Several hemoglobinopathies (e.g., Hb S, C, Graz, Sherwood Forest, D, and Padova) and chemically modified derivatives of hemoglobin interfere with some assay methods, independent of any effects attributable to shortened erythrocyte survival [*(168–170)*; for a review, see Ref. *(171)*]. Depending on the particular hemoglobinopathy and assay method, results can be either falsely increased or decreased. Some methods may give a value in the reference interval for a nondiabetic patient with a hemoglobin variant, but this is not an assurance that no interference is present; the interference may be subtle in the reference interval, but may increase steadily with increasing GHb. Boronate affinity chromatographic assay methods are generally considered to be less affected by hemoglobinopathies than methods that separate glycated and nonglycated components based on charge differences. In some cases, such as with most cation-exchange HPLC methods, manual inspection of chromatograms can alert the laboratory to the presence of either a variant or a possible interference. Alternative nonhemoglobin-based methods for assessing long-term glycemic control may be useful in these situations *(171)*.

Because interferences are method specific, product instructions from the manufacturer should be reviewed before use of the GHb assay method. In selecting an assay method, the laboratory should take into consideration characteristics of the patient population served, i.e., high prevalence of hemoglobinopathies.

Sample collection, handling, and storage. Blood can be obtained by venipuncture or by fingerprick capillary sampling *(172, 173)*. Blood tubes should contain anticoagulant as specified by the manufacturer of the GHb assay method (EDTA can be used unless otherwise specified by the manufacturer). Sample stability is assay method specific *(174, 175)*. In general, whole blood samples are stable for up to 1 week at 4 °C. For most methods, whole blood samples stored at −70 °C or colder are stable long term (at least 1 year), but specimens are not stable at −20 °C. Improper handling of specimens, such as storage at high temperatures, can introduce large artifacts that may not be detectable, depending on the assay method.

Recently, several convenient blood collection systems have been introduced, including filter paper and small vials containing stabilizing/lysing reagent *(176–178)* These systems are designed for field collection of specimens with routine mailing to the laboratory. These systems are generally matched to specific assay methods and should be used only if studies have been performed to establish comparability of test results using these collection systems with standard sample collection and handling methods for the specific assay method used.

Analytical

Recommendations: Laboratories should use GHb assay methods with an interassay CV<5% (ideally <3%). At least two control materials with different mean values should be analyzed as an independent measure of assay performance. Laboratories should verify specimens below the lower limit of the reference interval or >15% by repeat testing. If Schiff base (labile pre-Hb A_{1c}) interferes with the assay method, it should be removed before assay.

Level of evidence: C

Performance goals and quality control. Several expert groups have presented recommendations for assay performance. Early reports recommended that the interassay CV be <5% at the GHb concentrations found in apparently healthy and diabetic individuals *(179)*. More recent reports suggested lower CVs, e.g., intralaboratory <3% and interlaboratory <5% *(180)*. These recommendations are reasonable: intraindividual CVs are very small (<2%), and many current assay methods can achieve CVs <3%. We recommend intralaboratory CV <3%.

The laboratory should include two control materials with different mean values (high and low) at the beginning and end of each day's run. Frozen whole blood controls stored at −70 °C or colder in single-use aliquots are ideal and are stable for months or even years depending on the assay method. Lyophilized controls are commercially available but, depending on the assay method, may show matrix effects when new reagents or columns are introduced. It is recommended that the laboratory consider using both commercial and in-house controls to optimize performance monitoring.

Reference intervals. The laboratory should determine its own reference interval according to NCCLS guidelines (NCCLS Document C28A) even if the manufacturer has provided one. Test participants should be nonobese and have FPG <6.1 mmol/L (110 mg/dL). For NGSP-certified assay methods, the SD for the reference interval is generally ≤0.5% GHb, producing a 95% CI of ≤2% GHb (e.g., mean Hb A_{1c} ± 2 SD = 5.0% ± 1.0%). For NGSP-certified assay methods, reference intervals should not deviate significantly (e.g., >0.5%) from the 4–6% range. Note that ADA target values derived from the DCCT and UKPDS *(9)*, not the reference values, are used to evaluate metabolic control in patients.

Out-of-range specimens. The laboratory should repeat testing for all sample results below the lower limit of the reference interval, and if confirmed, the physician should be informed to see whether the patient has an abnormal hemoglobin or evidence of red cell destruction. In addition, sample results >15% GHb should be repeated, and if confirmed, the possibility of a hemoglobin variant should be considered *(171)*.

Removal of labile GHb. Formation of GHb includes an intermediate Schiff base that is called "pre-A_{1c}" or labile A_{1c} *(181, 182)*. This material is formed rapidly with hyperglycemia and interferes with some GHb assay methods, primarily those that are charge based. For methods that are affected by this labile intermediate, manufacturer's instructions should be followed for its removal.

INTERPRETATION

Laboratory—physician interactions

The laboratory should work closely with physicians who order GHb testing. Proper interpretation of test results requires an understanding of the assay method, including its known interferences. For example, if the assay method is affected by hemoglobinopathies (independent of any shortened erythrocyte survival) or uremia, the physician should be made aware of this.

An important advantage of using a NGSP-certified assay method is that the laboratory can provide specific information relating GHb test results to both mean glycemia and outcome risks as defined in the DCCT and UKPDS *(12, 90, 122)*. This information is available on the NGSP website. For example, each 1% change in GHb is related to a change in mean plasma glucose of ~2 mmol/L (35 mg/dL).

Some studies suggest that immediate feedback to patients at the time of the clinic visit with GHb test results improves their long-term glycemic control *(183)*. However, additional studies are needed to confirm these findings before this strategy can be recommended. It is possible to achieve the goal of having GHb test results available at the time of the clinic visit by either having the patient send in a blood sample shortly before the scheduled clinic visit or by having a rapid assay system convenient to the clinic.

Clinical application

> Recommendations: Treatment goals should be based on ADA recommendations, which include maintaining GHb concentrations <7% and reevaluation of the treatment regimen for GHb values >8%. (Note that these values are applicable only if the assay method is certified as traceable to the DCCT reference.) GHb testing should be performed at least biannually in all patients and quarterly for patients whose therapy has changed or who are not meeting treatment goals.
>
> Level of evidence: B

Treatment goals. GHb measurements are now a routine component of the clinical management of patients with diabetes mellitus. Principally on the basis of the results of the DCCT, the ADA has recommended that a primary goal of therapy is a GHb value <7% and that physicians should reevaluate the treatment regimen in patients with GHb concentrations consistently >8% *(9, 10)*. These GHb values apply only to assay methods that are certified as traceable to the DCCT reference, with a reference interval of ~4–6% Hb A_{1c} or Hb A_{1c}-equivalent. In the DCCT, each 10% reduction in GHb (e.g., 12% vs 10.8% or 8% vs 7.2%) was associated with ~45% lower risk for the progression of diabetic retinopathy *(184)*. Similar risk reductions were found in the UKPDS *(133)*. It should also be noted that in the DCCT and UKPDS, decreased GHb was associated with increased risk for serious hypoglycemia.

Testing frequency. There is no consensus on the optimal frequency of GHb testing. The ADA recommends that "for any individual patient, the frequency of GHb testing should be dependent on the judgment of the physician. In the absence of well-controlled studies that suggest a definite testing protocol, expert opinion recommends GHb testing at least two times a year in patients who are meeting treatment goals (and who have stable glycemic control) and more frequently (quarterly assessment) in patients whose therapy has changed or who are not meeting glycemic goals" *(14)*. These testing recommendations are for patients with either type 1 or type 2 diabetes. Diabetes quality assurance programs, e.g., ADA Provider Recognition Program and HEDIS 2000 *(134, 135)*, have generally required documentation of the

percentage of patients with diabetes who have had at least one GHb determination during the preceding year. Studies have established that serial (quarterly for 1 year) measurements of GHb produce large improvements in GHb values in patients with type 1 diabetes *(185)*.

Interpretation. GHb values in patients with diabetes are a continuum: they range from normal, in a small percentage of patients whose mean plasma glucose concentrations are close to those of nondiabetic individuals, to markedly increased values, e.g., two- to threefold increases in some patients, reflecting an extreme degree of hyperglycemia. Multiple comparisons between the two systems are required to generate this equation, which will be used only by manufacturers (not by individual clinical laboratories) to establish traceability. Proper interpretation of GHb test results requires that physicians understand the relationship between GHb values and mean plasma glucose, the kinetics of GHb, and specific assay limitations/interferences *(90)*. Small changes in GHb (e.g., ± 0.5% GHb) over time may reflect assay variability rather than a true change in glycemic status.

EMERGING CONSIDERATIONS

Use of GHb for diabetes screening/diagnosis

At present, the ADA does not recommend GHb for diabetes screening or diagnosis *(186)*. There is considerable controversy surrounding this issue, and further studies are needed to determine whether GHb is useful for screening and/or diagnosis of diabetes *(187–190)*. Harmonization of GHb assays has obviated one of the most commonly stated reasons for not using GHb for screening and/or diagnosis. Optimal clinical utility of GHb for screening and/or diagnosis will also require highly precise assay methods.

Use of other glycated proteins, including advanced glycation end-products for routine management of diabetes mellitus

Further studies are needed to determine whether other glycated proteins, such as fructosamine, are clinically useful for routine monitoring of patients' glycemic status. Further studies are also needed to determine whether measurements of advanced glycation end-products are clinically useful as predictors of risk for chronic diabetes complications *(191)*. None of these analytes was evaluated in the DCCT or UKPDS.

Global harmonization of GHb testing

In 1995, the IFCC formed a Working Group on HbA_{1c} Standardization (IFCC-WG). This committee, which includes members from the NGSP Steering Committee and Laboratory Network, has been evaluating several candidate reference methods and purified GHb materials (purified Hb A_{1c}) that potentially could provide firm links between the NGSP and GHb standardization programs in other countries *(192)*. Such a scheme is particularly attractive because it would allow GHb test results worldwide to be comparable to those in the DCCT and UKPDS. The IFCC has established a laboratory network, using both mass spectroscopy and capillary electrophoresis as candidate reference methods. The candidate reference material is a mixture of highly purified Hb A_{1c} and Hb A_0 *(193–195)*. Initial comparisons between samples analyzed by the IFCC Laboratory Network and the NGSP Laboratory Network are encouraging; there appears to be a linear relationship between the two reference systems (personal communication from Kor Miedema, Chairperson, IFCC-WG, January 17, 2000). If further studies confirm a consistent relationship between the two networks, it will be possible to use one of the IFCC reference methods to replace the current NGSP anchor (a designated comparison method with far less specificity for Hb A_{1c} than either the mass spectroscopy or capillary electrophoresis methods).

Assuming that the IFCC reference system is adopted by the NGSP and other standardization programs, an important issue that would need to be addressed is the different values obtained between the networks. The IFCC reference system yields GHb concentrations lower than those measured in the DCCT and UKPDS. Therefore, the question is whether the lower IFCC-based values should be adopted along with the new reference system or whether the current values, which are traceable to the DCCT and widely used, should be retained. In the latter event, the results obtained with the IFCC reference system would be converted into DCCT-equivalent concentrations by an equation. Multiple comparisons between the two systems are required to generate this equation, which will be used only by manufacturers (not by individual clinical laboratories) to establish traceability. Proper resolution of this important question will require international consensus with a process that includes both clinicians and laboratorians.

Genetic Markers

USE

Diagnosis/Screening

Type 1 diabetes

> Recommendation: Routine measurement of genetic markers is not of value at this time for the diagnosis or management of patients with type 1 diabetes. For selected diabetic syndromes, valuable information can be obtained with definition of diabetes-associated mutations.
>
> Level of evidence: E

Genetic markers are currently of limited clinical value in the evaluation and management of patients with diabetes. However, they hold promise for the future. For immune-mediated (type 1A) diabetes (IMD), HLA typing can be useful to indicate absolute risk of diabetes (see Table 6), as extended by insulin (*INS*) gene typing (and in some populations by CTLA-4 gene typing), and can assist

Table 6. Lifetime risk of type 1 diabetes in first-degree relatives (proband diagnosed before age 20).[a]

Relative	Risk, %
Parents	2.2 ± 0.6
Children	5.6 ± 2.8
Siblings	6.9 ± 1.3
HLA-nonidentical sibling	1.2
HLA-haploidentical sibling	4.9
HLA-identical sibling	15.9
Identical twin	30–40
General population	0.3

[a] From Harrison *(205)*.

in assigning a probability of the diagnosis of IMD to diabetes of uncertain etiology *(196)*. As indicated below, HLA-DR/DQ typing can be useful to indicate modified risk of IMD in persons with positive islet cell autoantibodies because protective alleles do not prevent the appearance of islet cell autoantibodies (most often as single autoantibodies), but do protect against clinical diabetes. Typing of class II major histocompatibility antigens or HLA-DRB1, -DQA1, and -DQB1 is not diagnostic for IMD. However, some haplotypes form susceptibility, whereas others provide substantial protection. Thus, HLA-DR/DQ typing can be used only to increase or decrease the probability of IMD presentation and cannot be recommended for routine clinical diagnosis or classification *(197)*.

It is possible to screen newborn children to identify those at increased risk of developing IMD *(198, 199)*. This strategy cannot be recommended until there is a proven intervention available to delay or prevent the disease *(200)*. The rationale for the approach is thus placed below under emerging considerations.

Type 2 diabetes and maturity onset diabetes of youth (MODY)

> Recommendation: There is no role for routine genetic testing in patients with type 2 diabetes. These studies should be confined to the research setting and evaluation of specific syndromes.
>
> Level of evidence: E

Type 2 diabetes: Fewer than 5% of patients with type 2 diabetes have been resolved on a molecular genetic basis, and not surprisingly, most of these have an autosomal dominant form of the disease or very high degrees of insulin resistance. Type 2 diabetes is a heterogeneous polygenic disease with both resistance to the action of insulin and defective insulin secretion *(3, 4)*. Multiple genetic factors interact with exogenous influences (e.g., environmental factors such as obesity) to produce the phenotype. Identification of the affected genes is therefore highly complex.

MODY: Mutation detection for MODY patients and their relatives is technically feasible. However, because of the high cost of establishing a facility to detect mutations and the high degree of technical skill required for analysis, few laboratories perform these assays. As direct automated sequencing of genes becomes standard, it is likely that detection of specific diabetes mutations will become more common.

Monitoring/Prognosis

Although genetic screening may provide information about prognosis and could be useful for genetic counseling, genotype may not correlate with the phenotype. In addition to environmental factors, interactions among multiple quantitative trait loci expressions may be involved. Genetic identification of a defined MODY will have value for anticipating the prognosis.

RATIONALE

The HLA system, which has a fundamental role in the adaptive immune response, exhibits considerable genetic complexity. The HLA complex on chromosome 6 contains class I and II genes that code for several polypeptide chains *(201)*. The major (classic) class I genes are *HLA-A*, *-B*, and *-C*. The loci of class II genes are designated by three letters: the first (D) indicates the class, the second (M, O, P, Q, or R) the family, and the third (A or B) the chain. Both classes of molecules are heterodimers: class I consists of an α chain and β_2-microglobulin, whereas class II has α and β chains. The function of the HLA molecules is to present short peptides, derived from pathogens, to T cells to initiate the adaptive immune response *(201)*. Genetic studies have revealed an association between certain HLA alleles and autoimmune diseases. These diseases include, but are not confined to, ankylosing spondylitis, celiac disease, Addison disease, and type 1 diabetes *(201)*.

Genetic testing for syndromic forms of diabetes is the same as that for the underlying syndrome itself *(1)*. Such diabetes may be secondary to the obesity associated with Prader–Willi syndrome, which maps to chromosome 15q, or to the absence of adipose tissue inherent to recessive Seip–Berardinelli syndrome of generalized lipodystrophy, which maps to chromosome 9q34 *(1, 202)*. There are >60 distinct genetic disorders associated with glucose intolerance or frank diabetes. Most forms of type 2 diabetes (which are usually strongly familial) will probably be understood in defined genetic terms, but this is far from realized at present. Some genes for MODY have been identified, but there are large numbers of individual mutants. Persons at risk within MODY pedigrees can be identified through genetic means. Depending on the specific MODY mutation, the disease can be mild (e.g., glucokinase mutation) and not usually associated with long-term complications of diabetes or as severe as typical type 1 diabetes [e.g., hepatocyte nuclear factor (HNF) mutations] *(203)*. The interest in the genetics of MODY is

the hope that insight will be obtained into type 2 diabetes. (Note that MODY is not a form of type 2 diabetes.)

Five different MODY types have been identified. MODY-1, -3, -4, and -5 all result from mutations in the genes encoding transcription factors that regulate the expression of genes in pancreatic β cells. These genes are *HNF-4α* in MODY-1, *HNF-1α* in MODY-3, *HNF-1β* in MODY-5, and insulin promoter factor-1 (*IPF-1*) in MODY-4. It has been shown that homozygous mutations of the *IPF-1* gene lead to pancreatic agenesis and that heterozygous mutations of *IPF-1* gene lead to MODY-4 *(202)*. The modes of action of the HNF lesions in MODY are still not clear. It is likely that mutation in *HNF-1α*, *-1β*, and *-4α* cause diabetes because they impair insulin secretion. MODY-2 is caused by mutations in the glucokinase gene. The product of the gene is an essential enzyme in the glucose-sensing mechanism of the β cells, and mutations in this gene lead to partial deficiencies of insulin secretion.

ANALYTICAL CONSIDERATIONS

A detailed review of analytical issues will not be attempted here because genetic testing for diabetes outside of a research setting is currently not recommended for clinical care. Serologic HLA typing should be replaced by molecular methods, such as sequence-specific priming, because antibodies with a mixture of specificities and cross-reactivities have been estimated to give inaccurate results in ~15% of typings.

Preanalytical

Detection of mutations is performed using genomic DNA extracted from peripheral blood leukocytes. Blood samples should be drawn into test tubes containing EDTA, and the DNA preparations should be harvested within 3 days; longer periods both lower the yield and degrade the quality of the DNA obtained. Genomic DNA can be isolated from fresh or frozen whole blood by lysis, digestion with proteinase K, extraction with phenol, and then dialysis. The average yield is 100–200 μg of DNA from 10 mL of whole blood. DNA samples are best kept at −80 °C in Tris-EDTA solution, where the integrity of the sample lasts virtually indefinitely.

Analytical

Methods for the detection of mutations differ for different types of mutation. The different MODY types have substitution, deletion, or insertion of nucleotides in the coding region of the genes. These are detected by PCR. The detailed protocols for the detection of specific mutations are beyond the scope of this review.

EMERGING CONSIDERATIONS

To screen for the propensity for IMD in general populations, *HLA-D* genes are the most important, contributing as much as 50% of the genetic susceptibility *(196)*. *HLA-DQ* genes appear to be central to the HLA-associated risk of IMD, albeit *HLA-DR* genes may be independently involved [for a review, see Refs. *(204, 205)*]. The heterodimeric proteins that are expressed on antigen-presenting cells, B lymphocytes, platelets, and activated T cells, but not other somatic cells, are composed of cis- and trans-complemented α- and β-chain heterodimers. Thus, in any individual, four possible DQ dimers are encoded. Positive risks for IMD are associated with α chains that have an arginine at residue 52 and β chains that lack an aspartic acid at residue 57. Persons at the highest genetic risk for IMD are those in whom all four HLA-DQ combinations meet this criterion. Thus, persons heterozygous for *HLA-DRB1*04-DQA1*0301-DQB1*0302* and *DRB1*03-DQA1*0501-DQB1*0201* are the most susceptible, with an absolute lifetime risk for IMD in the general population of ~1:12. Persons who are protected from IMD are those with *DRB1*15-DQA1*0201-DQB1*0602* (Asp 57+) haplotypes in particular *(206)*, albeit those with *DRB1*11* or **04* who also have *DQB1*0301* (Asp 57 +) are protected. HLA-DR is also involved in susceptibility to IMD in that the *B1*0401* and **0405* subtypes of *DRB1*04* are susceptible, whereas the **0403* and **0406* subtypes are protective, even when found in HLA haplotypes of the susceptible *DQA1*0301-DQB1*0302*. DR molecules are also heterodimers, but the DRα chain is invariant in all persons. Additional DRβ chains (B3, B4, and B5) are not important.

Class II MHC is involved in antigen presentation to CD4 helper cells, and the above associations are likely to be explained by defective affinities to islet cell antigenic peptides, leading to persistence of T-helper cells that escape thymic ablation. Class I HLA is also implicated in IMD. Multiple non-HLA loci also contribute to susceptibility to type 1 diabetes *(204)*. For example, the variable nucleotide tandem repeat upstream from the insulin (*INS*) gene on chromosome 11q is also useful for predicting the development of IMD, with alleles with the longest variable nucleotide tandem repeat having protective effects. Typing newborn infants for both *HLA-DR/DQ*, and to a lesser degree the *INS* gene, improves prediction of IMD to better than 1:10 in the general population. The risk of IMD in HLA-identical siblings of a proband with IMD is 1:4, whereas siblings who have HLA haplotype identity have a 1:12 risk and those with no shared haplotype a 1:100 risk *(205)*. The numerous other putative genomic intervals suggested to be linked to IMD remain to be confirmed in multiple data sets, and discussion of these is outside the scope of this report. The sequencing of the human genome and the formation of consortia should lead to advances in the identification of the genetic bases for both type 1 and type 2 diabetes. This progress should ultimately lead to family counseling, prognostic information, and the selection of optimal treatment *(202, 207)*.

Autoimmune Markers

USE

> Recommendation: Islet cell autoantibodies are recommended for screening of nondiabetic family members who wish to donate part of their pancreas for transplantation to a relative with end stage, immune-mediated (type 1) diabetes. Islet cell autoantibodies are not recommended for routine diagnosis of diabetes or for screening.
>
> Level of evidence: E

No therapeutic intervention has been identified that will prevent diabetes *(204, 205)*. Therefore, although several autoantibodies have been detected in individuals with type 1 diabetes, measurement of these has very limited use outside of clinical studies. Because of the minimal indication for use of autoantibodies in routine management of patients with diabetes, this section will focus on the pragmatic aspects of clinical laboratory testing for autoantibodies at present and briefly address some areas of controversy.

Diagnosis/Screening

Diagnosis. In type 1 diabetes, the insulin-producing β cells of the pancreas are destroyed. In the vast majority of these patients, the destruction is mediated by T cells *(1)*. This is termed type 1A diabetes or IMD (Table 1). Islet cell autoantibodies comprise autoantibodies to islet cell cytoplasm (ICAs); to native insulin, referred to as insulin autoantibodies (IAAs) *(208)*; to glutamic acid decarboxylase ($GAD_{65}A$) *(209–211)*; and to two tyrosine phosphatases [insulinoma-associated antigens IA-2A *(212)* and IA-2βA *(213)*]. Autoantibody markers of immune destruction are usually present in 85–90% of individuals with IMD when fasting hyperglycemia is initially detected *(1)*. Autoimmune destruction of the β cells has multiple genetic predispositions and is modulated by undefined environmental influences. The autoimmunity may be present for months or years before the onset of symptoms. Patients with type 1A diabetes have a significantly increased risk of other autoimmune disorders, including celiac disease, Graves disease, thyroiditis, Addison disease, and pernicious anemia *(63)*. As many as 1 in 4 females with IMD have autoimmune thyroid disease, whereas 1 in 280 patients develop adrenal autoantibodies and adrenal insufficiency. A minority of patients with type 1 diabetes (type 1B; idiopathic) have no known etiology and no evidence of autoimmunity. Most of these patients are of African or Asian origin.

Approximately 10–15% of Caucasian adult patients who present with the type 2 diabetes phenotype also have islet cell autoantibodies *(214)*, particularly $GAD_{65}A$, which predict insulin dependency. This has been termed latent autoimmune diabetes of adulthood *(215)*. Although ICA- or $GAD_{65}A$-positive diabetic patients progress faster to absolute insulinopenia than do antibody-negative patients, many antibody-negative (type 2) diabetic adults also progress (albeit more slowly) to insulin dependency with time. There is no role for islet cell autoantibody testing in patients with type 2 diabetes because the institution of insulin therapy is based on glucose control.

Screening

> Recommendation: Screening of relatives of patients with type 1 diabetes or of persons in the general population for islet cell autoantibodies is not recommended at present.
>
> Level of evidence: E

The risk of developing IMD in relatives of patients with type 1 diabetes is ~5%, which is 15-fold higher than the risk in the general population (1:250–300 lifetime risk). Screening relatives of patients with IMD for islet cell autoantibodies can identify those at high risk of IMD. However, as many as 1–2% of healthy individuals have a single autoantibody and are at low risk of developing IMD *(216)*. Because of the low prevalence of IMD (~0.3% in the general population), the positive predictive value of a single autoantibody will be low *(205)*. The presence of multiple islet cell autoantibodies (IAA, $GAD_{65}A$, and IA-2A/IA-2βA) is associated with a risk of IMD >90% *(216, 217)*. However, until cost-effective screening strategies can be developed for young children and effective intervention therapy to prevent the clinical onset of the disease become available, such testing cannot be recommended outside of a research setting.

Children and young adults with certain HLA-DR and/or DQB1 chains (**0602/*0603/*0301*) are mostly protected from IMD, but not from developing islet cell autoantibodies *(218)*. Because islet cell autoantibodies in these individuals have substantially reduced predictive significance, consideration should be given to excluding them from prevention trials.

Monitoring/Prognosis

> Recommendation: There is currently no role for measurement of islet cell autoantibodies in the monitoring of patients in clinical practice. Islet cell autoantibodies are measured in research protocols and some clinical trials as surrogate end-points.
>
> Level of evidence: E

No acceptable therapy has been demonstrated to prolong survival of islet cells once diabetes has been diagnosed or to prevent the clinical onset of diabetes in islet cell autoantibody-positive individuals *(204)*. Thus, repeated testing for islet cell autoantibodies to monitor islet cell autoimmunity is not clinically useful at present. In

islet cell or pancreas transplantation, the presence or absence of islet cell autoantibodies may clarify whether subsequent failure of the transplanted islets is attributable to recurrent autoimmune disease or to rejection *(219)*. When a partial pancreas has been transplanted from an identical twin or HLA-identical sibling, appearance of islet cell autoantibodies may raise consideration of the use of immunosuppressive agents to try to halt recurrence of diabetes. Notwithstanding these theoretical advantages, the value of this therapeutic strategy has not been established.

Some experts have proposed that testing for islet cell autoantibodies may be useful in the following situations: (*a*) to identify a subset of adults initially thought to have type 2 diabetes but who have islet cell autoantibody markers of type 1 diabetes and progress to insulin dependency *(220)*; (*b*) to screen nondiabetic family members who wish to donate a kidney or part of their pancreas for transplantation; (*c*) to screen women with GDM to identify those at high risk of progression to type 1 diabetes; and (*d*) to distinguish type 1 from type 2 diabetes in children to institute insulin therapy at the time of diagnosis *(221, 222)*. For example, some pediatric diabetologists are now treating children thought to have type 2 diabetes with oral medications but treat autoantibody-positive children immediately with insulin. However, it is possible to follow patients who are islet cell autoantibody-positive to the point of metabolic decompensation and then institute insulin therapy. A small pilot trial from Japan suggests that insulin therapy in islet cell autoantibody-positive patients may preserve C-peptide (a measurement of insulin secretion) compared with oral medications *(223)*, but this observation requires confirmation.

During the review of this manuscript by a panel of experts, it became evident that there is wide variability in clinical practice regarding the use of islet cell autoantibodies. Although some indicate that the results of autoantibody assays are clinically useful, others point to a lack of evidence. Although some clinicians, particularly those who treat pediatric patients, use autoantibody assays as outlined in the preceding paragraph, clinical studies are necessary to provide outcome data to validate this approach. There is no systematic review that addresses these questions.

RATIONALE

The presence of autoantibodies suggests that insulin therapy is the most appropriate therapeutic option, especially in a young person. Conversely, in children or young people without islet cell autoantibodies, consideration may be given to a trial of oral agents and lifestyle changes other than insulin therapy. There is no unanimity of opinion, but the presence of autoantibodies may alter therapy for subsets of patients, including Hispanic and African-American children with a potential diagnosis of non-IMD, adults with autoantibodies but clinically classified as having type 2 diabetes, and children with transient hyperglycemia. The majority of nondiabetic individuals who have only one autoantibody will never develop diabetes. Although expression of multiple anti-islet cell autoantibodies is associated with greatly increased diabetes risk *(216, 217)*, ~20% of individuals presenting with new-onset diabetes express only a single autoantibody.

ANALYTICAL CONSIDERATIONS

> Recommendation: It is important that autoantibodies be measured only in an accredited laboratory with an established quality-control program and participation in a proficiency-testing program.
>
> Level of evidence: E

ICAs are determined by indirect immunofluorescence on frozen sections of human pancreatic tails *(224)*. ICAs measure the degree of binding of immunoglobulin to the islets and are compared with a standard serum of the Immunology of Diabetes Workshop group *(225)*. The results are reported in Juvenile Diabetes Foundation (JDF) units. Positive results depend on the study or context in which they are used, but many laboratories use 10 JDF units determined on two separate occasions or a single result of ≥20 JDF units as significant titers that may convey an increased risk of IMD.

For IAAs, a radioisotopic method that calculates the displaceable insulin radioligand binding after the addition of excess nonradiolabeled insulin *(226)* is recommended. Results are reported as positive when the specific antibody binding is greater than the mean + 2 (or 3) SD for healthy persons. Each laboratory needs to assay at least 100 healthy individuals to determine its own values. Many laboratories use a cutoff value between 80 and 110 milliunits/L. An important caveat concerning IAA determination is that insulin antibodies develop after insulin therapy even in those persons who use human insulin.

For IA-2A and $GAD_{65}A$, a dual micromethod and RIA performed with ^{35}S-labeled recombinant human IA-2 and ^{3}H-labeled human recombinant GAD_{65} in a rabbit reticulocyte expression system is currently used by many laboratories *(216)*. Methods for both $GAD_{65}A$ and IA-2A have recently become commercially available. $GAD_{65}A$, IA-2A, and IA-2βA are reported as positive when the signal is >99.7% (3 SD) of values in healthy controls *(216)*. Comparison by multiple laboratories worldwide of a small number of quality-control sera sent out from the laboratory of one of the authors (N.M.) revealed a concordance >90% for classification of individuals as antibody positive or negative. The CDC is working with the Immunology of Diabetes Society to develop the Diabetes Autoantibody Standardization Program. A limited pilot proficiency-testing program using samples obtained from patients with type 1 diabetes was begun recently. It is not yet clear whether this program will become generally available.

INTERPRETATION

In newly diagnosed patients with type 1 diabetes, ICAs are found in ~75–85%, GAD_{65}As in ~60%, IA-2As in ~40%, and IA-2βAs in ~20%. IAAs are positive in >90% of children who develop type 1 diabetes before age 5, but in <40% in individuals developing diabetes after age 12.

In some laboratories, the ICA assay is considered to be the most sensitive and specific single test for the detection of type 1 diabetes. However, the ICA assay is labor-intensive and difficult to standardize, and marked inter-laboratory variability in sensitivity and specificity has been demonstrated in workshops *(207, 227)*. Few clinical laboratories are likely to implement this test. The immunoassays are more reproducible. Measurement of T-cell reactivity in peripheral blood is theoretically appealing (because T cells mediate islet destruction), but the variability of such assays precludes their use in a clinical setting *(228, 229)*.

Autoantibody-positivity is reported (by definition) in some healthy individuals despite an absence of family history of autoimmune diseases. Islet cell autoantibodies are no exception. If one autoantibody is found, the others should be assayed because the risk of IMD increases if two or more autoantibodies are positive *(205, 217)*. For the standardized ICA assay, replicate titers in excess of 10 JDF units predict an increased risk of diabetes. Similarly, IAA concentrations above the mean + 3 SD of healthy controls also predict an increased risk of diabetes, and when associated with ICA or another antibody, carry a risk of 20–50%.

The following suggestions have been proposed by Atkinson and Eisenbarth *(204)* as a rational approach to the use of autoantibodies in diabetes: (*a*) antibody assays should have specificity >99%; (*b*) proficiency testing should be documented; (*c*) multiple autoantibodies should be assayed; and (*d*) sequential measurements should be performed. These strategies will reduce false-positive and -negative results.

EMERGING CONSIDERATIONS

Because immunoassays for IAA, GAD, and IA-2A/IA-2βA are now available, it is likely that a panel of these autoantibodies will eventually be used for screening purposes, possibly with ICAs used for confirmatory testing. Because ICAs may represent either GAD_{65} or IA-2 autoantibodies and ICA assays are difficult to standardize, some experts in the field do not use ICA testing at all. Cost considerations aside, the best screening would be through all of the above autoantibodies, including ICAs. However, this recommendation is controversial and some experts disagree.

It is likely that other islet cell antigens will be discovered, which could lead to additional diagnostic and predictive tests for IMD. For example, GLIMA-38 *(230)* is associated with IMD, but its prognostic significance has not been established. Autoantibody screening on fingerstick blood samples appears quite feasible in the future. In those individuals who are islet cell autoantibody positive, *HLA-DR/DQ* genotyping can help define absolute risks of diabetes.

Several clinical trials to prevent IMD are being actively pursued *(205)*. Such trials can now be done in relatives of patients with type 1 diabetes or in the general population on the basis of the islet cell autoantibody and/or *HLA-DR/DQ* genotype status. Risk can be assessed by islet cell autoantibodies alone, without the need for evaluation of endogenous insulin reserves as was done for the US DPT-1 trial. Autoantibody positivity rates are distinctly lower in the general population than in relatives of individuals with IMD, so that trials in the latter group are more economical. Potential interventional therapies (for IMD) undergoing clinical trials include oral or nasal insulin given to patients at the time of diagnosis of diabetes or to nondiabetic, but islet cell autoantibody-positive, relatives of individuals with IMD. Trials of a vaccine based on immunization by an insulin β-chain peptide or GAD_{65} are scheduled to begin soon. Additional trials of other antigen-based immunotherapies, adjuvants, cytokines, and T-cell accessory molecule-blocking agents are likely in the future *(200)*. Decreased islet cell autoimmunity will be one important outcome measure of these therapies.

Microalbuminuria

USE

Diagnosis/Screening

Diabetes is the leading cause of end-stage renal disease in the US and Europe *(231)*. Early detection of diabetic nephropathy relies on tests for urinary excretion of albumin. Conventional qualitative tests (chemical strips or dipsticks) for albuminuria do not detect the small increases in urinary albumin excretion seen in early stages of nephropathy. For this purpose, tests for "microalbuminuria" are used. Microalbuminuria is defined *(231)* as excretion of 30–300 mg of albumin/24 h (or 20–200 μg/min or 30–300 μg/mg of creatinine; Table 7) on two of three urine collections.[10]

The ADA recommends periodic qualitative (dipstick) testing for urine albumin in adults with diabetes *(231)*. Positive tests represent "clinical albuminuria" or "overt nephropathy" in the ADA recommendations, corresponding to protein excretion >300 mg/24 h (>200 μg/min or >300 μg/mg of creatinine; Table 7). In these patients, quantitative measurement of urine protein excretion is used in the assessment of the severity of proteinuria and its progression, in planning treatment, and in determining the impact of therapy. Measurement of creatinine clearance as an index of glomerular filtration rate can be

[10] Although the term microalbuminuria is recognized as a misnomer (the albumin is not small), the term is well entrenched and not likely to be replaced by alternatives, e.g., paucialbuminuria or increased urinary albumin excretion (UAE) rate.

Table 7. Definitions of microalbuminuria and clinical albuminuria.[a]

	Albumin excretion		
	mg/24 h	μg/min	μg/mg of creatinine
Normal	<30	<20	<30
Microalbuminuria	30–300	20–200	30–300
Clinical albuminuria[b]	>300	>200	>300

[a] From ADA *(14)*.
[b] Also called "overt nephropathy".

performed on the same timed (usually 12-h or 24-h) urine collection. Negative dipstick tests for "clinical proteinuria" (albumin excretion <300 mg/day) should be followed with a test for microalbuminuria. For children with type 1 diabetes, testing for microalbuminuria is recommended to begin after puberty and after duration of diabetes for 5 years.

The recommendation to screen for microalbuminuria is based on expert opinion that considered such things as the natural history of diabetic nephropathy and the evidence from many randomized controlled clinical trials on the benefit of treatment of those patients found to have microalbuminuria.

In the ADA algorithm for urine protein testing *(231)*, the diagnosis of microalbuminuria requires the demonstration of increased albumin excretion (as defined above) in two of three tests repeated at intervals of 3–6 months as well as exclusion of conditions that "invalidate" the test.

Prognosis

Microalbuminuria has prognostic significance. In 80% of people with type 1 diabetes and microalbuminuria, urinary albumin excretion increases at a rate of 10–20% per year, with development of clinical proteinuria (>300 mg albumin/day) in 10–15 years. After development of clinical grade proteinuria, most (>80%) patients go on to develop decreased glomerular filtration rate and, given enough time, end-stage renal disease. In type 2 diabetes, 20–40% of patients with microalbuminuria progress to overt nephropathy, but by 20 years after overt nephropathy, only ~20% develop end-stage renal disease. In addition, patients with diabetes (type 1 and 2) and microalbuminuria are at increased risk for cardiovascular disease.

Monitoring

The roles of routine urinalysis and albumin measurements are less clear in patients with a diagnosis of microalbuminuria. Some have advocated urine protein testing to monitor treatment, which may include improved glycemic control, more assiduous control of hypertension, dietary protein restriction, and therapy with angiotensin inhibitors *(231)*. Therapy (e.g., with angiotensin-converting enzyme inhibitors) has been shown to slow the rate of increase of urinary albumin excretion or to prevent it in short-term studies, and intensive glycemic control is associated with delayed progression of urinary albumin excretion [for a recent study, see Ref. *(232)*]. Patients who were prescribed angiotensin-converting enzyme inhibitors are not being tested as frequently as others *(233)*. This finding points to an ambiguity in current guidelines because recommendations for renal screening in patients on angiotensin-converting enzyme inhibitors are not clearly defined.

RATIONALE

> Recommendation: Annual microalbumin testing of patients without clinical proteinuria should begin in pubertal or postpubertal individuals 5 years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes. The role of testing is unclear in patients under treatment with angiotensin-converting enzyme inhibitors and in those with a short life expectancy.
>
> Level of evidence: E

Early detection of microalbuminuria allows early intervention with a goal of delaying the onset of overt diabetic nephropathy. As stated earlier, microalbuminuria is a marker of increased risk of cardiovascular morbidity and mortality in both type 1 and type 2 diabetes. Thus, it is a signal for more intensive efforts to reduce cardiovascular risk factors.

Microalbuminuria rarely occurs with a short duration of type 1 diabetes or before puberty. Thus, testing is less urgent in these situations. Although the difficulty in precisely dating the onset of type 2 diabetes warrants initiation of annual testing early after the diagnosis of diabetes, older patients (age >75 years or life expectancy <20 years) may never be at risk for clinically significant nephropathy in view of a projected life span that is too brief for renal dysfunction to develop. In such patients, the role of treating microalbuminuria is far from clear, and the need to screen for it thus is uncertain at best.

ANALYTICAL CONSIDERATIONS

Analytical

> Recommendation: The analytical CV of methods to measure microalbuminuria should be <15%.
>
> Level of evidence: E

Analytical goals can be related to the degree of biological variation, with less precision required for analytes that vary widely in individuals to be tested. The within-person variation of albumin excretion is large in people without diabetes and even higher in patients with diabetes. Howey et al. *(234)* studied day-to-day variation, over 3–4 weeks, of the 24-h albumin excretion, the concentra-

tion of albumin, and the albumin:creatinine ratio. The latter two were measured in the 24-h urine sample and also in (*a*) the first morning void and (*b*) random untimed urine. In healthy volunteers, the lowest within-person CVs were found for the concentration of albumin in the first morning void (36%) and for the albumin:creatinine ratio in that sample (31%). They recommended use of the urine albumin concentration in the first morning void rather than 24-h urinary excretion of albumin, which had a higher within-person CV.

To keep the analytical CV less than one-half the biological CV, Howey et al. *(234)* proposed an analytical goal of CV = 18%. Alternatively, if the albumin:creatinine ratio is to be used, one may calculate the need for somewhat lower imprecision (i.e., a better precision) to accommodate the lower biological CV for the ratio and the imprecision contributed by the creatinine measurement. Assuming a CV of 5% for the measurement of creatinine, we calculate a goal of 14.7% for the analytical CV for albumin when it is used to estimate the albumin:creatinine ratio. A goal of 15% appears reasonable to accommodate use of the measured albumin concentration for calculation of either the timed excretion rate or the albumin:creatinine ratio.

In individuals with diabetes, the within-person variation (CV) was 61% for the albumin concentration in the first morning void and 39% for the albumin:creatinine ratio. Thus, the goals above appear more than adequate for use in individuals with diabetes.

Premeasurement

Recommendation: Acceptable samples to test for increased urinary albumin excretion are timed (e.g., 12 or 24 h) collections for measurement of albumin concentration and timed or untimed samples for measurement of the albumin:creatinine ratio. For screening, an untimed sample for albumin measurement (without creatinine) may be considered if a concentration cutoff is used that allows high sensitivity for detection of an increased albumin excretion rate.

Level of evidence: E

Collection of 24-h samples has advantages (e.g., possibility to measure creatinine clearance), but the albumin:creatinine ratio appears to be an acceptable alternative. The ratio has a within-person biological variation similar to that of the excretion rate and correlates well with timed excretion as well as with albumin concentration in a first morning void of urine *(234)*. A first morning void sample is somewhat preferable for the ratio because the ratio in a first morning void sample had a lower within-person variation than does the ratio in a random sample of urine during the day *(234)*. Although the ratio appears entirely acceptable for screening, limited data are available for its use in monitoring the response to therapy, and 12- or 24-h collections may be preferable.

Albumin is stable in untreated urine stored at 4 or 20 °C for at least 1 week *(235)*. Neither centrifugation nor filtration appears necessary before storage at −20 or −80 °C *(236)*. Whether centrifuged, filtered, or not treated, albumin concentration decreased by 0.27% per day at −20 °C but showed no decrease over 160 days at −80 °C *(236)*.

The urinary albumin excretion rate reportedly has no marked diurnal variation in diabetes, but it does in essential hypertension *(237)*.

Measurement: detection limit, imprecision

Commercially available quantitative methods for microalbuminuria have documented detection limits of ~20 μg/L or lower. Within-run imprecision and day-to-day (total) imprecision are well within the analytical goal of ~15%, and often are much lower. A recent study showed that most methods, but not all, agree well with each other and support a reference interval of 2–20 μg albumin/mg of creatinine *(238)*.

Recommendation: Semiquantitative or qualitative screening tests for microalbuminuria should be positive in >95% of patients with microalbuminuria to be useful for screening. Positive results must be confirmed by analysis in an accredited laboratory.

Level of evidence: E

Qualitative (or semiquantitative) tests for microalbuminuria have been proposed for use as screening tests for microalbuminuria. To be useful, screening tests must have high detection rates for abnormal samples, i.e., a high clinical sensitivity. Although many studies have assessed the ability of reagent strips (dipstick methods) for microalbumin to detect increased albumin concentrations in urine, the important question is whether the method can detect microalbuminuria, i.e., increased albumin excretion rate or its surrogate, increased albumin:creatinine ratio. We can find no published study in which the sensitivity for detection of an increased albumin excretion rate reached 95%.

In a large study *(239)*, the sensitivity for detection of an albumin excretion rate >30 mg/24 h was 91% when the test was performed by a single laboratory technician, 86% when performed by nurses, and 66% when performed by general practitioners. In two more recent studies *(240, 241)*, the sensitivities were 67–86%. False-positive results also appear to be common, with false-positive rates as high as 15% *(239)*. Thus it appears that at least

some of the tests, especially as used in practice, have the wrong characteristics for use in screening because of low sensitivity (high false-negative rates), and positive results must be confirmed by a laboratory method.

The available dipstick methods for microalbumin do not appear to lend themselves to viable screening strategies either in the physician's office or for home testing. The usual screening tests (e.g., for phenylketonuria) have low false-negative rates, and thus, only positive results require confirmation by a quantitative method. When a screening test has low diagnostic sensitivity, negative results also must be confirmed, a completely untenable approach. With semiquantitative tests, it may be possible (or indeed necessary) to use a cutoff <20 mg/L to ensure detection of samples with albumin >20 mg/L as measured by laboratory methods.

We recommend evaluation of chemical strip methods by testing of samples with albumin concentrations in the range of 20–50 mg/L because it is insufficient to show that the methods can detect albumin at higher concentrations.

Further studies are needed before the dipstick tests for microalbuminuria can be recommended as replacements for the quantitative tests. The use of the qualitative tests at the point of care is reasonable only when it can be shown to avoid quantitative testing in a sizeable proportion of patients and to ensure detection of those patients who have early renal disease.

INTERPRETATION

Nonanalytical sources of variation

Transient increases of urinary albumin excretion have been reported with short-term hyperglycemia, exercise, urinary tract infections, marked hypertension, heart failure, and acute febrile illness *(231)*.

Frequency of measurement

The ADA recommends annual measurement for microalbumin in patients with negative (dipstick) results for overt proteinuria. After the documentation of a diagnosis of microalbuminuria (i.e., with results as defined above in two of three tests performed within a period of 3–6 months), repeated testing is reasonable to determine whether a chosen therapy is effective. It may also be useful in determining the rate of progression of disease and thus support planning for care of end-stage renal disease. Although the ADA recommendations suggest that such testing is not generally needed before puberty, testing may be considered on an individual basis if it appears appropriate because of early onset of diabetes, poor control, or family history of diabetic nephropathy. A recent study indicated that the duration of diabetes before puberty is an important risk factor in this age group and thus can be used to support such testing in individual patients *(232)*.

Miscellaneous Potentially Important Analytes

INSULIN AND PRECURSORS

Recommendation: There is no role for routine testing for insulin, C-peptide, or proinsulin in most patients with diabetes. Differentiation between type 1 and type 2 diabetes may, in most cases, be made based on the clinical presentation and subsequent course. There is no role for measurement of insulin concentration in the diagnosis of the metabolic syndrome because knowledge of this value does not alter the management of these patients.

These assays are useful primarily for research purposes and, in rare cases, to identify patients with an absolute requirement for insulin before switching to oral agents, or to assist patients in obtaining insurance coverage for continuous subcutaneous infusion pumps.

A possible role for measurement of fasting insulin or the assessment of insulin resistance is in the evaluation of patients with polycystic ovary syndrome who may be candidates for treatment aimed at lowering insulin resistance in the absence of overt diabetes or glucose intolerance.

Level of evidence: E

Use

In the last several years, interest has increased in the possibility that measurements of the concentration of plasma insulin and its precursors might be of clinical benefit. In particular, evidence has been published that increased concentrations of insulin and/or proinsulin in nondiabetic individuals predict the development of CAD *(242)*. Although this possibility may be scientifically valid, its clinical utility is questionable. An increased insulin concentration is a surrogate marker that can be used to estimate resistance to insulin-mediated glucose disposal and can identify individuals at risk for developing syndrome X, also known as the insulin resistance syndrome *(243)*. Accurate measurement of insulin sensitivity requires the use of complex methods, such as the hyperinsulinemic euglycemic clamp technique, which are generally confined to research laboratories *(244, 245)*.

However, important as these changes may be in identifying such individuals, it is not clear that they are responsible for the increased risk of CAD. Consequently, it seems of greater clinical utility to quantify the consequences of the insulin resistance and hyperinsulinemia (or hyperproinsulinemia) rather than the hormone values themselves, i.e., by measuring blood pressure, degree of glucose tolerance, and plasma triglyceride and HDL-cholesterol concentrations. It is these changes that are the

focus of clinical interventions, not plasma insulin or proinsulin concentrations.

The clinical utility of measuring insulin, C-peptide, or proinsulin concentrations to help select the best antihyperglycemic agent for initial therapy in patients with type 2 diabetes is a question that arises from consideration of the pathophysiology of type 2 diabetes. In theory, the lower the pretreatment insulin concentration, the more appropriate might be insulin, or an insulin secretagogue, as the drug of choice to initiate treatment. Although this line of reasoning may have some intellectual appeal, there is no evidence that measurement of plasma insulin or proinsulin concentrations will lead to more efficacious treatment of patients with type 2 diabetes.

In contrast to the above considerations, measurement of plasma insulin and proinsulin concentrations is necessary to establish the pathogenesis of fasting hypoglycemia *(246)*. The diagnosis of an islet cell tumor is based on the persistence of inappropriately increased plasma insulin concentrations in the face of a low glucose concentration. In addition, an increase in the ratio of fasting proinsulin to insulin in hypoglycemic patients who have difficulty maintaining euglycemia strongly suggests the presence of an islet cell tumor. The absence of these associated changes in glucose, insulin, and proinsulin concentrations in an individual with fasting hypoglycemia makes the diagnosis of an islet cell tumor most unlikely, and alternative explanations should be sought for the inability to maintain fasting euglycemia.

Measurement of the C-peptide response to intravenous glucagon can aid in the rare cases in which it is difficult to differentiate between the diagnosis of type 1 and type 2 diabetes *(247)*. However, even in this clinical situation, the response to drug therapy will provide useful information, and measurement of C-peptide is not clinically necessary. In rare cases, it may be helpful to measure C-peptide concentrations before discontinuation of insulin. An example would be an obese adolescent presenting with DKA who may have type 2 diabetes and could be safely managed with an oral agent after resolution of glucotoxicity *(248)*. Measurement of C-peptide is essential in the investigation of possible factitious hypoglycemia attributable to surreptitious insulin administration *(249)*.

Finally, an emerging use for insulin assays is in the evaluation and management of patients with the polycystic ovary syndrome. Women with this syndrome manifest insulin resistance by androgen excess as well as by abnormalities of carbohydrate metabolism; emerging evidence suggests that both abnormalities may respond to treatment with metformin or thiazolidinediones. Although clinical trials have generally used the hyperinsulinemic euglycemic clamp to evaluate insulin resistance, fasting glucose-to-insulin ratios, and other modalities, the optimal laboratory evaluation of these patients is not yet clearly defined. It is certainly reasonable to document insulin resistance in a patient with polycystic ovary syndrome who does not have diabetes or IGT before beginning an insulin-sensitizing agent such as metformin or a thiazolidinedione *(10)*.

Analytical considerations

Although assayed for >40 years, there is no standardized method available to measure serum insulin *(248)*. Measurement of insulin, proinsulin, and C-peptide is accomplished by immunometric methods. Reference intervals have not been firmly established. Proinsulin reference intervals are dependent on methodology, and each laboratory should establish its own reference interval. Although it has been suggested by some, insulin measurement should not be used in an OGTT to diagnose diabetes. In the case of C-peptide, there is a discrepancy in reliability because of variable specificity among antisera, lack of standardization of C-peptide calibration, and variable cross-reactivity with proinsulin. Of note is the recent requirement of the US Centers for Medicare and Medicaid Services (CMS) that Medicare patients must have C-peptide measured to be eligible for coverage of insulin pumps. Initially, the requirement was that the C-peptide be ≤ 0.5 μg/L; however, because noncomparability of results from different assays led to denial of payment for some patients with values >0.5 μg/L, the requirement now states that the C-peptide should be less than the lower limit of the reference interval for the particular assay, plus 10% to account for the imprecision of the test *(250)*.

INSULIN ANTIBODIES

> Recommendation: There is no published evidence to support the use of insulin antibody testing for routine care of patients with diabetes.
>
> Level of evidence: E

Given sufficiently sensitive techniques, insulin antibodies can be detected in any patient being treated with exogenous insulin *(248)*. In the vast majority of patients, the titer of insulin antibodies is low, and their presence is of no clinical significance. Very low values are seen in patients treated exclusively with human recombinant insulin *(251)*. However, on occasion, the titer of insulin antibodies in the circulation can be quite high and associated with dramatic resistance to the ability of exogenous insulin to decrease plasma glucose concentrations. This clinical situation is quite rare and usually occurs in insulin-treated patients with type 2 diabetes, and the cause-and-effect relationships between the magnitude of the increase in insulin antibodies and the degree of insulin resistance is unclear. There are several therapeutic approaches for treating these patients, and a quantitative estimate of the concentration of circulating insulin antibodies does not appear to be of substantial benefit.

AMYLIN

Recommendation: Assays for amylin are not clinically useful in the management of diabetes. These studies should be confined to the research setting.

Level of evidence: E

Amylin is a 37-amino acid pancreatic peptide first described in 1987 *(252, 253)*. Amylin is cosecreted and colocated with insulin by the pancreatic β cells in response to nutrient intake. The peptide appears to help regulate glucose metabolism by delaying gastric emptying and decreasing glucagon production. Amylin deficiency may occur in insulinopenic type 2 patients. Trials of an amylin analog, pramlintide, are currently underway. At the present time, there is no clinical utility in measuring amylin.

LEPTIN

Recommendation: Routine measurement of plasma leptin concentrations is not of value at this time for the evaluation or management of patients with diabetes or obesity.

Level of evidence: E

Leptin is a recently discovered 167-amino acid protein synthesized by adipose tissue that appears to play a role in regulating appetite and energy intake via the hypothalamus, as well as influencing thermogenesis and reproductive functions *(254, 255)*. Although certain strains of genetically obese mice have a deficiency of leptin and lose weight when leptin is replaced, many obese humans have increased leptin concentrations.

Aside from rare cases of leptin deficiency, plasma leptin concentrations seem to vary directly with adiposity and plasma insulin concentrations. At this stage of knowledge, the only situation in which knowing the leptin concentration would be useful is in suspected cases of leptin deficiency, characterized by early-onset, massive obesity *(256)*. Obese persons usually have increased serum concentrations of leptin and appear to be resistant to its thermogenic and appetite suppressant effects.

LIPIDS

Recommendation: All adults with diabetes should receive annual lipid profiles. Individuals at low risk, i.e., those with LDL <2.6 mmol/L (100 mg/dL) and HDL >1.15 mmol/L (45 mg/dL) for men and >1.4 mmol/L (55 mg/dL) for women, may be screened less frequently. Because many patients with diabetes are candidates for lipid-lowering therapy, more frequent measurements may be required until control is achieved.

Level of evidence: A

Use

CAD is the major cause of morbidity and mortality in patients with type 2 diabetes *(257, 258)*, and attempts to ameliorate this situation must emphasize the diagnosis and treatment of dyslipidemia when present. Consequently, measurement of lipids is an important clinical practice recommendation for people with diabetes, especially type 2, although type 1 patients are also at increased risk for cardiovascular disease. Because this topic is covered in detail elsewhere *(257, 260)*, only brief mention of it is made here.

Small, dense LDL particles, hypertriglyceridemia, and low HDL concentrations characterize diabetic dyslipidemia. Generally speaking, diabetic patients can have lipid profiles measured in the same manner as the general population of patients appropriate for lipid screening.

The clinical evaluation of patients with type 2 diabetes should include quantification of plasma cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations. The ADA categorizes patients as high risk with LDL ≥3.35 mmol/L (130 mg/dL), HDL ≤0.90 mmol/L for men and <1.15 mmol/L for women (35 mg/dL for men and 45 mg/dL for women), and triglycerides ≥4.5 mmol/dL (400 mg/dL); intermediate risk as LDL ≥2.60–3.35 mmol/L (100–129 mg/dL), HDL concentrations of 0.9–1.15 mmol/L (35–45 mg/dL), and triglyceride concentrations of 2.30–4.5 mmol/L (200–399 mg/dL); and low risk as LDL ≤2.6 mmol/L (100 mg/dL), HDL >1.15 mmol/dL (45 mg/dL) for men and >1.40 mmol/L (55 mg/dL) for women *(259)*. These guidelines are also in agreement with the new Adult Treatment Panel III (ATP-III) guidelines recently issued by the National Cholesterol Education Program *(260, 261)*.

Analytical considerations

Preanalytical. Lipid profiles should be performed in the fasting state because LDL and especially triglyceride concentrations are dramatically affected by food intake.

Analytical. In most cases, accurate measurement can be accomplished by the usual clinical laboratory approach of directly measuring total plasma cholesterol and triglyceride concentrations, precipitating HDL and measuring the cholesterol concentration of the precipitate, and calculating the LDL-cholesterol concentration. This approach is satisfactory under most conditions, but it is inadequate if the plasma triglyceride concentrations are ≥4.5 mmol/L (400 mg/dL). In this situation, ultracentrifugation separation and measurement of the cholesterol and triglyceride concentrations in the specific lipoprotein fractions will be necessary to ensure accurate quantification of LDL- and HDL-cholesterol concentrations. Methods for the direct analysis of LDL are also available.

Extensive national and international programs exist to ensure the accuracy and reliability of lipid and lipoprotein assays. The Lipid Standardization Program of the CDC and National Heart, Lung, and Blood

Institute provides standardization for lipid and lipoprotein measurements. The CDC has established a Cholesterol Reference Laboratory Network to facilitate access to the National Reference System for Cholesterol and provide a means for clinical laboratories and manufacturers to verify traceability to the CDC reference method *(259)*.

Emerging considerations: new cardiovascular risk factors

> Recommendations: Measurement of nontraditional cardiovascular risk factors, such as C-reactive protein, fibrinogen, apolipoprotein (apo) B, and homocysteine, is not recommended for routine assessment of risk in patients with diabetes because the results would not lead to changes of therapy. Should ongoing trials support the use of folic acid to lower CAD by lowering homocysteine concentrations, or the use of other specific therapies aimed at one or more nontraditional risk factors, this recommendation may change.
>
> Level of evidence: E

Recently, evidence has been emerging that nontraditional risk factors may play an important role in the pathogenesis of CAD. Traditional laboratory risk factors, including hyperlipidemia, decreased HDL and an increased ratio of total cholesterol to LDL, clearly do not explain all of the variance in cardiovascular event rates. These nontraditional or novel risk factors include plasma homocysteine, fibrinolytic capacity, fibrinogen, and C-reactive protein *(262)*.

Lipid fractions that have been studied include HDL_2 and HDL_3, lipoprotein(a), apo A-1, and apo B. In particular, apo B has been shown in prospective observational studies to be strongly associated with cardiovascular events *(263)*. However, the therapeutic implications of this association are unclear because therapy that decreases LDL-cholesterol concentrations reduces event rates without altering apo B concentrations. Current recommendations of the ADA, the National Cholesterol Education Program, and the American Heart Association are that treatment decisions should be based on results of conventional lipid profiles, including total cholesterol, LDL, HDL, and triglycerides, as well as consideration of other risk factors. There are no published studies showing that measurement of additional lipid fractions is associated with improved treatment outcomes.

Inflammatory processes may play a role in the pathogenesis of atherosclerotic disease. C-reactive protein is a sensitive marker for inflammation and adds to the predictive value of total and HDL-cholesterol in predicting the risk of a future coronary event *(264)*. Several immunometric assays are commercially available, but reference intervals vary among assays *(264)*. The clinical value of these assays has yet to be established, but it is possible that measurement of C-reactive protein may eventually be helpful in risk stratification of persons at average risk based on lipid determinations; patients with average risk based on the ratio of total to HDL-cholesterol but who have higher than normal C-reactive protein concentrations may be more likely to benefit from aspirin or hydroxymethylglutaryl-CoA reductase inhibitors than those with low or normal concentrations. For patients with diabetes, all of whom are categorized as high risk, measurement of C-reactive protein may be less informative.

A hypercoagulable state may contribute to cardiovascular risk in diabetes. Fibrinogen has been shown, in several prospective studies, to be positively associated with cardiovascular events. Other thrombogenic factors that may be associated with cardiovascular disease include plasminogen activator inhibitor-1, factor VII, and tissue-type plasminogen activator *(262)*. The clinical utility of these analytes has not been established.

Homocysteine has also received considerable attention as a possible modifiable risk factor for CAD. A recent systematic review concluded that there is strong epidemiologic evidence of a link between homocysteine concentrations and CAD *(265, 266)*. Homocysteine may also be associated with increased mortality after a coronary event and with microvascular complications. Increased total homocysteine concentrations are associated with increased cardiovascular mortality in patients with type 2 diabetes *(260)*. Although relatively simple and inexpensive measures, such as supplemental folate, vitamin B_6, and vitamin B_{12} therapy, may reduce homocysteine concentrations, it is unclear whether this will produce a reduction of CAD. Clinical trials are currently underway to resolve this issue. Additionally, the fortification of all enriched grain products with folic acid, mandated in the US since 1998, has lowered homocysteine concentrations in the general population *(267)*. Until the effectiveness of lowering homocysteine concentrations is established, it is uncertain what additional benefit may be achieved by measuring homocysteine.

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