MISCELLANEOUS POTENTIALLY IMPORTANT ANALYTES

I. 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 be in most cases 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, as knowledge of this value does not alter the management of these patients.

These assays are useful primarily for research purposes, and in rare instances to identify patients with an absolute requirement for insulin prior to 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

1. 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 coronary artery disease (CAD) (242). Although this possibility may be scientifically valid its clinical utility is questionable. Increased insulin concentration is a surrogate marker which 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 high density lipoprotein (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 pre-treatment insulin concentration, the more appropriate might be insulin, or an insulin secretagogue, as the drug of choice to initiate treatment. While 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 patients with hypoglycemia difficulty maintaining eug-

lycemia 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 instance 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 instances, it may be helpful to measure C-peptide concentrations prior to 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 due 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 abnormalities of carbohydrate metabolism; emerging evidence suggests that both abnormalities may respond to treatment with metformin or thiazolidinediones. While clinical trials have generally evaluated insulin resistance using the hyperinsulinemic euglycemic clamp, 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 overary syndrome who does not have diabetes or impaired glucose tolerance prior to beginning an insulin-sensitizing agent such as metformin or a thiazolidinedione (10).

2. Analytical Considerations

Although assayed for over 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 United States Health Care Financing Administration (HCFA) that Medicare patients must have C-peptide measured in order to be eligible for coverage of insulin pumps. Initially, the requirement was that the C-peptide be ≤ 0.5 ng/mL; however because of non-comparability of results from different assays resulting in denial of payment for some patients with values above 0.5 ng/mL, 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).

II. 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 lower plasma glucose concentrations. This clinical situation is quite rare, 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 significant benefit.

III. AMYLIN

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

Amylin is a 37-amino acid pancreatic peptide first described in 1987 (252, 253). Amylin is co-secreted and co-located with insulin by the pancreatic beta 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.

IV. 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.
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> 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 instances 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.

V. LIPIDS

> **Recommendation:** All adults with diabetes should receive annual lipid profiles. Individuals at low risk, i.e., low density lipoprotein (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. Since many patients with diabetes are candidates for lipid lowering therapy, more frequent measurements may be required until control is achieved.
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> Level of evidence: A

1. 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, measure-

ment 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. As 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 0.9-1.15 mmol/L (35-45 mg/dL), and triglycerides 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).

2. Analytical Considerations

A. Preanalytical

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

B. 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 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 insure 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 accuracy and reliability of lipid and lipoprotein assay. 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).

5. EMERGING CONSIDERATIONS: NEW CARDIOVASCULAR RISK FACTORS

Recommendation: Measurement of non-traditional cardiovascular risk factors, such as C-reactive protein, fibrinogen, apolipoprotein-B, and homocysteine, is not recommended for routine assessment of risk in patients with diabetes since the results would not lead to changes of therapy. Should ongoing trials support the use of folic acid to lower coronary artery disease by lowering homocysteine concentrations, or the use of other specific therapies aimed at one or more non traditional risk factors, this recommendation may change.

Level of evidence: E

Recently, evidence has been emerging that non-traditional 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 non-traditional 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), apolipoproteins (apo) A-1 and apoB. In particular, apoB has been shown in prospective observational studies to be strongly associated with cardiovascular events (263). However, therapeutic implications of this association are unclear because therapy that decreases LDL-C concentrations reduces event rates without altering apoB 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 ranges 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 prove 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 concentration may be more likely to benefit from aspirin or HMG-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 a number of prospective studies, to be positively associated with cardiovascular events. Other thrombogenic factors which may be associated with cardiovascular disease include plasminogen activator inhibitor-1 (PAI-1), factor VII, and tissue-type plasminogen activator (262). 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 epidemiological evidence of a link between homocysteine levels 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 result in 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 reduced 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.