Insulin Assay Standardization

Leading to measures of insulin sensitivity and secretion for practical clinical care

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Diabetes, primarily type 2 diabetes, has increased in prevalence throughout the world and current projections suggest a continued rise worldwide for at least the next quarter century. Insulin resistance, which frequently accompanies obesity, is known to be a key factor in the pathogenic development of type 2 diabetes (1–6). Type 2 diabetes occurs when insulin secretion is no longer sufficient to compensate for the resistance to the actions of insulin.

Measurements of insulin sensitivity and secretion are currently done only for research purposes and are only comparable in individual studies. There are no clinical applications for these measures. In fact, there are no criteria by which an individual could be classified as being insulin sensitive or resistant or as having mild, moderate, or severe impairment of insulin secretion. In theory, one could envision that knowledge of an individual's response to insulin or the ability to secrete insulin might be useful for selecting patients for intensified prevention efforts, in the choice of initial therapy upon onset of overt hyperglycemia, or in evaluating the response to therapy beyond glycemia. For example, if a person newly diagnosed with diabetes could be determined to be very insulin resistant, the choice of initial therapy could be a drug that primarily improves insulin sensitivity. On the other hand, if the person was only moderately insulin resistant but had more of a defect in insulin secretion, a drug that improves insulin secretion might be a better choice. However, these are goals for the future without current clinical utility.

One of the barriers for conducting the extensive research needed to determine the clinical utility of measures of insulin sensitivity and secretion is the lack of standardized insulin assays. Results reported from one study to the next are not comparable, making only qualitative comparisons between studies possible. Larger epidemiology studies have been limited to populations in which the same laboratory was used for all measures of insulin, making it impossible to conduct any quantitative summarization or meta-analysis of the results.

The usual progression from research findings to clinical guidelines has been that initially considerable research is done in intensive studies in small populations, and epidemiology studies are conducted with simple measures that generate hypotheses. Subsequently larger prospective, population-based trials are conducted to establish clinical outcomes and determine the sensitivity and specificity of outcome measures to predict clinical status. To date, only a few trials have investigated measures of insulin sensitivity and secretion over a period of years in a large number of individuals to be able to determine their predictive power. Even when trials of this type are done, the results are limited to that specific insulin assay and are not translatable to other laboratories or studies due to the lack of standardization of insulin assays.

An effort is underway by the American Diabetes Association to standardize insulin assays so that this barrier to advancing research is removed. Once the insulin assays are standardized, research can be done to establish measures of insulin sensitivity and secretion that can be used by various research laboratories to build scientific consensus on how to define these measures for use in research. If the research provides strong evidence for the ability of measures of insulin sensitivity and secretion to monitor or predict clinical outcomes, then experts could begin to draft guidelines for the use of these measures in the clinical setting.

Reasonably accurate glucose assays have been available for decades. For insulin, despite the efforts of the previous workgroup (7), little progress was sustained following that group's report to stimulate enhanced harmonization among both commercial and research assays. Thus, substantial work remained to bring all clinical and research assays into harmony with one another. Current Food and Drug Administration–approved commercially available assays for insulin produce a range of values for the same samples (8,9) based on the use of different insulin standard preparations, variable protocols, and different approaches for derivation of reporting units. There clearly is a need to standardize the reference system and protocols to enable all available assays to achieve consistent and uniform results and to report insulin in identical units.

The Insulin Standardization Workgroup was established by the American Diabetes Association in 2004 to address these issues in conjunction with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the Centers for Disease Control and Prevention (CDC), the European Association for the Study of Diabetes (EASD), and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The workgroup submitted recommendations on the measurement of circulating insulin including the assay specificity, optimal type of sample, standardization of the assay, and manner of reporting results. The

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results of this work and that of others have recently been published (9–11).

The workgroup recommended that concentrations of insulin be reported in Systeme Internationale (SI) units: pmol/l, avoiding all references to traditional insulin units based on insulin biological activity per milligram of standard preparation. As insulin preparations have increased in purity, the original value of 25 units/mg no longer applies. The highly purified, recombinantly produced insulin standards generously donated to the workgroup permitted accurate assignment of molar units of insulin to the calibrators in each of the participating assays, assuming a molecular weight of monomeric insulin of 5,808 Da. In the most recent publication (11), the Insulin Standardization Workgroup investigated alternative preparations for insulin reference materials. It demonstrated an important fact: most assays can achieve consistent performance with calibration traceability based on individual serum samples with insulin concentrations set by isotope dilution mass spectrometry, a reference measurement procedure for insulin that greatly improves the assessment of accuracy of measurement (12,13). The investigation showed that not all insulin assays had acceptable performance characteristics at concentrations as low as 12 pmol/l (~2 μU/ml in the old units), which is necessary for clinical use. The workgroup concluded that several, but not all, commercial assays were able to measure insulin with acceptable precision, accuracy, and cross-reactivities (11). Reasons for discrepancies in the results among commercially available assay methods were likely multifactorial and thus not explained by a single analytical performance characteristic. Improvement in standardization of insulin assay results will require an ongoing effort to achieve traceability to the isotope dilution mass spectrometry high-level reference measurement procedure calibrated with pure recombinant insulin and for manufacturers to address immunoassay specificity and response characteristics over the measuring interval when necessary (11).

We call for the introduction of a sustainable insulin assay standardization program. A standardized insulin assay will encourage research leading to measures of insulin sensitivity that will be practical for clinical care in a general population. Cutoff values of insulin concentrations and insulin sensitivity can then be determined that are associated with known magnitude of risk for developing type 2 diabetes. Additional criteria could be developed, perhaps based on fasting or stimulated insulin concentrations, which would guide therapy for type 2 diabetes and assist in confirming the diagnosis of type 1 diabetes. Insulin secretion and action are central to the pathophysiology of diabetes. It is time to address the chaos in the measures of these key processes.

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