Computer Assisted Analysis of Insulin Response to Glucose Stimulus

CHARLES R. McREYNOLDS, M.D., SHARFUDDIN SHAH, M.D., AND ROBERT P. STOFFER, M.D.

The Hertzler Research Foundation, The Hertzler Clinic, Halstead, KS 67056

ABSTRACT

Concomitant measurements of plasma insulin levels were performed on all samples of blood obtained from 1,408 patients undergoing a diagnostic oral glucose tolerance test. These patients had no prior diagnosis of diabetes but qualified for the study because of either suspicious history, family history, or signs and symptoms. Graphic display of the findings, with insulin plotted as percent increase over basal level and glucose as milligrams per 100 ml, resulted in recognizably distinct patterns of response in both nondiabetic and diabetic patients. Computer assisted analysis of these, along with correlation of the factor of obesity, confirmed the recognizably separate patterns. The concept of the initial lesion of insulin deficiency of diabetes as one of rate rather than magnitude tends to be confirmed.

It was concluded that the routine and simultaneous performance of plasma insulin levels during oral glucose tolerance tests was of value in the diagnosis of various stages of diabetes and in selection of the proper treatment regime.

Introduction

The diagnosis of diabetes depends on the proper use and interpretation of clinical laboratory procedures that detect elevated blood glucose levels. Of the variety of tests available for this purpose, the oral glucose tolerance test (OGGT) is of singular value. Since the advent of the radioimmunoassay (RIA) technique for measuring circulating insulin in 1960, a wealth of new information has re-established the importance of insulin deficit as the primary but not the only underlying defect in diabetes. In view of the accepted value of these two laboratory tests, a survey was undertaken in a large series of patients to determine whether or not the routine performance of the tests might adduce information that could be used to facilitate the diagnosis and/or management of all types and stages of diabetes.

Method and Material

Patient Population

The participants in this prospective survey were derived from a large patient-population that, during the latter half of 1972, underwent diagnostic work-ups at a
large group-practice facility. Admission to the survey was on the basis of one or more of the following: (1) an increased fasting blood glucose; (b) an increased two-hour glucose test; (3) a family history of diabetes and (4) signs and symptoms indicative of diabetes. Patients with an established diagnosis of diabetes were excluded from the series. Of the 1,554 patients originally included in the survey, 146 of them subsequently had to be excluded as a result of missing demographic data and/or questionable laboratory results. The remaining group of 1,408 patients was comprised of 839 females and 569 males. The females ranged in age from 13 to 92 years (average age was 51.9), and the males from 12 to 85 years (average age was 49.6). Virtually all the patients were seen on an in-patient basis.

**Laboratory Procedures**

**Oral Glucose Tolerance Test (OGTT).** Standard OGTTs were performed on all in-patients and whenever possible on out-patients. Despite out-patients being instructed in a diet containing approximately 250 g of carbohydrates to be ingested for three days prior to the test day, rigid insistence on this was not always enforced, since it has been shown that a diet containing 150 g would not invalidate the test results. All patients fasted for 12 hours prior to the start of the test. Venous blood, taken from the antecubital vein, was employed for all analyses. After a fasting specimen was drawn, 100 g of a flavored glucose meal was given. Venous samples were subsequently drawn at 60, 90, 120, and 180 minutes in all patients, and at 240 and 300 minutes in selected patients (those suspect of reactive hypoglycemia). The data obtained from these latter two samples are not considered in this report. All samples were centrifuged within 30 minutes after being withdrawn, and glucose determinations were made the same day on plasma by the potassium ferricyanide (Auto-Analyzer) method. Results of the OGTT were reported as mg of glucose per 100 ml of plasma. Samples were collected in a seven ml vacutainer containing five mg of thymol and 50 mg of sodium fluoride.

**Plasma Insulin Levels.** An aliquot of the plasma used for each glucose determination was either immediately assayed for insulin or frozen and assayed later (within 24 to 48 hours). A radioimmunoassay (Phadebas Insulin Test) noted for its technical simplicity was utilized throughout the survey. In this test, specific antibodies to insulin are covalently coupled to a solid phase immunosorbent (cross-linked dextran), and the insulin in an unknown sample is allowed to compete with a fixed amount of I\(^{\text{125}}\)-insulin for the binding sites on the insulin antibodies. This competitive capacity is then compared with that of standard insulin preparations of known concentration. With this technique, the time-consuming steps required by the older double-antibody techniques is considerably shortened (minimum incubation time is three hours). Free and bound radiolabeled antigen are easily separated by conventional centrifugation. The results of this test were reported as micro-units of insulin per ml of plasma. The optimal conditions under which this assay should be performed have recently been described, as have its exceptional reproducibility, sensitivity and reliability.

**Data Collection and Processing.** Demographic and laboratory test results were recorded on forms suited to graphic analysis and conventional data-reduction techniques. Demographic data included body weight, height, age, sex and a series of questions relative to the history and treatment of diabetes.

**Graphic Analysis.** Individual blood glucose and plasma insulin levels were plotted on the same graph as a simple means of classifying patients as either nondiabetic
<table>
<thead>
<tr>
<th>Classification of Patients According to Comparative Secretory Responses</th>
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<tbody>
<tr>
<td><strong>Number of Patients in Group (females/males)</strong></td>
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<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Nondiabetic</td>
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<tr>
<td>Malabsorption type of curve</td>
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<tr>
<td>Malabsorption type of curve</td>
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<tr>
<td>Transient hypoglycemia with slightly elevated insulin</td>
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<td>Normal glucose tolerance with high insulin output</td>
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<td>Normal glucose tolerance with low insulin output</td>
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<td>Altered glucose tolerance with high insulin output</td>
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<td>Altered glucose tolerance with low insulin output</td>
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<tr>
<td>Adult onset diabetes with high insulin output</td>
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<td>Adult onset diabetes with low insulin output</td>
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<td>Moderately severe diabetes with high insulin output</td>
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* Standard error of mean.
† Nonobese = actual weight < 115 percent of adjusted ideal weight; obese = actual weight > 115 percent of adjusted ideal weight.
COMPUTER ANALYSIS OF INSULIN RESPONSE

A classification of diabetic was given to patients whose glucose was greater than 130 mg per 100 ml, or whose 60, 90, and 120 minute levels were over 185, 160, and 140 mg per ml. Patients were categorized as "borderline" when one or two of the OGTT values were abnormal. Glucose values were plotted linearly, i.e., as mg per 100 ml, whereas insulin values were plotted as a percent change from the basal level. This latter technique, which has been reported to exclude effects due to obesity, served to "amplify" the insulino-genic response to the glycemic stimulus and thereby facilitate the classification of patients into subgroups according to their comparative secretory responses (table I).

Computer Assisted Analysis. The programming and computer (IBM 360 65) facilities of a large university were employed to process the data that had been collected during the survey. Segments of the demographic data were utilized to calculate body surface area (DuBois formula) and ideal body weight. The ideal body weight obtained was then individually adjusted for age, height, and sex. A classification of nondiabetes or diabetes and the type of diabetes was made by one of the authors (usually a practicing clinician) on the basis of adjusted Fajans-Conn Criteria. All pertinent data was displayed for each individual within the group classifications listed in table I. Within each classification, individual data was broken down according to the sex of the patient and whether or not he was obese. Patients whose actual weight exceeded 115 percent of their adjusted ideal weight were considered obese. Biostatistical routines were employed to calculate means, standard deviation of the mean and standard error of the mean (SEM).

Results

On the combined evaluation of the blood glucose and plasma insulin levels they exhibited it was possible to classify the 1,408
participants in the survey into eight fairly distinct groups (table I). Five of the eight groups enumerated in this table are comprised of 574 (369 females and 205 males) "nondiabetics." The three remaining groups, which total 834 patients (470 females and 364 males) are comprised of borderline diabetics (243 patients), adult onset diabetics (433 patients) and moderately severe diabetics with low insulin output (158 patients). Comparisons of the mean glucose and insulin levels for males and females failed to show any significant differences, regardless of whether or not the individuals comprising the group were diabetic or nondiabetic. Since the comparisons confirmed the fact that glucose tolerance and insulin concentration during glucose tolerance tests are independent of sex, no further attempt will be made to distinguish possible differences due to this factor. Considerable attention will be directed toward discovering differences between obese and nonobese diabetics, since it has been shown that obesity and not carbohydrate intolerance is associated with elevated fasting insulin levels.  

**Nondiabetic Patients**

Although 574 of the participants in this survey were classified as being "nondiabetics," only 405 of them qualified as such on the basis of having glucose values throughout the entire OGTT within normal limits and having plasma insulin values that reflected a proportional and parallel response to the corresponding stimulus (figure 1a). On the whole, the mean glucose values for the 87 (21.5 percent) obese patients in this group were insignificantly higher than corresponding values for the nonobese patients (figure 1b). While the mean fasting
insulin levels in both groups are only slightly different, considerably higher mean levels occur at 60 and 90 minutes. These differences are statistically significant (p 0.05) whereas the differences at 120 and 180 minutes are not. It is doubtful that such transient differences have any clinical significance. More important, perhaps, is the fact that the shapes of the insulin curves in this figure are entirely parallel, and devoid of any delay in the insulinogenic response to the glycemic stimulus.

Of the remaining 169 patients classified as "nondiabetic," 96 of them presented glucose tolerance curves indicative of malabsorption syndrome. The absence of any significant glycemic stimulus in this group of patients is accurately reflected in the mean immunoreactive insulin test-values presented in figure 2a. The remainder of the "nondiabetic" groups are, by comparison, quite small in number. Except for displaying the average mean glucose and insulin curves for the two larger groups (figure 2b), no further attention will be accorded these patients. As for the data presented in figure 2b, suffice it to say that, despite normal glucose tolerance curves, it was possible to show that the input of insulin was fairly high in one group (34 patients) and fairly low in the other group (31 patients).

**Diabetic Patients**

The mean glucose and insulin values for the three groups of patients classified as diabetic are presented graphically in figures 3a, b and c. Upon comparing the three glucose tolerance curves in this figure, two distinct trends are even more dramatic when the glucose curves are compared with the one depicted in figure 1a.

The corresponding insulin curves reflect a somewhat similar trend on the one hand (figures 3a and b), and a completely different picture on the other (figure 3c). The shift upward and to the right in the insulin curves suggests a progressive worsening in the degree of diabetes exhibited by these patients. The insulin curve in figure 3a can be interpreted as lacking any major sign of
pancreatic decompensation. There is, however, some delay in the release of insulin to the glycemic stimulus. A much longer delay in the pancreatic response to circulating glucose is evident from the insulin curve presented in figure 3b. Of equal importance is the fact that the insulin values in this figure are far less proportional to the corresponding glucose values than are the ones in figure 3a. This lack of proportionality has been interpreted by others to signify a decreasing insulin response to glucose.\(^1\)\(^6\) The curves presented in figure 3c need little if any amplification. The mean fasting blood sugar is only slightly elevated, whereas the glycemia following the glucose load is fairly exaggerated throughout the remaining intervals of the GTT. The mean fasting insulin level is higher in this group of patients than in any other, and the virtual absence of any insulinogenic response to high circulating levels of glucose is striking.

**Obesity**

In contrast to the accepted belief that most diabetics (or at least most adult-onset diabetics\(^1\)) are obese, it should be noted that the overall incidence of obesity in the 1,408 patients included in this survey was only 27.3 percent. The incidence of obesity in 834 patients classified as having some form of diabetes was only slightly higher, i.e., 31.2 percent. The selection of patients may account for this discrepancy, as known or previously diagnosed diabetics tended to be excluded from this study.

The effect of obesity on the glucose tolerance and serum insulin values of obese and nonobese nondiabetic and diabetics is
summarized in table II and in figures 4a and b. By inspection of the mean curves presented in these figures, the fasting plasma insulin in the obese adult-onset diabetics is neither elevated nor significantly different from the fasting value in their nonobese counterparts. This finding is in agreement with that of others but disagrees with the findings of at least two other groups of workers. The hyperinsulinemia at all times subsequent to the fasting value is an expected finding in obese and nonobese adult onset diabetics.

**Comment**

The findings of the present survey have at least two interpretations. One is that they lend additional support to several concepts held by leading diabetologists. The other, and perhaps the more important of the two, is that they leave little doubt that the routine determination of plasma insulin levels with all OGTTs does indeed adduce information that has considerable value in the diagnosis and management of diabetes. The details surrounding these two interpretations are given.

The overall findings of the survey and, more specifically, the mean glucose and insulin levels depicted in figures 3a, b, and c, lend support to the fairly widely held concept that the natural history of diabetes involves a series of stages, starting with prediabetes or potential diabetes and continuing through latent diabetes to manifest diabetes. It should be emphasized, however, that the lines of demarcation between these stages are rarely clear-cut. Instead, there is a good deal of overlap between them which can make diagnosis a most difficult task. This is particularly true in the face of concomitant obesity. As can be seen from the distribution of the population in this survey in each of the three categories of diabetes (tables I and II), far fewer patients are in the stage of manifest diabetes than are in the stage of borderline diabetes or mild onset maturity diabetes. The basis for the transition from one stage to another is as yet, unclear.

The present findings also support the concept that diabetes mellitus is fundamentally a disease in which the underlying cause is a deficient secretion of insulin. In the initial stages of the disease, the deficiency is not necessarily absolute but, as has been suggested, a deficiency in the rate of insulin secretion. Sooner or later, however, insulin output is obtunded and the deficiency becomes absolute. Evidence for this from the present survey can be found by comparing the mean insulin curves presented in figures 3a, b and c. The first of these three curves reflects what has been called a biochemical inertia in that the release of insulin in response to hyperglycemia is excessive as well as delayed. In the second of the three figures, an even greater glycemic stimulus is required to overcome the inertia of the most severely decompensated beta cell. The prolonged postprandial hyperglycemia is, of course, the pathophysiologic condition of major concern. Finally, the last of the three curves reflects a reduced insulin secretory reserve, which is generally taken to represent the step in the natural history of diabetes that preceded unrelenting, around-the-clock hyperglycemia.

Since the regulation of glycemia resides primarily in the release of insulin by the pancreas, and since it is both the speed and degree of the release that are usually impaired in diabetics, the routine performance of plasma insulin levels in such patients should really need no recommendation. The present understanding of diabetes, while far from complete, has progressed to the point where the disorder can no longer be defined in terms of high blood glucose. Instead, it must be defined on the basis of whether or not the high blood glucose exists in the presence of an adequate insulin response and poor insulin
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<th>Glucose Tolerance (mg/100 ml)</th>
<th>Plasma Insulin μ Units/ml</th>
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<tr>
<td></td>
<td>Fasting</td>
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<tr>
<td>Nondiabetics (n = 405)</td>
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<tr>
<td>Nonobese (n = 318)</td>
<td>Mean</td>
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<td>122</td>
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<tr>
<td></td>
<td>±SEM</td>
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utilization, i.e., insulin resistance, as in the case of grossly obese patients, or whether or not it exists in the presence of a delayed insulin release (figure 3b), or in the absence of a significant release of insulin (figure 3c). Unfortunately, the cause of the development of insulin resistance in obese patients is still not known. Patients of this type, as well as those in whom the release of insulin is delayed, rarely require the administration of insulin. Instead, a reducing diet and, if necessary, the temporary administration of an oral hypoglycemic agent often result in satisfactory regulation. In the absence of any significant insulino-genic response to hyperglycemia, treatment with insulin may be the only effective form of treatment.

Summary

A prospective survey is described in which the objective was to determine whether or not the routine radioimmunoassay of plasma insulin levels during standard oral glucose tolerance tests (OGTTs) might adduce information that could be used to facilitate the diagnosis and/or management of all types and stages of diabetes.

Glucose determinations were made on plasma by the potassium ferricyanide (Auto-Analyser) method. A radioimmunoassay noted for its reproducibility and technical simplicity (Phadebas Insulin Test) was employed for measuring plasma insulin levels. Glucose was reported in mg per 100 ml and insulin in micro-units per ml. Data analysis was computer-assisted.

The findings of this survey lend support to the concept that the natural history of diabetes involves a series of stages, and that the lines of demarcation between them are not clear-cut, especially when clinical findings include obesity. Obesity was not, however, as frequent a complication in the diabetic patient-population studied as it is usually reported in the literature.

The findings relative to plasma insulin levels also lend support to the concept that, at least initially, the insulin deficiency of diabetes is one of rate rather than magnitude. As measured by the radioimmunoassay employed in this large series, insulin levels in the later stages of diabetes indicate not only a deficiency in the rate of secretion, but also a deficiency in the amount of hormone being secreted.

Overall, the information gained from the simultaneous and routine performance of a standard OGTT and immunoreactive plasma insulin test in this survey of 1,408 patients aided in the diagnosis of various types of diabetes. Having knowledge of each patient's insulin response to the glycemic stimulus also provided an important basis for selecting an individualized treatment regimen.

Acknowledgment

The authors would like to thank Richard Mautner for his valuable assistance with the programming and data processing aspects of the survey.

References

7. Seltzer, H. S. and Harris, V. L.: Exhaustion of insulogenic reserve in maturity-onset diabetic patients during prolonged and contin-

THE CHIEF'S BASIC RULES

Rule 1. The Chief is right.
Rule 2. In the impossible hypothesis that a subordinate may be right, rule 1 becomes immediately operative.
Rule 3. The Chief does not sleep: he rests.
Rule 4. The Chief is never late: he is delayed elsewhere.
Rule 5. The Chief never leaves his work: his presence is required elsewhere.
Rule 6. The Chief never reads the paper in his office: he studies.
Rule 7. The Chief never takes liberties with his secretary: he educates her.
Rule 8. Whoever may enter the Chief's office with an idea of his own must leave the office with his Chief's ideas.
Rule 9. The Chief is always Chief even in bathing togs.

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