The Assessment of Insulin, Glucose and Lipids in Ischemic Thrombotic Cerebrovascular Disease

BY MENARD M. GERTLER, M.D., HILLAR E. LEETMA, M.D., RUSSELL J. KOUTROUBY, B.A., AND ELYSE D. JOHNSON, B.A.

Abstract:
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Sixty-one male patients with ischemic thrombotic cerebrovascular disease (ITCVD) and 61 age-matched controls were studied to determine the interrelationships between the primary risk factors of ITCVD. Impaired carbohydrate metabolism in ITCVD was reflected in the significantly greater number of abnormal oral glucose tolerance tests (GTT) and type IV lipoprotein abnormalities when compared to controls. Elevated uric acid and triglyceride levels also were observed in ITCVD. Glucose and immunoreactive insulin (IRI) response curves in ITCVD were elevated and exhibited delayed peaks despite the normal or abnormal classification of GTT, indicating that insulin is ineffective in restoring glucose to normal levels. In the ITCVD and control groups with abnormal GTT the free fatty acid (FFA) levels were elevated at fasting and lacked the characteristic rebound at two hours observed in subjects with normal GTT, supporting the theory of a glucose-FFA cycle and its role in carbohydrate disturbances.

Additional Key Words
- Immunoreactive insulin
- Risk factors
- Glucose tolerance test
- Stroke
- Lipoproteins
- Free fatty acids

Introduction

In recent years several studies have attempted to identify the factors contributing to risk of ischemic thrombotic cerebrovascular disease (ITCVD). However, there has been no unanimity of opinion as to the degree of contribution of each risk factor nor has any attempt been made up to this time to structure the risk factors of ITCVD into a biochemical causal relationship.

In 1972 a stroke profile was developed by this laboratory in order to identify individuals prone to ITCVD. This study was based on the analyses of 36 biochemical, metabolic and clinical variables including blood pressure, glucose tolerance, immunoreactive insulin (IRI), free fatty acids (FFA), blood lipids, lipoprotein electrophoretic patterns, uric acid, electrocardiographical abnormalities and excessive cigarette smoking. The four primary risk factors which were found to discriminate best between the prone and nonprone individual are: (1) the three-hour IRI level, (2) systolic blood pressure, (3) lipoprotein abnormality, and (4) the two-hour glucose level.

The application of these four risk factors in the stroke profile will enable physicians to screen their patient populations for covert ITCVD and to initiate appropriate primary preventive measures in order to forestall or prevent the acute thrombotic event.

Due to the strong association of impaired carbohydrate metabolism with ITCVD, it was felt that an in-depth analysis of the interrelationships between the risk factors was warranted. This communication presents the results of these analyses.

Methods

Sixty-one ITCVD men and 61 healthy age-matched male controls were selected from a retrospective study in which clinical and biochemical evaluations were made within three months after the acute event in the ITCVD group. The ITCVD patients were screened from more than 500 consecutive admissions to the Institute of Rehabilitation Medicine at the New York University Medical Center who were ambulatory and undergoing active rehabilitation at the Institute. All participants assigned to the study were free from associated diseases such as hyperthyroidism, hypothyroidism, gout, coronary heart disease or diabetes mellitus and were not on drugs which affected either carbohydrate metabolism (e.g., steroids, thiazides) or lipid metabolism (e.g., hypolipemic agents).

A complete clinical evaluation including a review of medical history was accomplished. The criteria for the diagnosis of ITCVD and the methods for the biochemical determinations are described elsewhere. Total cholesterol, lipid phosphorus, triglycerides, uric acid and lipoprotein...
electrophoretic patterns were determined on fasting serum. A three-hour oral glucose tolerance test (GTT) was administered to all subjects. Following the ingestion of the equivalent of 75 gm of glucose in the form of Glucola (Ames Co., Inc., Elkhart, Indiana), serum glucose, IRI and FFA were determined at the one-half, one, two and three-hour intervals.

For the analyses of the data the 61 ITCVD patients and 61 age-matched controls were subdivided by the normal or abnormal classification of GTT according to the criteria of Fajans and Conn. Thus, the following study groups form the basis for our further analyses: (a) a normal GTT group comprised of 18 ITCVD patients and 39 controls, and (b) an abnormal GTT group comprised of 43 ITCVD patients and 21 controls.

For the statistical analyses Student t-tests were employed to determine the significant differences in the means of the continuous variables between the groups, e.g., cholesterol, lipid phosphorus, uric acid, FFA and IRI. The mean response curves and statistically significant differences for glucose, IRI and FFA during the three-hour GTT are presented graphically. For the analysis of categorical variables, e.g., GTT classification, and lipoprotein patterns, the nonparametric Chi-square was applied.

Since the lipoprotein abnormality was one of the four significant risk factors in the stroke profile it was decided that the ITCVD and control subjects with normal and abnormal lipoproteins should be analyzed in relation to normal and abnormal GTT response. Type IV was the predominant lipoprotein abnormality found in both the ITCVD and control groups. Only two of the 36 patients who were typed for lipoproteins had type II abnormalities and, therefore, they were excluded from further analyses involving lipoproteins.

The number of controls with type II and type IV lipoprotein patterns is found in the ITCVD group when compared to the controls (table 1). There is also a significantly greater number of type IV lipoproteins in ITCVD patients (77%) than in the control subjects (23%) whether or not the GTT classification is normal or abnormal, with significance at \( P < 0.001 \).

### Normal Glucose Tolerance — ITCVD and Control Groups

As seen in table 2 the 18 ITCVD men and the 39 controls with normal GTT have virtually the same mean age (60 years). The ITCVD group has a significantly higher mean systolic blood pressure (\( P < 0.05 \)) and mean triglyceride level (\( P < 0.05 \)) than the controls, although both are within normal limits. No significant differences are found in height, weight, diastolic blood pressure, cholesterol, lipid phosphorus or uric acid levels between the two groups, although the difference in uric acid levels approaches statistical significance (\( P < 0.09 \)).

The mean GTT and IRI response curves of the ITCVD and control groups are compared in figure 1. The half-hour glucose level is significantly higher in the ITCVD group than in the controls (\( P < 0.05 \), \( P < 0.001 \), respectively). It is noteworthy that the glucose curve of the ITCVD group peaks at one hour while the curve of the control group peaks at one-half hour, although both groups are classified as normal GTT. At the three-hour interval the glucose level of more than 90% of the control subjects had returned to fasting level or below as compared to only 50% of the ITCVD patients, with statistical significance at \( P < 0.001 \) (fig. 2). The mean IRI response curve for the ITCVD group has greater values than the controls at all time intervals during the GTT with statistical significance at fasting (\( P < 0.05 \), two hours

### Results

A significantly greater prevalence of both abnormal GTT and type IV lipoprotein patterns is found in the ITCVD group when compared to the controls (table 1).

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**TABLE 1**

<table>
<thead>
<tr>
<th>Variables</th>
<th>ITCVD</th>
<th>Controls</th>
<th>( P &lt; )</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTT response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>18 (30%)</td>
<td>39 (66%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Abnormal</td>
<td>43 (70%)</td>
<td>21 (34%)</td>
<td></td>
</tr>
<tr>
<td>Lipoproteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>12 (33%)</td>
<td>24 (67%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Abnormal</td>
<td>24 (77%)</td>
<td>7 (23%)</td>
<td></td>
</tr>
<tr>
<td>Normal GTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal lipoproteins</td>
<td>5 (39%)</td>
<td>18 (86%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Abnormal lipoproteins</td>
<td>8 (61%)</td>
<td>3 (14%)</td>
<td></td>
</tr>
<tr>
<td>Abnormal GTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal lipoproteins</td>
<td>7 (31%)</td>
<td>6 (60%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Abnormal lipoproteins</td>
<td>16 (69%)</td>
<td>4 (40%)</td>
<td></td>
</tr>
</tbody>
</table>

*Type IV.
**TABLE 2**

Summary of Variables in ITCVD Men and Age-Matched Controls With Normal GTT

<table>
<thead>
<tr>
<th>Variables</th>
<th>ITCVD (N = 18), mean ± SD</th>
<th>Controls (N = 19), mean ± SD</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60.4 ± 10.79</td>
<td>61.0 ± 9.3</td>
<td>NS</td>
</tr>
<tr>
<td>Height</td>
<td>67.5 ± 3.03</td>
<td>68.5 ± 2.63</td>
<td>NS</td>
</tr>
<tr>
<td>Weight</td>
<td>160.6 ± 24.3</td>
<td>170.4 ± 21.3</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>147.7 ± 25.4</td>
<td>132.3 ± 16.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>85.8 ± 15.3</td>
<td>82.7 ± 8.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>224.0 ± 46.9</td>
<td>225.0 ± 41.3</td>
<td>NS</td>
</tr>
<tr>
<td>Lipid phosphorus</td>
<td>9.8 ± 1.56</td>
<td>9.5 ± 1.48</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid</td>
<td>5.9 ± 1.25</td>
<td>5.3 ± 1.14</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>137.0 ± 37.3</td>
<td>111.0 ± 51.1</td>
<td>0.05</td>
</tr>
</tbody>
</table>

(P < 0.001) and three hours (P < 0.001). In addition, the IRI curve of the ITCVD group has a delayed peak at two hours.

The mean FFA response curves of the ITCVD and control groups with normal GTT in figure 3 are almost identical, reaching their nadir at two hours and rebound at three hours.

**ABNORMAL GLUCOSE TOLERANCE — ITCVD AND CONTROL GROUPS**

As shown in table 3 the mean ages are virtually identical (65 years) for the 43 ITCVD men and the 21 control subjects with abnormal GTT. In the ITCVD group the systolic and diastolic blood pressure and triglyceride and uric acid levels are significantly higher when compared to the controls, although they do not attain abnormal levels. No significant differences are seen in height, weight, cholesterol or lipid phosphorus levels. This confirms our earlier findings that cholesterol is normal in ITCVD and is uninfluenced by normal or abnormal GTT classification.

It is observed that the triglyceride and uric acid levels are higher in ITCVD subjects with abnormal GTT when compared to both the ITCVD and control groups with normal GTT.

The mean glucose and IRI response curves in figure 4 indicate that the two-hour and three-hour glucose levels and the three-hour IRI level are significantly higher in the ITCVD group than in the controls. It is demonstrated in figure 2 that in the abnormal GTT group, the glucose level of only 26% of the ITCVD patients had returned to fasting level or below at three hours, as compared to 80% of the controls. This is statistically significant at P < 0.001. It should be recalled that 50% of the ITCVD group with normal GTT had three-hour glucose values above their fasting level as well, indicating that despite the normal or abnormal GTT classification, the mean glucose curves of the ITCVD patients exhibit a significant lag at three hours. Therefore, it may be concluded that the abnormal GTT classification alone is not sufficient to determine proneness to ITCVD, but it is the overall change within the glucose response.
TABLE 3
Summary of Variables in ITCVD Men and Age-Matched Controls With Abnormal GTT

<table>
<thead>
<tr>
<th>Variables</th>
<th>ITCVD (N = 43), mean ± SD</th>
<th>Controls (N = 21), mean ± SD</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>65.2 ± 8.06</td>
<td>65.9 ± 7.10</td>
<td>NS</td>
</tr>
<tr>
<td>Height</td>
<td>67.6 ± 2.41</td>
<td>67.8 ± 2.00</td>
<td>NS</td>
</tr>
<tr>
<td>Weight</td>
<td>157.8 ± 18.05</td>
<td>156.8 ± 19.84</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>146.8 ± 22.48</td>
<td>134.3 ± 16.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>83.6 ± 13.01</td>
<td>76.6 ± 9.25</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>230.0 ± 36.8</td>
<td>233.0 ± 41.7</td>
<td>NS</td>
</tr>
<tr>
<td>Lipid phosphorus</td>
<td>10.1 ± 1.54</td>
<td>10.2 ± 1.23</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid</td>
<td>6.15 ± 1.04</td>
<td>5.45 ± 1.13</td>
<td>0.05</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>154.0 ± 42.0</td>
<td>113.0 ± 43.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

curve, although it may be within normal limits, which should be evaluated. Thus, the recognition of a delayed peak in conjunction with the other risk factors in the stroke profile will identify more accurately the ITCVD-prone individual.

The mean FFA curves of the ITCVD and control groups with abnormal GTT are similar in figure 5 in that neither curve shows a rebound phenomenon, but the FFA curve in the ITCVD group shows a lower response at all time intervals, with significant differences at two and three hours (P < 0.05).

INTERRELATIONSHIPS OF VARIABLES IN ITCVD
The strong association of abnormal lipoproteins in ITCVD patients and the interrelationship with glucose, IRI and FFA response6 prompted an in-depth analysis of these particular variables. The flow chart below depicts the manner in which these interrelationships were analyzed.

ITCV

ABNORMAL OR NORMAL GTT

NORMAL LIPOPROTEINS

ABNORMAL LIPOPROTEINS

GTT  IRI  FFA  FFA  IRI  GTT

Normal GTT and Lipoproteins in ITCVD
The mean glucose response curve in figure 6 for ITCVD patients with normal lipoproteins shows a significantly higher three-hour glucose level than in the abnormal lipoprotein group (P < 0.05). The mean IRI response curve for patients with abnormal lipoproteins exhibits a significantly higher fasting IRI level than the patients with normal lipoproteins (P < 0.05), but at the third hour the values for both groups are similar. However, the IRI curves for both the normal and abnormal lipoprotein groups show the characteristic delay at two and three hours. The mean FFA response curves for both lipoprotein groups in figure 7 are almost identical at fasting. The FFA curve of the abnormal lipoprotein group shows a rebound at one hour whereas the curve of the normal lipoprotein group rebounds at two hours. The FFA levels in the abnormal lipoprotein group are higher at all time intervals with significant differences at two hours (P < 0.05) and three hours (P < 0.01).

Abnormal GTT and Lipoproteins in ITCVD
The mean glucose response curves for the ITCVD patients with abnormal and normal lipoproteins in figure 8 are identical at fasting, one-half hour, and one hour, but in the patients with normal lipoproteins the two-hour glucose level is significantly higher (P < 0.05).

The fasting and three-hour IRI levels are similar

GLUCOSE AND IRI RESPONSE IN ITCVD MALES AND AGE-MATCHED CONTROLS WITH ABNORMAL GTT

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Summary of IRI Response

The comparison of IRI responses in the ITCVD and control groups by the GTT classification is illustrated in figure 10. The overall features of the curves show a progressive impairment of IRI response related to glucose intolerance and ITCVD, i.e., the controls with normal GTT exhibit the most normal IRI response and the ITCVD patients with abnormal GTT exhibit the most abnormal response. The abnormal IRI responses are reflected in the delayed two-hour peaks and in the failure of the three-hour levels to return to fasting values.

Glucose/IRI Ratio

When the glucose/IRI ratio is determined for both ITCVD and age-matched control groups during the three-hour GTT, it is observed that the control group has less insulin per unit of glucose than the ITCVD group at fasting and at three-hour intervals.

Canonical Correlations

Canonical analysis considers two sets of variates with a joint distribution and transformations on these sets create two linear functions which correlate maximally. Canonical correlations were obtained for the following three pairs of serial variates: GTT-IRI, GTT-FFA, IRI-FFA. A chi-square analysis was employed to determine the significance of any of the sets of canonical variates, the results of which are shown in table 4. A significant correlation was found between glucose and IRI and glucose and FFA, but no significant correlation was found between IRI and FFA.
Discussion

The results of this study reconfirm the existence of an impaired carbohydrate metabolism in ITCVD patients. The preponderance of abnormal GTT and elevated and delayed IRI response in ITCVD patients following an oral glucose load points to either an overproduction or a lack of utilization of insulin. The impairment of carbohydrate metabolism in ITCVD is further supported by the high incidence of type IV hyperlipoproteinemia associated with (a) hypertriglyceridemia by either excessive carbohydrate intake or faulty carbohydrate metabolism, (b) diabetes mellitus and (c) hyperuricemia. The interrelationships between glucose, insulin and FFA response in this study are similar to those observed by Hales and Randle in a study of diabetic patients and healthy individuals on a low carbohydrate diet. These investigators suggested that an increased release of FFA causes insulin to be ineffective, further suggesting that the release of FFA from triglycerides in adipose and muscle tissue is a result of faulty triglyceride metabolism which could lead to early diabetes mellitus. The data of our study support the theory of a glucose-insulin-FFA cycle proposed by Hales and Randle which in part is evidenced by the high percentage of abnormal GTT found in ITCVD patients. It should be emphasized that none of the ITCVD patients had a history of overt diabetes mellitus prior to the study.

A more severely impaired carbohydrate metabolism in ITCVD patients is demonstrated by the significantly higher glucose and insulin levels, despite the normal or abnormal classification of GTT, when compared to their respective healthy controls. The elevated glucose levels in the ITCVD groups reflect the ineffectiveness of insulin to restore a glucose balance despite the elevated IRI response.

It is significant that the glucose and IRI responses in the ITCVD group with normal GTT exhibit delayed peaks at one and two hours, respectively, when compared to the controls with normal GTT.

SUMMARY OF IRI RESPONSE IN ITCVD MALES AND AGE-MATCHED CONTROLS BY GTT CLASSIFICATION
TABLE 4

<table>
<thead>
<tr>
<th>Canonical Correlations in ITCVD Men and Age-Matched Controls</th>
<th>r</th>
<th>X²</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTT-IRI</td>
<td>0.696</td>
<td>152.4</td>
<td>25</td>
<td>0.005</td>
</tr>
<tr>
<td>GTT-FFA</td>
<td>0.667</td>
<td>56.9</td>
<td>25</td>
<td>0.005</td>
</tr>
<tr>
<td>IRI-FFA</td>
<td>0.501</td>
<td>35.0</td>
<td>25</td>
<td>NS</td>
</tr>
</tbody>
</table>

while the peaks of the glucose and IRI response curves of the ITCVD and control groups with abnormal GTT are similar at one and two hours, respectively. The delayed glucose and IRI peaks in the ITCVD group with normal GTT suggest, again, an impaired carbohydrate metabolism, although the impairment is not as severe as it is in the abnormal GTT groups.

It is generally accepted that the FFA response in healthy subjects with normal GTT has a characteristic rebound at two hours. In this study a similar rebound phenomenon beginning at the two-hour nadir was also found in the ITCVD patients with normal GTT. The main difference noted between the ITCVD and age-matched control groups with abnormal GTT is the significantly lower FFA response curve of the ITCVD group, although both response curves fail to demonstrate a rebound at two hours. In addition, the fasting FFA levels were more elevated in the ITCVD and control groups with abnormal GTT than in the groups with normal GTT. The elevated fasting levels in the groups with abnormal GTT could be attributed to the failure of insulin to suppress effectively FFA release, possibly via the mechanism of dibutyryl cyclic AMP.11

It is observed that in the ITCVD group with normal GTT and abnormal lipoproteins the glucose, IRI and FFA levels are higher than in the ITCVD group with normal lipoproteins. It is interesting to note that the FFA response shows no rebound in the ITCVD group with abnormal GTT despite the normal or abnormal lipoproteins, while the ITCVD group with normal GTT and either lipoprotein classification shows a rebound phenomenon characteristic of normal GTT.

Free fatty acids are: (1) derived from exogenous and intermediate metabolic sources, (2) found in the serum as triglycerides and partially stored in tissue, and (3) attached to other substances, e.g., cholesterol and phospholipids. FFA may be increased by hydrolysis of triglycerides in either the serum, liver or adipose tissue by the action of lipoprotein lipase which is influenced by insulin and cyclic AMP.12 The release of FFA, in turn, may account for the insulin ineffectiveness resulting in the elevated glucose levels in the ITCVD and control groups with abnormal GTT.

The evidence points to a special relationship among the variables uric acid, triglycerides and diabetes mellitus in ITCVD. Uric acid resembles alloxan in its chemical structure. It has been established that alloxan may be involved in diabetogenesis through the destruction of pancreatic beta cells. This hypothesis is reinforced by an association of high incidence of abnormal GTT and diabetes mellitus in patients with gout, reported in the literature.13

Abnormal GTT in ITCVD and age-matched controls results in a depression of FFA response without a rebound, indicating a great similarity between these two groups. Thus, the age-matched controls with abnormal GTT may be incubating ITCVD. This finding reinforces the importance of impaired carbohydrate metabolism as a risk factor of ITCVD. In order to detect these biochemical abnormalities in putatively healthy individuals, a three-hour oral GTT should be administered concomitant with the determination of IRI, FFA, lipid profile and lipoprotein typing. This should become a routine office and screening procedure in the detection of high-risk individuals to ITCVD. The detection of these abnormalities or risk factors in nonsymptomatic middle-aged and elderly subjects is of paramount importance so that the acute thrombotic event can be delayed or prevented through the application of primary preventive measures which include diet, weight reduction, and hypoglycemic, hypolipidemic, and uricosuric agents. These data also indicate a need for controlled randomized prospective studies which would determine the efficacy of primary prevention on morbidity and mortality from ITCVD through the intervention of the multiple risk factors.

References

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