Variations in Blood ATP after Oral Administration of Glucose, in Individuals Diagnosed as Normal, Equivocal, or Diabetic According to the Glucose Tolerance Sum Principle

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Blood adenosine triphosphate was determined for 35 individuals at various times after they had ingested glucose. They were divided into four groups, categorized according to the "glucose tolerance sum" principle as normal, equivocal, or abnormal. Patients showing glucose intolerance also had supranormal concentrations of ATP as compared to the fasting level. Data are presented which suggest that the increase in ATP is a regulatory mechanism for insulin secretion.

Additional Keyphrases diabetes • possible mechanism of effect on ATP

The most characteristic metabolic disturbance in diabetes mellitus is in carbohydrate metabolism (1). In the metabolism of carbohydrates, the importance of adenosine triphosphate (ATP) in oxidative phosphorylation and as a source of high-energy phosphate has been well established (2). Both the glycolytic and tricarboxylic acid cycles are regulated by the ATP:ADP (AMP) ratios. The first enzyme of the glycolytic pathway, phosphofructokinase, is inhibited by ATP, the end-product of energy metabolism (3).

Data are available indicating that ATP participates in the events leading to the release of insulin from pancreatic cells. Experiments performed with rabbit pancreas in vitro indicate that insulin secretion increases when ATP is added to the cells (4). Hellman et al. (5), using obese hyperglycemic mice, found considerably more ATP in the islets than in the rest of the pancreas after stimulation with glucose, and suggest that this arrangement might ensure a ready source of energy for insulin secretion.

The importance of ATP in insulin secretion in relation to 3',5'-cyclic AMP should also be noted. ATP, under the influence of adenylcyclase, is converted to cyclic AMP, which has been shown to stimulate insulin secretion (6). Studies of cyclic AMP indicate that it exerts two insulin-like effects: increased glucose uptake and decreased cellular space (7).

Because ATP is so important in various aspects of carbohydrate metabolism, we studied the variations in blood ATP concentrations in normal and diabetic subjects after an oral glucose load. Previous studies do not agree. Blood ATP was determined in normal and diabetic patients after injection of adrenaline: It decreased more in the diabetics than in normal individuals (8). Another study (9) showed that ingestion of glucose produced an increase in blood ATP in both normal and diabetic patients, but no significant difference in ATP production could be demonstrated between them. In contrast, Szczecinska and Leyko (10) found no significant increase in erythrocyte content of ATP in normal individuals after glucose ingestion, but a significant increase in diabetics.

We measured blood ATP at various times after the ingestion of 100 g of glucose. The "glucose tolerance sum" principle of Danowski et al. (11, 12), which incorporates the four most widely used sets of criteria for classifying glucose tolerance curves, was used to evaluate the results of the oral glucose tolerances.

Materials and Methods

Reagents

Reaction mixture. pH 7.6, containing 0.1 mol of triethanolamine buffer, 4 mmol of magnesium sulfate, and 6 mmol of 3-phosphoglycerate per
liter. (Contents of bottle 1 dissolved in 50 ml of redistilled water.)

**Reduced DPNH solution.** 0.012 mol of DPNH per liter. (Contents of bottle 2 dissolved in 1 ml of redistilled water.)

**Indicator reaction mixture.** Four milligrams of glyceraldehyde-3-phosphate dehydrogenase and 1 mg of phosphoglycerate kinase per ml of solution. (Crystalline suspension contained in bottle 3 is used as such and inverted gently several times before use.)

**Perchloric acid.** Five milliliters of perchloric acid (700 g/liter) diluted to 100 ml with redistilled water.

### Preparation of Patient and Collection of Specimens

After patients fasted overnight, blood specimens (about 10 ml) were collected from 35 non-obese subjects by antecubital venipuncture before and 0.5, 1, 2, and 3 h after they had ingested 100 g of glucose in 200 ml of water. The subjects were inactive during this period.

### Analytical Procedures

**Blood ATP determination.** One milliliter of blood is immediately transferred to a 12-ml centrifuge tube containing 1.0 ml of cold perchloric acid. The tube is stoppered and mixed vigorously for about 15 s, placed in crushed ice, and taken to the laboratory. It is centrifuged for about 7 min, and the supernatant fluid is carefully removed to a clean, cold centrifuge tube for recentrifugation. ATP is determined on the clear filtrate by a modification of the enzymatic determination in which phosphoglycerate kinase is used. Two and a half milliliters of reaction mixture, 50 μl of reduced DPNH solution, and 0.2 ml of filtrate are added to a 1-cm square glass cuvet, mixed by inversion, and incubated in a water bath at 25°C for 15 min. An absorbance reading (A₁) is then taken. Indicator reaction mixture, 50 μl, is then added, mixed by several gentle inversions, and absorbance is measured (A₂) after exactly 5 min. The value for A₂ is subtracted from A₁ to obtain ΔA. ATP in mg/100 ml of blood is calculated from the absorbivity (3.03 × 10³) for DPNH at 25°C and 366 nm.

**Blood glucose determination.** The clotted blood specimens are centrifuged and serum glucose is determined by the ferricyanide method.

**Interpretation of glucose tolerance results.** Oral glucose tolerance tests were interpreted by use of the principle of "glucose tolerance sum" (11, 12) for 2 h (GTSₙ₋₃h): the sum of the blood glucose levels at 0, 0.5, 1, and 2 h. These values are then related to the criteria used by WHO, the British Diabetic Association, Fajans and Conn (15), and the USPHS as suggested by Danowski et al. (11). We separated the GTS into four groups: <500, 500 to 650, 650 to 800, and >800. For GTS values of 500 or less, few tolerances meet the criteria of any of the above four sets of standards for abnormality. For values of 800 or above, all of the criteria are met for defining the test as abnormal. Values between 500 and 800 are equivocal (12). From the data of Danowski et al. (12) we calculate that an average of about 67.1% of the tolerances with a GTS between 500 and 800 will be classified as abnormal when the results of all four standards are averaged; 47.8% will be between 500 and 650, and 86.5% between 650 and 800.

### Results

**Reproducibility of ATP procedure.** This was tested by running 36 duplicate analyses on consecutive days. The standard deviation was ±0.70. The precision (95% limits) in the range of 25.5–41.7 mg/100 ml of blood was 4.42%.

**Normal values for blood ATP.** Normal values for a nonpatient population were determined by using 15 volunteers (seven men and eight women, 19 to 46 years old). They fasted overnight, and blood was taken before and 1 h after they ingested 100 g of glucose. The means, in mg of ATP per 100 ml of blood, were as follows: fasting, 31.3 ± 3.47 (SD); at 1 h, 31.1 ± 4.21. The fasting glucoses of these individuals were within the normal range of 60–100 mg/100 ml of blood; all were less than 160 mg/100 ml at 1 h after glucose ingestion.

**Glucose tolerance tests.** A mixed population of 35 individuals was studied. They consisted of 13 hospital patients, four laboratory volunteers, and 18 out-patients.

Table 1 summarizes GTS ranges and mean glucose values for the four GTS groups.

**ATP results.** The mean ATP values, in mg/100 ml of blood, at 0, 0.5, 1, 2, and 3 h for the four GTS groups are shown in Figure 1.

Average maximum increases of ATP in mg/100 ml and changes for each GTS group were as follows: group <500 was 0.7 at 3 h (p > 0.20); group 500 to 650 was 3.2 at 0.5 h (p > 0.01) and at 1 h was p > 0.20; group 650 to 800 was 4.8 at 1 h (p > 0.10).

### Table 1. Results of Glucose Tolerance Tests

<table>
<thead>
<tr>
<th>GTS (mg/l)</th>
<th>No. of samples</th>
<th>GTS (range)</th>
<th>Mean glucose values, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fastings</td>
<td>0.5 h</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>&lt;500</td>
<td>14</td>
<td>334–457</td>
<td>75</td>
</tr>
<tr>
<td>500–650</td>
<td>9</td>
<td>507–645</td>
<td>92</td>
</tr>
<tr>
<td>650–800</td>
<td>5</td>
<td>686–732</td>
<td>105</td>
</tr>
<tr>
<td>&gt;800</td>
<td>7</td>
<td>812–1340</td>
<td>173</td>
</tr>
</tbody>
</table>

* Glucose tolerance sum.

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2 Parenthetical remarks refer to the complete reagent kit, available from C. F. Boehringer and Soehne GmbH, Mannheim, Germany.
and at 2 h was $p > 0.10$, at 3 h was $p > 0.20$; and group $> 800$ was 3.7 at 0.5 h ($p > 0.025$), and other changes were at 1 h ($p > 0.01$) and 3 h ($p > 0.1$).

**Discussion**

Our normal mean fasting value for blood ATP was 31.3 ± 3.47 (sd) mg/100 ml. Using the phosphoglycerate kinase procedure, Dennemann (16) found a mean of 25 mg of ATP per 100 ml of blood (range, 19 to 32). Our different values for the means may be explained by the difference in subjects chosen. We used only individuals with a normal fasting blood glucose and a glucose concentration of $< 160$ mg/100 ml 1 h after oral ingestion of 100 g of glucose. The diabetic group, GTS $> 800$, had a lower fasting ATP level than the normal group ($p < 0.05$) (Figure 1).

In an experiment (16) on two individuals to determine the effect of ingestion of 100 g of glucose on blood ATP levels, fasting ATP was 32 mg/100 ml with a corresponding glucose value of 96 mg/100 ml in one individual and 31 mg/100 ml ATP with a corresponding glucose value of 82 mg/100 ml in the other. An hour later the blood glucose concentrations were 89 and 94 mg/100 ml for the two, respectively, and blood ATP concentrations were the same as when fasting. The ATP values in these two normal individuals agree exactly with our mean value, but are just within the upper range reported by Dennemann (16). Since Dennemann (16) gives no values for blood glucose in his subjects other than the two mentioned above, his lower range may be explained by the possible presence of diabetics.

All GTS groups of our patients increased their blood ATP level after ingesting 100 g of glucose. Goto et al. (9) also found increased ATP concentrations, both in diabetics and nondiabetics. These authors estimated blood ATP as “pyrophosphate” obtained by 10-min hydrolysis in boiling 1N hydrochloric acid and measurement of the liberated inorganic phosphorus (8). They found no significant difference in mean maximal increase rates for the two groups (9). For comparison, the diabetic subjects of Goto et al. (9) most closely resemble our GTS group designated as $> 800$.

Although the exact mechanism involved in insulin secretion is still unknown, energy expenditure is obviously necessary. The insulin stimulatory effect normally produced by glucose is abolished in the presence of cyanide, 2,4-dinitrophenol, or anoxia, all of which interfere with oxidative processes (17). It is therefore possible that insulin secretion may be induced by ATP. Experiments providing evidence for the secretion of insulin by ATP have been performed (4). Glucose increases ATP in pancreatic islet cells (5). When islet cells were exposed to hypoglycemic sulfonylurea compounds, ATP decreased (8). Pancreatic $\beta$-cells have been found to contain a considerably higher amount of ATP than the other cells of the pancreas (5). The histochemical demonstration of both extramitochondrial and mitochondrial ATPase (ATP phosphohydrolase, EC 3.6.1.3) in the $\beta$-cells also indicates that ATP is a source of energy for insulin secretion (18). ATPase is found in other pancreatic cells, although it is always located in the mitochondria. Extramitochondrial ATPase of the $\beta$-cells is located in the insulin-containing granules (18).

Increase in ATP with time in two of the three GTS groups exhibiting glucose intolerance may be a compensatory mechanism for insulin secretion caused by the hyperglycemic state. There are reported studies that contradict the statement that hyperglycemia is usually caused primarily by decreased insulin production (19, 20). Buchanan and McKiddie (19) reported that moderately carbohydrate-intolerant patients, compared with normals, had higher blood insulin fasting at 1.5 and 2 h after oral ingestion of 50 g of glucose. They found no difference in insulin levels at 0.5 h. Insulin levels in the severely carbohydrate-intolerant patients, compared to normals, were the same fasting and at 1.5 h, less than normal at 0.5 and 1 h, but greater than normal at 2 h (19). Yalow and Berson (20) found the diabetic average fasting concentration of insulin to be slightly greater than in the normal individuals. After the diabetics ingested 100 g of glucose, their average insulin concentration was less than normal at 0.5 h and greater than normal at 1 and 2 h.

More knowledge may be gained by relating the insulin concentration for each type of diabetic to their respective glucose concentrations, rather than comparing insulin in all types of diabetics to normal insulin concentrations. Using the data of
Yalow and Berson (20), we averaged the glucose in mg/100 ml for the diabetic and nondiabetic groups at fasting, 0.5, 1, and 2 h, and calculated the glucose : insulin ratio (g/i) by using the average insulin values for the various time intervals. The g/i values for the nondiabetic population at fasting, 0.5, 1, and 2 h were 4.28, 0.92, 0.86, and 0.95, respectively. Diabetic g/i values were 4.07, 1.89, 1.47, and 0.87, respectively, for the fasting, 0.5, 1, and 2 h intervals. It can be seen that the fasting and 2 h values are about the same in diabetic and nondiabetic. However, we may speculate that the diabetics are secreting a decreased amount of insulin at 0.5 and 1 h, since none of the g/i values were lower in the diabetic group than in the non-diabetic group.

However, if the patients presented by Yalow and Berson (20) are divided into the four cts groups, and if average glucose values in mg/100 ml and g/i values are calculated, some interesting data can be obtained. The mean g/i values for the various cts groups at fasting, 0.5, 1, and 2 h were: group <500, 4.28, 0.92, 0.86, and 0.95; group 500 to 650, 3.23, 1.29, 1.16, and 0.68; group 650 to 800, 4.20, 1.88, 1.51, and 0.79; and group >800, 5.54, 4.60, 2.02, and 1.55. If g/i values are calculated with use of glucose results from our patients at various cts levels and insulin results taken from Yalow and Berson (20), the following g/i values for the fasting, 0.5, 1, and 2 h levels were obtained: group <500, 3.57, 0.91, 0.87, and 0.87; group 500 to 650, 3.06, 1.31, 1.14, and 0.54; group 650 to 800, 4.20, 1.87, 1.47, and 0.73; and group >800, 6.65, 5.15, 2.17, and 1.74, respectively. Both sets of data for g/i are in agreement although one would not expect group >800 to agree exactly because cts results at this level could be extremely variable. The fasting and 2 h g/i values for cts group 500 to 650 and 2-h value for cts group 650 to 800 are lower than the normal group, <500, suggesting an excess secretion of insulin at these times. All other g/i values for the cts groups were higher than group <500, suggesting a decreased or insufficient insulin secretion.

We are indebted to Margaret Brennan for her secretarial assistance and for her unending encouragement and cheerfulness.

References