Increased Activity of Some Enzymes in Serum in Cases of Severely Decompensated Diabetes, with and without Ketoacidosis

Francesco Belfiore, Elena Napoli, and Luigi Lo Vecchio

In each of 10 highly hyperglycemic decompensated diabetics with ketoacidosis, we found a markedly increased serum activity of two lysosomal hydrolases (N-acetyl-β-glucosaminidase and β-glucuronidase). This was also true to a lesser degree of five diabetics with less severe decomposition and without ketoacidosis. The activity of both enzymes and the degree of hyperglycemia were highly correlated. We think these enzymatic changes result from a process of activation and release of tissue lysosomal enzymes, probably occurring in connection with the increased catabolism present in decompensated diabetes. Nonlysosomal (cytoplasmic or mitochondrial) enzymes were less changed (aspartate and alanine aminotransferases) or normal (aldolase, lactate- and malate dehydrogenase, and creatine kinase). This indicates that tissue damage alone could not account for the increased activity of the two lysosomal hydrolases; it therefore seems primarily to be due to involvement of lysosomes.

Additional Keyphrases N-acetyl-β-glucosaminidase • β-glucuronidase • lysosomal enzymes • atherosclerosis • hepatic disease

In diabetes mellitus, a moderate elevation of serum N-acetyl-β-glucosaminidase (1) and β-glucuronidase (1–4) activities has been reported. This finding has been given conflicting interpretations. Some workers (1, 2) have regarded the enzyme change as being linked to the diabetic condition. Others (3, 4), on the ground of some observations (5, 6) showing a correlation between the activity of these enzymes and atherosclerotic disease, have postulated that the increased enzyme activity is linked to the increased susceptibility of diabetics to atherosclerosis. Because serum β-glucuronidase increases in hepatic diseases (7–9), its increased activity in diabetes has also been ascribed to underlying liver involvement (10).

We thought that the hypothesis of a relationship between the activities of these two enzymes in serum and diabetes mellitus could be verified by studying the enzyme activity in severely decompensated diabetes; a markedly elevated activity should be found if such a correlation actually exists.

Materials and Methods

The present study was carried out on 15 hospitalized patients with decompensated diabetes. Ten of these patients had marked hyperglycemia [average value of blood glucose, 640 ± 209 (sd) mg/100 ml] and various degrees of ketoacidosis. The other five patients had less severe compensation, with an average blood glucose concentration of 340 ± 54 (sd) mg/100 ml, and no ketoacidosis.

After compensation, clinical and laboratory examinations showed that these patients were free from diseases other than diabetes. Besides the more usual investigations—such as the determination of glycemia, glycosuria, azotemia, ketone bodies, and plasma electrolytes—several serum enzymatic activities were measured. In ketoacidotic patients, enzyme activities were first measured at about the 12th hour from the onset of mental clouding, and then repeated at about the 36th, 60th, 84th, 108th, and 132nd hours as shown in Figure 1. Enzyme studies were also done on a group of normal subjects, as indicated in Table 1.

N-acetyl-β-glucosaminidase was determined according to Findlay et al. (11), β-glucuronidase according to Fishman (12, 13), aldolase (ALT) according to Bruns and Bergmeyer (14), lactate dehydrogenase (LD) according to Wroblewski and La Due (15), malate dehydrogenase (MD) according to Bergmeyer and Bernt (16), aspartate aminotransferase (AST) according to Karmen (17), alanine aminotransferase (ALT) according to Wroblewski and La Due (18), and creatine kinase (CK) according to Tanzer and Gilvarg (19). The activity

From the Istituto di Patologia Medica e Metodologica Clinica, Università di Catania, 95123, Catania, Italy.

1 Trivial names used: N-acetyl-β-glucosaminidase (chitobiase; chitobiose acetamidoxyglocohydrolase, EC 3.2.1.29); β-glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31); aldolase (keto-1-phosphate aldolase; ketose-1-phosphate aldehydolysase, EC 4.1.2.7); lactate dehydrogenase (l-lactate:NAD oxidoreductase, EC 1.1.1.27); malate dehydrogenase (l-malate: NAD oxidoreductase, EC 1.1.1.37); aspartate aminotransferase (l-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1); alanine aminotransferase (l-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2); and creatine kinase (ATP: creatine phosphotransferase, EC 2.7.3.2).

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of all the investigated enzymes was expressed in International Units (U) per liter of serum. One U is defined as the activity that converts 1 μmol of substrate per minute under standard conditions (20).

Glucose was determined by the glucose oxidase method, with an AutoAnalyzer, electrolytes by flame spectrophotometry, and azotemia, ketone bodies, and glycosuria by routine methods. The results were statistically analyzed according to Cavalli-Sforza (21).

Results

Results for the ketoacidotic patients are summarized in Table 1 and in Figures 1 and 2. At about the 12th hour from the onset of mental clouding, of the enzymes studied, N-acetyl-β-glucosaminidase and β-glucuronidase activities showed statistically very significant \( P < 0.001 \) increase, with a variation of 140 and 283%, respectively. Aspartate- and alanine aminotransferase were increased by 54 and 44%, respectively, while the other enzymes changed only insignificantly (Table 1). N-acetyl-β-glucosaminidase and β-glucuronidase values did not overlap the normal ones, all values being above the upper normal limit, while for the aminotransferases, although the mean was appreciably increased, only two values were above the upper normal limit. As regards the other enzymes, values were in all instances within the normal range (Table 1).

As indicated above, both of the aminotransferases were determined by spectrophotometric methods. Therefore, results were not affected by serum acetoacetate, a compound that can interfere with transaminase determination when measurement is made by multi-channel chemical analysis (22) and that might be responsible for the apparently enhanced transaminase activity reported (23) for diabetic ketoacidosis.

After the first 24 h the patients were no longer

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**Table 1. Serum Enzyme Activities in Normal Subjects and in Ten Decompensated Diabetics with Ketoacidosis**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Normal subjects</th>
<th>Ketoacidotic patients*</th>
<th>No. increased values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD, U/liter</td>
<td>No. cases</td>
<td>Mean ± SD, Variation, %</td>
</tr>
<tr>
<td>N-acetyl-β-glucosaminidase</td>
<td>6.40 ± 1.61</td>
<td>73</td>
<td>15.25 ± 2.71</td>
</tr>
<tr>
<td>β-glucuronidase</td>
<td>341 ± 139</td>
<td>75</td>
<td>1311 ± 544</td>
</tr>
<tr>
<td>AST</td>
<td>8.42 ± 2.88</td>
<td>128</td>
<td>13.00 ± 7.20</td>
</tr>
<tr>
<td>ALT</td>
<td>6.23 ± 2.69</td>
<td>128</td>
<td>9.00 ± 5.00</td>
</tr>
<tr>
<td>CK</td>
<td>0.45 ± 0.42</td>
<td>44</td>
<td>0.75 ± 0.50</td>
</tr>
<tr>
<td>ALS</td>
<td>2.00 ± 1.05</td>
<td>57</td>
<td>1.00 ± 0.50</td>
</tr>
<tr>
<td>LD</td>
<td>100 ± 41</td>
<td>87</td>
<td>94 ± 42</td>
</tr>
<tr>
<td>MD</td>
<td>67 ± 20</td>
<td>54</td>
<td>70 ± 20</td>
</tr>
</tbody>
</table>

* At about the 12th hour from the onset of mental clouding.
ketoacidotic nor hyperglycemic, since, within some hours of admission, metabolic compensation was achieved by appropriate treatment. In fact, mean blood glucose values in mg per 100 ml (± sd) were 640 (± 209) at about the 12th hour from the onset of mental clouding, 168 (± 96) at the 36th hour, 159 (± 74) ml at the 60th hour, 149 (± 34) at the 84th hour, 120 (± 14) at the 108th hour and 129 (± 22) at the 132nd hour. However, serum enzyme activities were followed for several days, and we saw a rapid decrease in the activity of N-acetyl-β-glucosaminidase and β-glucuronidase (Figure 1), while the other enzymes showed moderate irregular variations.

Glycemia was statistically significantly correlated with N-acetyl-β-glucosaminidase (r = 0.774, P < 0.01) and β-glucuronidase (r = 0.668, P < 0.01), but not with the other enzymes investigated (P ranging between 0.10 and 0.50). Correlation with azotemia, ketone bodies, or plasma electrolytes was not significant for any of the enzymes studied.

The transaminases were elevated in only two patients with marked tachycardia, severely subnormal blood pressure, and liver enlargement. In these two subjects N-acetyl-β-glucosaminidase and β-glucuronidase activities were not higher than in the other patients. Figure 2 refers to a patient who had a relapse at about the 60th hour from the onset of mental clouding, and shows the close correlation of both N-acetyl-β-glucosaminidase and β-glucuronidase with glycemia, as well as the different behavior of these two enzymes from that of aspartate aminotransferase.

In the five decompensated diabetics without ketoacidosis we found an average value of 12.16 ± 2.40 U for N-acetyl-β-glucosaminidase and of 818 ± 270 U for β-glucuronidase. These values, compared to normal, were increased by 80 and 140%, respectively. Therefore, these diabetics had more moderate hyperglycemia compared to the ketoacidotic patients, and comparatively less accentuated increase in enzyme activity. The other enzymes investigated were within the normal range in these five subjects. In five normal sera with an average value of 5.80 ± 1.18 U for N-acetyl-β-glucosaminidase and of 302 ± 118 U for β-glucuronidase, determinations of these enzymes were repeated after addition of glucose to bring the glucose concentration to about 600 mg/100 ml, and a mean of 6.00 ± 1.26 U and 298 ± 129 U, respectively, was found for the two enzymes. This shows that hyperglycemia itself does not affect the activity of these enzymes.

Discussion

The results described above raise the problem of interpreting an observed marked elevation of serum N-acetyl-β-glucosaminidase and β-glucuronidase, with less pronounced and inconstant changes of transaminases and only minor variations of other enzymes, in a complex condition such as decompensated diabetes.

Since in our patients the activity of both N-acetyl-β-glucosaminidase and β-glucuronidase was correlated with glycemia, and promptly returned towards normal as metabolic compensation was achieved, the increased activity was clearly linked to the acute condition of severe diabetic decompensation. Therefore, this enzymatic change cannot be ascribed, as it has been, to a possible underlying chronic liver involvement (10) or to the increased susceptibility of diabetes to atherosclerosis (3, 4).

It seems that even the possibility of acute involvement of some organ being the cause of serum elevation of these two enzymes can be excluded, because, if this were so, the most pronounced changes should be in other enzymes—such as, for instance, the transaminases in the case of involvement of liver, or creatine kinase in the case of involvement of muscle. In fact, with the possible exception of some forms of chronic liver diseases (7–9), available data (7–9, 24) indicate that N-acetyl β-glucosaminidase and β-glucuronidase do not show elevations more pronounced than those of transaminases and other enzymes in diseases of various organs.

Perhaps the presence of tissue damage, most probably damage to liver, might have played a relevant role only in the two cases in which the transaminases were significantly increased (Table 1). An explanation for the enzymatic pattern found in this research can be envisaged if the intracellular localization of the enzymes investigated is considered. From this point of view, it is apparent that the two enzymes that showed more pronounced increase are contained mainly in lysosomes (25, 26), while the other enzymes studied, which were either normal or moderately increased, are located either in cytoplasm or mitochondria (25, 26). Therefore, the hypothesis can be put forward that serum elevation of N-acetyl-β-glucosaminidase and β-glucuronidase in severely decompensated diabetes could be the result of an activation of lysosomal enzymes in tissues. This hypothesis is supported by the observations that in experimental activation of lysosomes, lysosomal enzymes are released from cells (27) and increase in serum (28, 29). The postulated activation of tissue lysosomal enzymes might be connected with the increased catabolism present in decompensated diabetes. In fact, it is known that lysosomal enzymes can degrade most types of organic compounds (30), and that lysosomal enzymes are activated in conditions characterized by increased tissue catabolism, such as fasting (31). Acidosis per
se does not seem to play a significant role, since both N-acetyl-β-glucosaminidase and β-glucuronidase were increased also in decompensated diabetics without ketocidosis.

Which tissue is the major source of N-acetyl-β-glucosaminidase and β-glucuronidase in severe diabetes cannot be established with certainty. In man, the highest activity of these enzymes has been found in the thymus, liver, spleen, and adrenal gland (32). Of these organs, the liver, both because of its size and its metabolic importance—which causes it to be greatly involved in diabetic decompensation—appears to be the most probable source of lysosomal enzymes in serum.

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References


