The transketolase assay of thiamine in some diseases

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ABSTRACT Erythrocyte transketolase activity (ETKA) and the effect of adding thiamine pyrophosphate have been measured in a group of 27 healthy individuals and in 37 patients diagnosed as having diabetes mellitus, anemia, polyneuritis, or malnourishment secondary to vascular disease of the brain. The observed values for the malnourished group did not differ significantly from those for the control group. The low ETKA values in diabetes mellitus seem to be due to a reduced apoenzyme level resulting from the disease itself rather than thiamine deficiency. Polyneuritis patients had low values of ETKA. In the anemic group as a whole the values showed a difference of only marginal significance from those found in the control group, but the patients with pernicious anemia all had a highly significant elevation of the ETKA values. Although the absolute thiamine pyrophosphate effect differ, there are no significant differences in percentage of thiamine pyrophosphate effect between the groups. It appears that differences in the patient groups studied here reflect variations in apoenzyme levels rather than thiamine status. Am. J. Clin. Nutr. 30: 1591-1596, 1977.

The transketolase assay is one of a number of biochemical methods used to assess thiamine activity in mammalian tissues. The method is based on the conversion of intracellular pentose to sedoheptulose and glyceraldehyde, a process where transketolase acts as an apoenzyme and thiamine functions as coenzyme. Brin and coworkers, assuming thiamine to be the limiting factor, demonstrated a test system (1-3) based on this reaction, using erythrocytes as representative cells. Various modifications of the original method have been used with different designations of enzyme activity (4-7). The assay has been applied in population surveys (6, 8-10) and balance studies (2, 6, 11).

Literature reports of the use of the test in estimating thiamine status in disease are mainly limited to studies of beriberi and other neuropathies (4, 5, 8, 12), apart from a few more general studies (7, 14). The present study was thus undertaken to investigate the potential of the method to provide insight into the condition of certain other groups of patients.

Materials

A control group of 27 healthy individuals was chosen from the hospital staff. A total of 37 patients, diagnosed as suffering from diabetes mellitus, anemia, polyneuritis, or malnourishment were investigated. Seven of the diabetics were of the early onset insulin-dependent type, whilst the other 10 patients had a maturity onset diabetes with low or no insulin requirement. Macrocytic anemia was present in four of the nine patients in the anemia group. All four were typical cases of pernicious anemia with low levels of vitamin B12 in the blood. In the remaining five patients sideropenia was present, the anemia being microcytic and hypochromic in two of them, normocytic and hypochromic in two, the last one being within normal ranges in both these respects. Blood folic acid values, determined in eight of these patients, were found within normal limits in all but one, a case of normocytic anemia with a slightly reduced level. The polyneuritic patients, of whom one was an alcoholic, displayed typical clinical manifestations, and thiamine lack could be suspected as a cause from the anamnestic data. In the last group of patients malnourishment was due to a protracted lack of food intake secondary to vascular disease of the brain.

Methods

Venous blood samples were collected in heparinized glass tubes. The transketolase activity was then measured by a slightly modified version of the procedure used, for the Dreyfus micromethod (4), by Schouten and coworkers (5). The modification consisted of freeze-
ing to −25 C and thawing blood samples three times before assay. This was done because of our finding that when 17 samples were estimated after a single freeze-thaw cycle and the results compared with those obtained for the same samples after two and three freeze-thaw cycles, the latter values showed average increases in enzyme activity of 46 and 55%, respectively. All samples were adjusted to a hematocrit of 35% before assay.

Transketolase catalyzes the conversion of pentose-5-phosphate to sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. Transketolase activity is expressed in international units (IU) equivalent to the number of micromoles of sedoheptulose-7-phosphate formed per minute per liter of blood with the hematocrit adjusted to 35%. Thiamine pyrophosphate (TPP) is a cofactor for the enzyme, transketolase, and the percent increase in enzyme activity caused by adding TPP in vitro is denoted the TPP-effect.

Ribose-5-phosphate, thiamine pyrophosphate, sedoheptulose-7-phosphate and L-cysteine-HCl were obtained from Sigma Chemical Co.

Results

The coefficient of deviation from the mean for duplicate determinations of erythrocyte transketolase activity (ETKA) in 25 pairs of samples was 4.8%.

The results of measurements of ETKA in the control group and in the different groups of patients are shown in Figure 1.

No correlation was found between age or sex and transketolase activity.

ETKA-values for the group of malnourished individuals did not differ significantly from those found for the control group. Although ETKA-values for the group of anemic patients as a whole were raised (Fig. 1), it appears that this is due almost entirely to much raised values for individuals with pernicious anemia (Table 1).

The erythrocytes from the diabetics display a distinctly reduced transketolase activity, an average of 33 IU as against 46 IU for the healthy individuals. This difference is significant with a P < 0.01, employing Wilcoxon's two-sample test. An even more pronounced difference is obtained when the average ETKA is calculated for the group whose diabetes mellitus was of the early-onset, insulin-requiring type (Table 1). Correspondingly, the average ETKA for the patients with maturity-onset diabetes and

Table 1

Results and comparison of measurements in controls, in patients with anemias and in patients with diabetes mellitus. Erythrocyte transketolase activity, mean values with one standard deviation, before and after TPP addition, with significance by Wilcoxon's two-sample test (two-tailed), for differences between groups.

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>ETKA</th>
<th>ETKA with TPP</th>
<th>ETKA with TPP - ETKA</th>
<th>Percentage of TPP effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>27</td>
<td>46.2 ± 9.5</td>
<td>59.5 ± 9.8</td>
<td>12.3 ± 4.8</td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>4</td>
<td>82.5 ± 29.6</td>
<td>101.8 ± 26.5</td>
<td>19.2 ± 15.3</td>
</tr>
<tr>
<td>P values for differences from control group</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Other anemics</td>
<td>5</td>
<td>52.5 ± 17.8</td>
<td>62.7 ± 21.9</td>
<td>10.2 ± 7.0</td>
</tr>
<tr>
<td>P values for differences from control group</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>&gt;0.1</td>
<td>=0.1</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Early onset diabetes, insulin dependent</td>
<td>6</td>
<td>26.5 ± 7.8</td>
<td>33.2 ± 7.8</td>
<td>7.1 ± 2.3</td>
</tr>
<tr>
<td>P values for differences from control group</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Maturity onset diabetes, low or no insulin requirement</td>
<td>10</td>
<td>37.3 ± 9.3</td>
<td>44.3 ± 8.7</td>
<td>7.0 ± 5.4</td>
</tr>
<tr>
<td>P values for differences from control group</td>
<td>=0.02</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Early onset diabetes</td>
<td>&lt;0.05</td>
<td>=0.02</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>
The absolute increase in ETKA is lower in the diabetics than in healthy individuals, $P < 0.01$ (Fig. 3). No significant difference in percentage of TPP effects exists between the various groups, as is shown by Figure 4.

**Discussion**

Differences in results found for various analyses in normal reference populations and patient groups abound, and may reflect, at least partly, methodological variations. Erythrocyte enzyme studies are known to be particularly susceptible to changes in the method used to carry out hemolysis (13).

![Image](https://academic.oup.com/ajcn/article-abstract/30/10/1591/4649932)
groups. Although our average for the percentage of the TPP effect (27 ± 11) is considerably higher than in their work (17 ± 12), the range of values is similar. On the other hand, the average ETKA measured in our control subjects (46 ± 9 IU) is somewhat lower than found by Chong and Ho for laboratory personnel (50 ± 16 IU) and by Schouten and coworkers for blood donors (62.5 ± 14 IU).

There is an almost exact correspondence of ETKA values in the control group and in malnourished individuals in our study. Pongpanich and coworkers (12) have reported the same observation: ETKA and percentage of the TPP effects are virtually identical in normal and malnourished individuals.

In starvation, a large part of the total

While Dreyfus (4) executed three rapid freeze-thaw cycles to ensure hemolysis, Schouten and coworkers (5) used a single overnight freezing. Other workers have included buffy coat removal (1, 7) and a variety of washing and resuspension steps in their sample preparation for the same analysis (1, 7, 14). Against this background, the results of the present study are in reasonable accord with those found by earlier workers (5, 8), bearing in mind the difference in hemolytic procedure discussed in the methods section.

Schouten and coworkers (5) examining 22 blood donors found an average percentage of the TPP effect ± SD of 22.5 ± 9. Chong and Ho (8) noted large individual variations in both ETKA values and percentage of the TPP effect in all examined
caloric expenditure of the body is provided by fatty acids and ketone bodies. Our observations for the malnourished group may thus reflect a low thiamine demand as a result of reduced glycolysis. When the anemic group is taken as a whole, raised ETKA values are found \((P < 0.1)\). However, if the group is split into 1) patients with pernicious anemia and 2) with other anemias, it is apparent that ETKA-values are significantly raised in pernicious anemias \((P < 0.01)\) but not in other anemias. Elevated ETKA-values \((14)\) and increased erythrocyte enzyme activities \((17)\) have previously been found in pernicious anemia. We associate these findings with the fact that pernicious anemics have a larger population of young blood cells than either healthy individuals or other anemics.

Our findings in the patients with diabetes mellitus are in good agreement with the observations of Haugen \((15)\). Using the thiochrome method, he found a significantly lower thiamine content in the blood of young insulin-dependent diabetics, but normal thiamine levels in elderly diabetics without an insulin requirement \((15)\). Kimura and coworkers \((16)\) have previously noted reduced activities of glycolytic enzymes in erythrocytes from diabetics, the same activities being elevated in insulinomas. The nutritional supply of thiamine in our diabetes patients was considered adequate. The absolute increase in enzymatic activity after TPP addition being significantly lower than normal, indicates that a deficit in thiamine is not responsible for the low transketolase activity in the diabetics. A lack of apoenzyme, as an expression of diabetes per se, seems a more probable reason. Whether an enzymatic system works at a reduced level due to lack of either apoenzyme or cofactor, the result is the same. This may explain why many of the manifestations of diabetes are similar to those of thiamine deficiency.

The ETKA values registered in the polyneuropathic patients after TPP addition are all less than in the control group. A reduction in the level of apoenzyme present may explain this finding, as a continued low supply of thiamine may reduce the level of transketolase apoenzyme \((6)\).

There are no significant differences in percentage of the TPP effect between the groups. Although the patients with pernicious anemia have an average ETKA three times as high as the group with early onset diabetes, the percentage of the TPP effects are nearly equal for the two groups. As the percentage of the TPP effect often is regarded as the most reliable indication of the thiamine status \((2, 4)\) there is no indication of any group being more in deficit of thiamine than the others. Arbitrarily choosing 25% TPP effect as a logical limit for deficiency, Chong and Ho found 21% of their apparently healthy subjects to be above this limit. Judging from this, 50% both of our control subjects and patients would benefit from increasing their intake of thiamine.

As the ETKA values registered are due to the coworking of the apoenzyme and TPP, a change in either of these will affect the ETKA level. In certain diseases, a change in the level of apoenzyme seems to be the cause of ETKA values differing from control values. In pernicious anemia the transketolase apoenzyme appears to be increased. In polyneuropathy and diabetes, particularly in early onset insulin-dependent diabetics, the level of apoenzyme seems to be decreased, and thus mainly responsible for the low ETKA values registered. Bearing in mind these differences related to the diseases, it is interesting to note the changes in ETKA values following TPP addition. It is obvious that a high percentage of the TPP effect indicates that an increased intake of thiamine would be advantageous. This holds for some of the subjects in all groups, but seems particularly important for those with early onset diabetes. The manifestations of diabetes, similar to thiamine deficiency, may be reduced by giving sufficient thiamine to ensure optimal utilization of the patients reduced transketolase apoenzyme.

References

3. Brin, M. Defects in pyruvate and pentose metabolism in relationship to transketolase activity in rats and man and to the paroxysmal response in thiamine-deficient rats. In: Thiamine Deficiency, ed-