AN EVALUATION OF A MODIFIED ERYTHROCYTE TRANSKETOLASE ASSAY FOR ASSESSING THIAMINE NUTRITIONAL ADEQUACY

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Summary Transketolase (TK) activity was assessed by incubating hemolyzed whole blood with ribose-5-phosphate both in the presence (TKsat.) and in the absence (TK) of thiamine pyrophosphate (TPP). The amount of pentoses utilized between 5 and 60 min after the start of the incubation was taken as a measure of the TK activity. The TPP effect was expressed as (TKsat. - TK)/TK x 100. The coefficient of variation for the TPP effect determination was 9.6% within the TPP effect range from 6 to 22%. Within the TPP effect range from 24 to 46%, the coefficient of variation was 8.7%.

The mean TPP effect as measured in a group of healthy individuals amounted to 13.5%, with a range from 8.3 to 18.5%. In a group of obese women submitted to an energy-restricted diet, the TPP effect ranged from 12.7 to 30%. In patients suffering from Wernicke’s encephalopathy, the TPP effect varied from 28 to 67%.

Keywords erythrocyte transketolase activity, thiamine pyrophosphate (TPP) effect, sample preparation, normal figures, obese subjects, Wernicke’s encephalopathy

Various methods have been developed to evaluate thiamine status in humans. Both direct methods i.e., measuring the blood thiamine level (1) and urinary thiamine excretion (2) and indirect methods such as the evaluation of the carbohydrate index (3) and the erythrocyte transketolase (TK) activity (4) have been used. Although the latter assay, i.e. measuring the erythrocyte TK activity, is not a totally satisfactory method, its usefulness is, however, accepted by various authors (5).

TK catalyzes together with its coenzyme, thiamine pyrophosphate (TPP), and

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with magnesium as cofactor, the following steps in the metabolism of pentoses:

\[
\begin{align*}
\text{Xylulose-5-phosphate} + \text{ribose-5-phosphate} & \rightarrow \\
& \text{sedoheptulose-7-phosphate} + \text{glyceraldehyde-3-phosphate} \\
\text{Xylulose-5-phosphate} + \text{erythrose-4-phosphate} & \rightarrow \\
& \text{fructose-6-phosphate} + \text{glyceraldehyde-3-phosphate}.
\end{align*}
\]

The erythrocyte TK activity can be assayed by incubating hemolyzed erythrocytes with ribose-5-phosphate followed by the determination of the amount of ribose-5-phosphate utilized (6) or the amount of sedoheptulose-7-phosphate (7, 8), of glyceraldehyde-3-phosphate (9) or of hexoses (9) formed. Both the decrease in the TK activity and the increase in the stimulation in vitro of the TK activity by TPP, the so-called TPP effect, are used as a measure of the thiamine status. The TPP effect, however, is generally considered to be the most reliable measure.

In the present study, the method of Brin (6) for measuring the erythrocyte TK activity based on the disappearance of pentoses has been modified by taking the amount of pentoses utilized between 5 and 60 min after the start of the incubation as a measure of the erythrocyte TK activity. The reproducibility was assessed and the sensitivity of the assay tested in a control group, in a group of obese subjects submitted to an energy-restricted diet and in a group of alcoholics showing signs of Wernicke's encephalopathy.

**MATERIALS AND METHODS**

**Blood samples.** Blood was drawn by venipuncture into plastic tubes containing lithium or sodium salt of heparin as anticoagulant. The erythrocytes were hemolyzed by freezing at \(-18^\circ C\) overnight. The sample was then stored at \(-18^\circ C\) and the TK activity was assayed within two weeks.

**Subjects.** The control group consisted of medical and laboratory personnel who were apparently in a healthy condition. Their dietary history showed the usual pattern and there was no excessive consumption of alcohol. Blood samples were also obtained from obese women submitted for two months to an energy-restricted diet containing 3.4 to 5.9 megajoules (800 to 1,400 kcal) and 0.7 to 1.2 mg thiamine per day. Further, blood was also drawn from subjects suffering from Wernicke's encephalopathy.

**Procedure.** Two hundred \(\mu l\) of hemolyzed blood were incubated at \(37^\circ C\) either with 100 \(\mu l\) phosphate buffer pH 7.4, containing 4.8 \(\text{mM NaCl}\), 124 \(\text{mM KCl}\), 1.2 \(\text{mM MgSO}_4\cdot7\text{H}_2\text{O}\), 9.1 \(\text{mM K}_2\text{HPO}_4\) and 5.8 \(\text{mM KH}_2\text{PO}_4\) per liter or with 100 \(\mu l\) phosphate buffer pH 7.4, containing in addition 0.9 \(\text{mM thiamine pyrophosphoric acid chloride (Boehringer Mannheim GmbH, Mannheim, W. Germany)}\) per liter (Table 1). After 20 min, 200 \(\mu l\) 18 \(\text{mM ribose-5-phosphate (\(\delta\)-ribose-5-phosphate, disodium salt, obtained from Sigma Chemical Company, St. Louis, Mo, U.S.A.)}\) were added and the incubation was continued. The incubation was stopped by
Table 1. Incubation procedure of the erythrocyte transketolase assay.

<table>
<thead>
<tr>
<th></th>
<th>With TPP* stimulation (ml)</th>
<th>Without TPP stimulation (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP phosphate buffer</td>
<td>0.1</td>
<td>—</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>—</td>
<td>0.1</td>
</tr>
<tr>
<td>Hemolysate,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>incubation at 37°C for 20 min</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Ribose-5-phosphate,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>incubation at 37°C for 60 min</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* TPP, thiamine pyrophosphate.

adding 3 ml 7.5% trichloroacetic acid (TCA), either after 5 min, i.e. the reference value, or after 60 min. Each determination was run in triplicate. The reference value was measured once.

The tubes were centrifuged for 5 min at 1,000 g and the pentoses in the TCA supernatant were determined as described hereafter. To 200 µl of supernatant were added 5.8 ml of orcinol reagent, containing 12.5 mM orcinol (5-methyl resorcinol monohydrate obtained from Sigma Chemical Company, St. Louis, Mo, U.S.A.), 0.37 mM ferric chloride per liter of 24% hydrochloric acid. Two hundred µl of a ribose (E. Merck, Darmstadt, W. Germany) standard (100 mg ribose per liter) were treated in the same way. The mixture was heated in boiling water for 20 min, cooled and the color measured at 670 nm by means of a Bausch & Lomb Spectronic 88 spectrophotometer (Bausch & Lomb, Analytical Systems Division, Rochester, NY, U.S.A.). The color was stable for at least 2 hr.

The hemoglobin (Hb) concentration was determined by the cyanomethemoglobin method as described by van Kampen and Zijlstra (10).

Calculations. The TPP effect can be expressed as:

\[
\frac{\Delta OD_{\text{sat.}} - \Delta OD}{\Delta OD} \times 100\%.
\]

\(\Delta OD_{\text{sat.}}\) = difference between the optical density (OD) measured after 5 min incubation and the optical density measured after 60 min, for the incubation with addition of TPP; \(\Delta OD\) = difference between the optical density (OD) measured after 5 min incubation and the optical density measured after 60 min, for the incubation without addition of TPP.

The transketolase activity, expressed in µg pentose metabolized per min per g hemoglobin, is given by:

\[
TK = \Delta OD \times \frac{1}{OD_{\text{stand.}}} \times 87.5 \times \frac{1}{55 \text{ min}} \times \frac{1,000}{\text{Hb}}.
\]

ΔOD = difference between the optical density (OD) measured after 5 min incubation i.e. the reference value on the one hand, and the OD measured after 60 min incubation on the other; OD_{stand.} = optical density per microgram of pentose standard; 87.5 = dilution factor; Hb = g hemoglobin per liter hemolysate.

Statistical methods. Means, standard deviations, coefficients of variation and paired t-tests were computed using standard programmes (11).

RESULTS

The determination of the TK activity and the TPP effect

During the first minutes after the addition of the substrate, i.e. ribose-5-phosphate, to the hemolysate, the measured amount of pentoses decreased very rapidly. After 4 to 5 min the disappearance curve of pentose became linear up to 60 min (Fig. 1). The difference in pentoses measured after 5 and after 60 min was taken as the measure of the TK activity. The drop in the measured amount of pentoses observed during the 5 first min of the incubation corresponded to a TK activity of an almost constant value of 110 μg pentose/min/g Hb.

Influence of storing and preparation of the blood sample (Table 2)

Storing the blood sample at 4°C for 24 hr before deep-freezing at −18°C had no effect on the TPP effect. In the same experiment, the plasma and the buffy coat were removed by suction after centrifugation at 1,000g for 10 min. The aspirated volume was replaced by an equal volume of distilled water and the erythrocytes were hemolyzed by overnight freezing at −18°C. After this treatment, somewhat lower values for the TPP effect were obtained than those observed for the whole blood samples, the difference, however, being not significant. Removing the plasma

![Fig. 1. Disappearance of ribose-5-phosphate during the incubation with hemolyzed whole blood, as measured with orcinol.](image-url)
Table 2. Influence of storage and preparation of the blood samples (n=12) on the TPP\(^b\) effect of the transketolase activity.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>TPP effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>I)</td>
<td>Whole blood immediately deep-frozen</td>
<td>15.7</td>
</tr>
<tr>
<td>II)</td>
<td>Whole blood kept for 24 hr at 4(^\circ)C and then deep-frozen</td>
<td>15.6</td>
</tr>
<tr>
<td>III)</td>
<td>Hemolysate obtained after centrifugation and replacement of the plasma and buffy coat with distilled water</td>
<td>14.4</td>
</tr>
<tr>
<td>IV)</td>
<td>Same procedure as III), but also washing the erythrocytes with 0.9% NaCl</td>
<td>12.3</td>
</tr>
</tbody>
</table>

\(^a\) n, number of samples investigated; \(^b\) TPP, thiamine pyrophosphate; \(^c\) S.D., standard deviation; \(^d\) n.s., not significant.

Table 3. The erythrocyte transketolase activity (TKA) and the TPP\(^a\) effect (%) in 21 subjects after an overnight fast and in non-fasting conditions (mean \(\pm\) S.D.\(^b\)).

<table>
<thead>
<tr>
<th></th>
<th>After overnight fast</th>
<th>Non-fast</th>
<th>Paired (t)-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKA</td>
<td>(126 \pm 14.7)</td>
<td>(129 \pm 15.7)</td>
<td>n.s.(^e)</td>
</tr>
<tr>
<td>TPP effect</td>
<td>(15.4 \pm 2.90)</td>
<td>(15.1 \pm 3.07)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

\(^a\) TPP, thiamine pyrophosphate; \(^b\) S.D., standard deviation; \(^c\) n.s., not significant.

and theuffy coat and washing the erythrocytes with 0.9\% NaCl resulted in a significantly \((p<0.01)\) lower mean value for the TPP effect.

**Influence of food intake**

The TK activity and the TPP effect was measured in a group of 21 subjects in non-fasting conditions and after an overnight fast (14 hr). No difference between the TK activity and the TPP effect was found (Table 3).

**Reproducibility**

The standard deviation (S.D.) of the assay was calculated by the formula \(\sqrt{(x_1-x_2)^2/2N}\) for duplicate determinations, which were executed within 2 weeks after the blood sampling. For 48 duplicate determinations in the TPP effect range from 6 to 22\%, the S.D. was 1.33\% with a mean TPP effect of 13.8\%, corresponding to a coefficient of variation of 9.6\%. For 22 duplicate de-

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Fig. 2. Frequency distribution of the TPP effects of the transketolase activity in a control group (A), in subjects submitted to an energy-restricted diet (B), and in patients suffering from Wernicke's encephalopathy (C).

Terminations in the TPP effect range from 24 to 46%, the S.D. was 2.81% with a mean TPP effect of 32.3%, which corresponds to a coefficient of variation of 8.7%.

TPP effect in healthy adults, in obese women submitted to an energy-restricted diet, and in adults suffering from Wernicke's encephalopathy (Fig. 2)

The TPP effect was determined in 54 apparently healthy adults. The mean value obtained was 13.5%, with a range from 8.3 to 18.5%. In the group of 23 obese women submitted to an energy-restricted diet, the mean TPP effect amounted to 22.2%, with a range from 12.7 to 30.0%. For 18 alcoholics suffering from Wernicke's encephalopathy, the average TPP effect was 44.4% with a range from 28 to 67%.

DISCUSSION

The method proposed by Brin(6) for assessing the erythrocyte TK activity takes the amount of ribose-5-phosphate utilized during the first 60 min of the incubation with hemolyzed blood as the measure of activity. However, during the first minutes of the incubation, ribose-5-phosphate is converted to xylulose-5-phosphate and ribulose-5-phosphate by two non-thiamine-dependent enzymes, ribose-5-phosphate isomerase and xylulose-5-phosphate epimerase, the final ratio between both pentoses being 9:1 and the amount of ribose-5-phosphate being equal to that of xylulose-5-phosphate (12). The optical density at 670 nm of the orcinol products, formed by xylulose-5-phosphate and ribulose-5-phosphate with

the orcinol reagent, is 65% of that found for an equal amount of ribose-5-phosphate (13). It would appear therefore that Brin’s method, which takes this initial conversion of ribose-5-phosphate into account, overestimates the TK activity. In the present study, the initial drop in optical density was not taken into account and the change in the measured amount of pentoses between 5 and 60 min after the start of the incubation was taken as a measure of the TK activity.

Various methods have been used to prepare the hemolysate. Some workers executed rapid freeze-thaw cycles or a single overnight freezing of total blood (7, 14). Others removed both plasma and the buffy coat (6, 15) and included washing and resuspension steps in the preparation of the hemolysate (9). From our results it appears that the preparation of the hemolysate may influence the obtained value of the TPP effect. Smeets and Muller (9) found in this regard that washing the erythrocytes resulted in lower values for the TK activity as compared to the whole blood sample. They showed that this decrease was related to the removal of the leucocytes.

No aberrant (i.e. falsely high) values were obtained in the group of normal subjects. The figures for the range of the TPP effect determined in the control group are in agreement with the normal values found by most authors. However, the values of the TPP effect, as measured in a control group, may vary according to the method used and to the nutritional status of the subjects investigated. Compared with the orcinol pentose method described by Brin (6), the upper limit in the present investigation was slightly higher.

It was noted that a number of obese subjects submitted to an energy-restricted diet showed an increased TPP effect. They had no specific clinical signs of thiamine deficiency. Whether these increased values of the TPP effect are to be considered as marginal deficiency figures i.e. biochemical deficiency without overt or clear clinical signs, remains open for discussion. As a matter of fact, it has recently been stressed again that the early stages of thiamine deficiency may be accompanied by nonspecific symptoms which can easily be overlooked or misinterpreted (16).

With regard to the patients suffering from Wernicke’s encephalopathy, the TPP effect figures were in general grossly abnormal, though a few subjects showed figures situated within the range of figures in the obese subjects, the latter, as mentioned before, showing no specific clinical signs of thiamine deficiency. To explain this phenomenon, several possibilities exist. First, the alcoholics may have consumed a somewhat more appropriate diet during the period immediately preceding the measurement of the TPP effect. Also, the possibility cannot be excluded that their symptoms may have been the result of a combination of several nutritional deficiencies, thereby accentuating the signs of thiamine deficiency. Another possibility are the host characteristics i.e. that some subjects are more prone to develop Wernicke’s encephalopathy. Further, the effect of alcohol as such and/or of other substances eventually present in alcoholic beverages has also to be considered.
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REFERENCES