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Nutrition

Vitamin B_1 Status Assessed by Direct Measurement of Thiamin Pyrophosphate in Erythrocytes or Whole Blood by HPLC: Comparison with Erythrocyte Transketolase Activation Assay

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Background: **The concentration of thiamin diphosphate (TDP) in erythrocytes is a useful index of thiamin status. We describe an HPLC method for TDP and its results in patients at risk of thiamin deficiency.**

Methods: **We used reversed-phase HPLC with postcolumn derivatization with alkaline potassium ferricyanide and fluorescence detection. Samples were deproteinized and injected directly onto a C_{18} column. TDP concentrations in erythrocytes were compared with those in whole blood. Reference intervals for erythrocyte TDP (n = 147; 79 males and 68 females; mean age, 54 years) and whole blood TDP (n = 124; 68 males and 56 females; mean age, 54 years) were determined in an apparently healthy population. We compared erythrocyte TDP with results of the erythrocyte transketolase activation test in 63 patients who were considered at risk of thiamin deficiency.**

Results: **The method was linear to at least 200 μg/L. The between-run CV was <8%. The lower limit of quantification for both whole blood and packed erythrocytes was 300 pg on column with a detection limit of 130 pg on column. Recovery of TDP from blood samples was >90%. TDP in erythrocytes correlated strongly with that in whole blood (r = 0.97). Reference intervals for erythrocyte and whole blood TDP were 280–590 ng/g hemoglobin and 275–675 ng/g hemoglobin, respectively. Of the 63 patients suspected of thiamin deficiency, 46 were normal by both TDP and activation tests, 13 were deficient by both tests, 1 was deficient by the activation test but had normal erythrocyte TDP concentrations, and 4 were normal by the activation test but had low TDP.**

Conclusions: **The HPLC method is precise and yields results similar to the erythrocyte activation assay.**

The roles of thiamin and its phosphate esters as a coenzyme in carbohydrate metabolism and in the process of nerve conduction are now well established *(1, 2)*. The free form of thiamin occurs mainly in plasma, whereas the coenzyme thiamin diphosphate (TDP)[1] predominates intracellularly. The concentration of total thiamin (free thiamin plus its phosphate esters in whole blood) is 60–120 μg/L, with 90% of the vitamin in erythrocytes and leukocytes. The erythrocytes contain ~80% of the total thiamin in whole blood *(3)*, predominately in the form of diphosphate *(4)*.

The most widely used method to detect thiamin deficiency is the indirect measurement of TDP in erythrocytes with either the transketolase activation test or the transketolase activity assay *(5, 6)*, but these tests are functional rather than a direct measurement of thiamin status and therefore may be influenced by factors other than thiamin deficiency. These include loss of reactivatable apoenzyme during chronic deficiency in vivo *(7, 8)*, altered binding of apoenzyme and coenzyme because of the presence of isoenzymes of transketolase *(9)*, and reduced synthesis of apoenzyme in patients with diabetes *(10)* and liver disease *(11)*. Other factors that have reduced the usefulness of the transketolase assays have been the relatively poor interassay precision of the assay, difficulty with standard-

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[1] Nonstandard abbreviations: TDP, thiamin diphosphate; TMP, thiamin monophosphate; TCA, trichloroacetic acid; QC, quality control; and Hb, hemoglobin.

ization, and sample storage instability because of rapid inactivation of the transketolase enzyme *(7)*. These factors contribute to the lack of agreement over the upper limit of the reference range for the activation assay, with values ranging from 15.5% to 40% *(12, 13)*.

The TDP concentration in erythrocytes has been shown to be a good indicator of body stores because it depletes at a rate similar to those of other major organs *(14)*. Studies by Warnock et al. *(15)* and Baines and Davies *(16)* have suggested that erythrocyte TDP concentrations determined by the apoenzyme recombination technique or by HPLC are a more sensitive index of thiamin status than the measurement of erythrocyte transketolase activity. The use of HPLC for the direct determination of TDP has clear advantages in terms of sensitivity, specificity, precision, and robustness *(15–17)*.

We describe a simple, robust reversed-phase HPLC method with postcolumn derivatization that is suitable for the routine measurement of TDP in erythrocytes or in whole blood. The method is fast and has adequate sensitivity and precision for use in the assessment of thiamin status in clinical practice and for research purposes. Measurement of TDP in erythrocytes is compared to that in whole blood. Direct measurement of TDP in erythrocytes is compared with the indirect measurement of TDP by the transketolase activation assay as an indicator of thiamin status in patients at risk of thiamin deficiency.

Materials and Methods

REAGENTS

All reagents were of analytical grade. Drabkins reagent, potassium ferricyanide, TDP, thiamin monophosphate (TMP), thiamin, and bovine serum albumin were obtained from Sigma Chemical Company. Trichloroacetic acid (TCA) and AnalaR-grade acetonitrile were purchased from BDH, and HPLC columns were purchased from Jones Chromatography.

CHROMATOGRAPHIC CONDITIONS

The HPLC system consisted of a Waters solvent delivery system and a Waters 470 fluorometer (Waters) The isocratic mobile phase for chromatography was 20 mmol/L sodium dihydrogen phosphate buffer (pH 4.6) containing 10 g/L acetonitrile. The mobile phase was filtered through a 0.45 μm nylon filter and pumped through a 5 μm reversed-phase column (Apex ODS; 4.6 $\times$ 250 mm, protected with a 10-mm guard column) at a flow rate of 1.5 mL/min.

The mobile phase was optimized with respect to stability of signal, column life, analysis time, reproducibility of results, and separation by varying its pH and the concentration of acetonitrile and phosphate buffer.

DETECTION

For postcolumn derivatization of TDP, the column effluent was reacted with 2 mol/L sodium hydroxide containing 600 mg/L potassium ferricyanide at a flow rate of 0.5 mL/min. The effluents were excited at 375 nm; the emission was measured at 430 nm and quantified by measuring the peak height.

PREPARATION OF BLOOD SAMPLES

Venous blood was collected into heparin-containing tubes. The packed erythrocytes were washed three times with 9 g/L NaCl, with care being taken to remove the buffy coat because leukocytes are a rich source of TDP. To hemolyze the cells, we added 1 mL of distilled water to 1 mL of packed cells and vortex-mixed the mixture for 1 min. The hemolysates were stored at −70 °C until analysis. For the measurement of TDP in whole blood, 1 mL of heparinized whole blood was stored at −70 °C until analysis.

Blood samples for population reference values were obtained from laboratory staff and from people attending a cardiovascular risk clinic at Glasgow Royal Infirmary. None of the subjects were taking any vitamin supplements or had any significant medical history. Although their dietary data were not recorded, all subjects indicated having reasonably typical diets. Erythrocyte TDP concentrations were measured in 147 subjects, 79 males and 68 females, with an average age of 54 years (range, 22–71 years). The reference range for whole blood TDP was established on 124 subjects, 68 males and 56 females.

PREPARATION OF CALIBRATORS

A stock solution of TDP was prepared by dissolving 100 mg of TDP in 100 mL of 0.1 mol/L HCl. This solution was stable for 4 months when stored at −70 °C. The stock solution was diluted 1:1000 in distilled water to prepare the intermediate stock solution. Calibrators were prepared by adding the intermediate stock solution to a solution of 80 g/L bovine serum albumin in distilled water to provide a range of 20–80 μg/L TDP. Although the working solution of TDP in 0.1 mol/L HCl was stable for <2 weeks when stored at −70 °C, its stability was improved when prepared in 80 g/L bovine serum albumin solution. Calibrators stored were stable for 4 weeks when stored at −70 °C.

QUALITY CONTROL

Internal quality control (QC). Hemolysates were prepared as described above. QC1 was the hemolysate, and QC2 was prepared by addition of the intermediate TDP solution to the hemolysate. The QC hemolysates were aliquoted and were stable for at least 12 months when stored at −70 °C.

External quality assessment. Currently there is no external quality assessment scheme available in the United Kingdom for measurement of TDP by HPLC.

SAMPLE PREPARATION

Samples and QC hemolysates were thawed, vortex-mixed, and centrifuged at 1000*g* for 10 min to remove cell

debris. Distilled water (500 μL) was added to 500 μL of whole blood, red cell, or QC hemolysate in a small centrifuge tube and vortex-mixed for 1 min; 500 μL of this diluted hemolysate was transferred to another tube, and 75 μL of 500 g/L TCA was added to precipitate the protein. The precipitated sample was thoroughly vortex-mixed and centrifuged for 10 min. A 200-μL aliquot of the supernatant was transferred to conical autosampler vials, and 50 μL was injected onto the HPLC column via an autosampler.

The remainder of the diluted hemolysate was used for hemoglobin (Hb) estimation using Drabkins reagent (Sigma Diagnostics), which contains potassium ferricyanide and potassium cyanide. Hb is oxidized and converted to stable cyanmethemoglobin by ferricyanide and cyanide, respectively. The absorbance of cyanmethemoglobin was measured at 540 nm using the Cobas Mira analyzer (Roche Products).

CALCULATION OF RESULTS

The calibration curve was prepared on the day of analysis by the addition of 75 μL of 500 g/L TCA to 500 μL of calibrators (containing 20, 40, and 80 μg/L TDP), which were treated in the same way as the sample hemolysates. Six calibrators were run with each batch of 30 samples: two at the start, two in middle, and two at the end of the run.

STABILITY STUDIES

To check the stability of TDP in whole blood at room temperature, five different whole blood samples were treated freshly and after 24, 48, and 72 h. Each blood sample was analyzed three times to obtain mean TDP values at the times specified above. The long-term storage stability of hemolysates and whole blood was checked by aliquoting a pooled hemolysate and a whole blood sample. The aliquots were stored at −20 °C and −70 °C and analyzed over a period of 7 months.

TRANSKETOLASE ACTIVATION ASSAY

The transketolase activation effect was measured by adapting the method of Bayoumi and Rosalki *(18)* for use on the Cobas Bio centrifugal analyzer (Roche Products) as described by Mount et al. *(19)*. The activation result was calculated by expressing the two activities of transketolase enzyme (with and without addition of TDP cofactor) as a percentage. The upper reference limit for the activation assay was obtained by determining the activation effect on blood samples from apparently healthy subjects (n = 79). The upper cutoff established in our laboratory was 25% (mean + 2 SD).

The within-batch imprecision (CV) of the assay, which was obtained by analyzing 17 aliquots of the same hemolysate, was 5.9%. The between-batch CV was 21%, as assessed by analyzing a hemolysate aliquoted and stored at −70 °C on 10 occasions over 2 weeks. Others have reported similar CVs, which are a reflection of sample instability during storage of hemolysates and show deterioration of transketolase activity over a 2-week period when stored at −70 °C *(7, 19)*.

Results

CHROMATOGRAPHIC CONDITIONS

The chromatographic profiles corresponding to a calibrator, a red cell hemolysate extract, and a whole blood extract are shown in Fig. 1, A–C. The TDP peak was well resolved and identified by comparing peak retention time with that of an albumin solution with added TDP. The TDP peak eluted after 4 min, and because there were no later eluting peaks, further injections were made at intervals of 6 min. The chromatographic profile of a blank

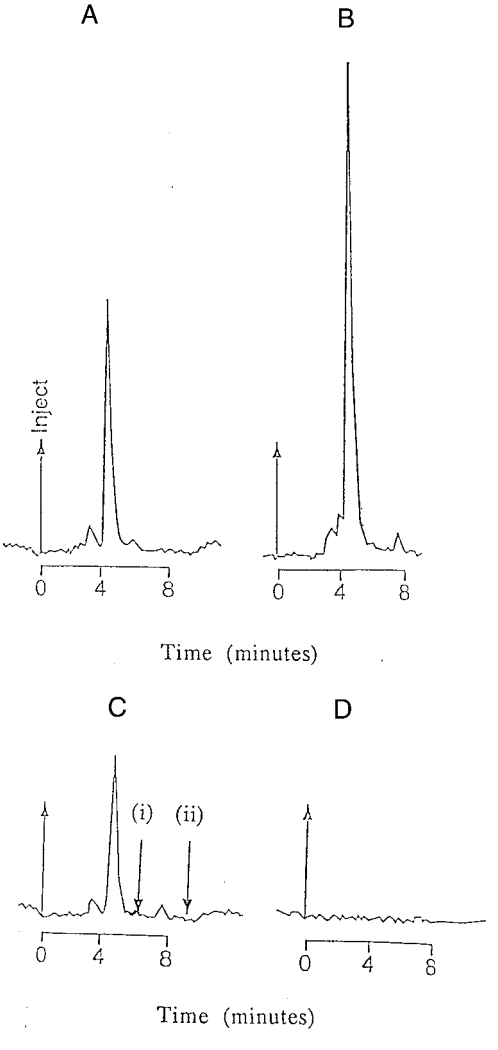

Fig. 1. Chromatographic profiles obtained from an albumin-based 20 μg/L TDP calibrator (*A*), a red cell hemolysate extract (*B*), a whole blood extract (*C*), and from an underivatized whole blood sample extract (*D*).

TDP concentrations in the hemolysate (*B*) and whole blood (*C*) were 725 and 269 ng/g Hb, respectively. In *C*, (*i*) and (*ii*) indicate the elution times for TMP and thiamin, respectively, which may be present in very low concentrations in whole blood.

injection prepared by omitting the thiochrome reaction suggested a lack of interference in sample extracts (Fig. 1D). Under the chromatographic conditions described, there was no interference from either TMP or thiamin, which together with thiamin triphosphate are present in very low concentrations in whole blood (<5% of total thiamin). TMP has a retention time of 6.2 min, with thiamin eluting after 9.0 min. With this chromatographic system, we detected neither thiamin nor TMP in either whole blood or red cells.

The concentration of the phosphate buffer in the mobile phase affected column stability and performance. At concentrations >100 mmol/L, the column was unstable, and at <10 mmol/L, the TDP peak was broader and retention times were longer. A phosphate buffer concentration of 20–50 mmol/L avoided both problems. The intensity and reproducibility of the fluorescence signal was found to depend on acetonitrile as an organic modifier in the mobile phase. A final concentration of 10 g/L acetonitrile in the mobile phase yielded a TDP peak that was sharp and well resolved with reproducible retention time and signal response. Varying the pH of the mobile phase between 3.0 and 6.0 did not affect the retention time, resolution, or intensity of the TDP signal.

For postcolumn derivatization of TDP, 0.6 g/L potassium ferricyanide in 2 mol/L sodium hydroxide solution delivered at a flow rate of 0.5 mL/min produced an optimal signal response with minimum peak broadening.

ANALYTICAL VALIDATION

Linearity and detection limit. The calibration curve was linear up to least 200 μg/L. The minimum detectable concentration, defined as the lowest concentration of the analyte in the calibrator that could be detected from zero with 97% confidence (n = 9) was 3 μg/L TDP (130 pg on column). The limit of quantification, defined as 10 times the signal-to-noise ratio, was 7 μg/L TDP (300 pg on column) for both whole blood and packed red cells.

Recovery studies. When 50 and 100 μg/L TDP was added to a pool of washed red cells, the recoveries were 95% ± 6% (mean ± SD; n = 5) and 97% ± 4.8% (n = 5), respectively. The corresponding recoveries for a whole blood sample were 97% ± 5.0% (n = 5) and 93% ± 6.7 (n = 5), respectively.

Precision studies. Precision data are shown in Table 1. The within-batch imprecision for the measurement of TDP in red cells was calculated by analyzing QC1 and QC2 10 times on the same day. The between-batch imprecision data were obtained from QC1 and QC2 material analyzed 23 times over a period of 5 months.

The within-batch precision data for the measurement of TDP in whole blood were obtained from a pooled whole blood sample analyzed 10 times on the same day. Between-batch imprecision was obtained by analyzing the pooled whole blood sample aliquoted and stored at −70 °C over a period of 3 months (n = 14).

Table 1. Precision of TDP measurements in red cells and in whole blood.

	Intrabatch (n = 10)			Interbatch[a]		
	Mean[b]	SD[b]	CV, %	Mean[b]	SD[b]	CV, %
QC1	297	17.9	6.1	298	24.2	8.1
QC2	477	24.6	5.2	477	30.0	6.3
WB[c]	411	26.2	6.3	403	30.1	7.5

[a] Interbatch imprecision data were obtained by analyzing QC1 and QC2 over a period of 5 months (n = 23) and a pooled whole blood sample over a period of 3 months (n = 14).
[b] Means and SD are expressed as ng of TDP/g Hb.
[c] WB, whole blood.

Recalibration of assay. Within the same HPLC batch, the assay was recalibrated after every 15 samples to correct for any drift and to monitor the stability of the chromatographic system. No significant drift was observed in calibrators at the start and end of a batch containing 30 samples.

STABILITY STUDIES

Hemolysates and whole blood stored at −70 °C were stable for at least 7 months. The mean value of a pooled hemolysate at the start of a 7-month storage period was 372 ng TDP/g Hb (range, 355–391 ng/g Hb; n = 5), and at the end, it was 384 ng TDP/g Hb (range, 368–405 ng/g Hb; n = 5). The corresponding mean values for whole blood were 404 ng TDP/g Hb at the start (range, 377–421 ng/g Hb; n = 5) and 387 ng TDP/g Hb after 7 months (range, 370–407 ng/g Hb; n = 5). Although hemolysates were stable at −20 °C for at least 7 months, the mean TDP value in whole blood stored at −20 °C was significantly different ($P < 0.01$) from that at the start (mean, 331 ng TDP/g Hb; range, 258–377 ng/g Hb; n = 5). We therefore stored all blood samples at −70 °C. At room temperature, whole blood was stable for TDP for up to 48 h. After 72 h, a variability of −10.3% to 13.7% was observed. Differences between mean TDP values in whole blood after 72 h and when freshly treated were significantly different for all five blood samples analyzed ($P < 0.05$ to < 0.01).

TDP in deproteinized samples was stable in the autosampler for up to 12 h at room temperature and for up to 24 h when the autosampler was refrigerated at 10 °C.

POPULATION REFERENCE VALUES

Whole blood and red cell TDP values showed a gaussian distribution. The Kolmogorov–Smirnov test revealed no significant departures from a gaussian distribution ($P > 0.15$). The mean concentration of TDP in red cells was 433 ng/g Hb (n = 147) with a 95% reference interval of 280–590 ng/g Hb (mean ± 2 SD). There was no significant difference in TDP concentrations between males and females. The corresponding reference interval for whole

blood TDP was 275–675 ng/g Hb with a mean of 460 ng/g Hb (n = 124).

COMPARISON STUDIES

Transketolase activation assay vs HPLC red cell TDP assay. Sixty-three acute and chronic medical and surgical patients who were considered to be at risk of thiamin deficiency were assessed for thiamin status by both HPLC and transketolase activation assays.

Clinical assessments by the two methods agreed in 58 of the 63 patients (Fig. 2). Fourteen patients were considered to be thiamin deficient by the transketolase activation test, and 13 of these patients also had low red cell TDP by the HPLC method. However, four patients with low TDP concentrations had transketolase activation values within the reference range. Activation values in these patients were 21–23%, which is close to the upper cutoff for biochemical deficiency established by the transketolase activation assay (>25%).

Chronic renal failure patients are at risk of thiamin deficiency as a consequence of dietary restrictions and loss of thiamin during dialysis. The thiamin status of six chronic renal failure patients was assessed by both assays in an attempt to establish whether thiamin supplements were adequate. All patients were undergoing intermittent dialysis and thiamin supplementation. Both the activation and HPLC assays showed that no patients were thiamin deficient either pre or post dialysis. However, the HPLC assay provided the additional information of thiamin accumulation in the red cells of these patients, which is cleared during dialysis (Table 2).

Red cell TDP assay vs whole blood TDP assay. TDP concentrations in red cells and in whole blood were measured by HPLC in 76 patients who were receiving nutritional support. Measurement of TDP in red cells correlated strongly with that in whole blood (r = 0.97). The regression equation was: whole blood TDP (ng/g Hb) = 11.2 + 0.97 × red cell TDP (ng/g Hb). A difference plot *(20)* also showed that there was good agreement between red cell and whole blood TDP concentrations (Fig. 3). The mean difference ±2 SD was 27 and −25 ng/g Hb, which represents an 8% difference between the two assays at the lower end (300 ng TDP/g Hb) and 3% at the upper end (800 ng TDP/g Hb). The 95% confidence interval for the mean difference was 4.4 to −1.8 ng TDP/g Hb, indicating that there was no detectable bias.

Table 2. Pre- and postdialysis red cell TDP concentrations in six chronic renal failure patients undergoing dialysis.

Patient number	TDP, ng/g Hb		Transketolase activation, %
	Predialysis	Postdialysis	
1	716	545	2
2	965	756	2
3	945	340	11
4	859	404	10
5	1334	586	2
6	867	455	7

Discussion

Because of the limitations of the erythrocyte transketolase assay, several workers have found the direct measurement of TDP in erythrocytes by HPLC a more useful index of thiamin status *(15, 16)*. In our experience, the HPLC method has several advantages over the transketolase activation assay. The HPLC assay has an acceptable between-batch precision and, because of the availability of pure TDP, the assay is easy to standardize. More importantly, we found that samples stored at −70 °C were stable for TDP for at least 7 months. In addition, TDP was stable in whole blood for 48 h at room temperature.

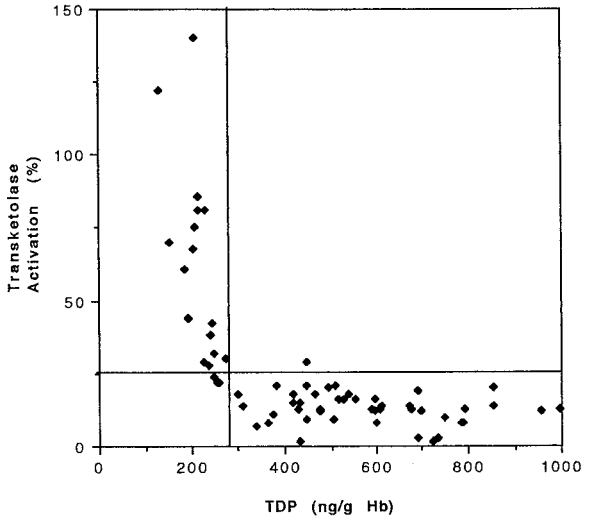

Fig. 2. Comparison of the transketolase activation assay with the HPLC red cell TDP assay.

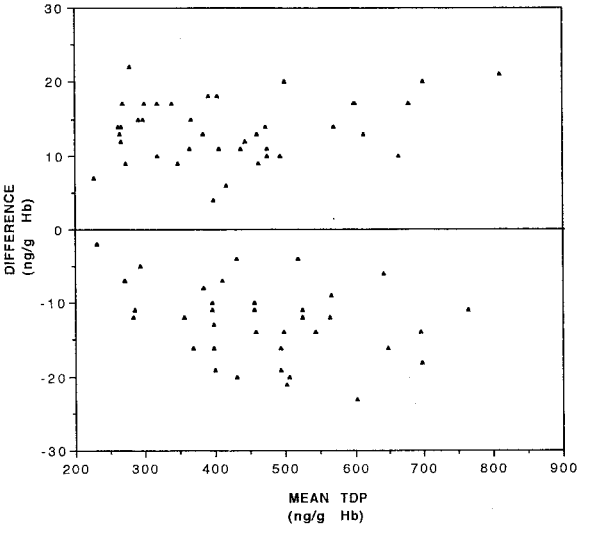

Fig. 3. Altman-Bland plot of the differences between red cell and whole blood TDP concentrations vs their means.

We found that erythrocyte TDP concentrations measured by HPLC compared well with the erythrocyte transketolase activation assay when assessing patients with suspected thiamin deficiency. Other workers who used HPLC with a precolumn derivatization procedure have reported similar results *(15, 16)*. However, four of our patients who had activation values just below the upper cutoff (<25%) were clearly deficient by HPLC (Fig. 2), which may indicate that measurement of TDP in erythrocytes is a more sensitive indicator of thiamin status than the transketolase activation assay. This has also been suggested by Warnock et al. *(15)* and Baines and Davies *(16)*. Unlike the activation test, the HPLC assay can also detect tissue thiamin accumulation, as demonstrated in patients on hemodialysis for chronic renal failure.

Blood and tissue concentrations of thiamin TDP are not subject to any known physiological regulation but are dependent on dietary intake. Diets are very variable globally, being influenced by local agriculture and economy and increasingly, in affluent societies, by fashion. Thus, the concepts of intra- and interindividual biological variation are not applicable. Recommendations for optimal intake of vitamins to maintain health such as the Recommended Dietary Allowances in the US *(21)* and the Reference Nutrient Intake values in the United Kingdom *(22)* have been published, but although similar, they have some significant differences and are not universally accepted. It is therefore essential that a local reference range be established. Our population reference range for TDP in red cells is comparable with those reported previously *(17, 23, 24)*. However, we related TDP concentrations in erythrocytes to Hb rather than to volume of packed red cells because accurate pipetting of packed red cells was difficult because of their viscosity, which adversely affected the precision of the HPLC assay. Relating the erythrocyte TDP concentration to Hb in the sample improved the reproducibility of the assay.

Measurement of TDP concentrations in red cells correlated strongly with that in whole blood. TDP concentrations in red cells and in whole blood showed good agreement when corrected for Hb. This is not surprising because TDP is present predominately in red cells (>90%). Because whole blood is convenient to use and preparation of washed red cells is time-consuming and has safety implications, we recommend the measurement of TDP in whole blood rather than washed red cells for the laboratory assessment of thiamin status. However, whole blood analysis should be carried out only if accompanied by Hb estimation to correct for any cell volume variability.

HPLC methods for the measurement of total thiamin or TDP in blood can be divided into pre- and postcolumn derivatization techniques with or without hydrolysis of TDP *(3, 6, 16, 17, 24–31)*. Measurement of total thiamin in whole blood requires time-consuming enzymatic hydrolysis of TDP to free thiamin before HPLC analysis *(3, 6, 27)*. The sensitivity and precision of the postcolumn derivatization method described here for the determination of TDP in erythrocytes or whole blood compare favorably with precolumn methods that have been described previously *(16, 17, 23, 24)*. Although both techniques are simple to perform, the postcolumn procedure is more robust and less labor-intensive and time-consuming (analysis time <15 min) than precolumn methods, which usually require removal of TCA from the acid extracts with ether *(15, 23)* or its neutralization with sodium hydroxide *(24, 28)* before derivatization with alkaline potassium ferricyanide. In addition, the precolumn derivatization procedure uses relatively harsh chromatographic conditions because of the need to use an alkaline mobile phase (pH 7.5–8.0) for the thiochrome derivatives to fluoresce. In our experience, use of such mobile phases leads to fairly rapid loss of column performance because of the dissolution of silica at high pH. Other disadvantages include poor yield of reaction (<67%) and instability of thiochrome derivatives at pH values below 8.0 *(32)*. These problems are largely eliminated with the use of postcolumn derivatization, as described here and by Kimura and Itokawa *(25)*, where the sample is deproteinized and injected directly onto a reversed-phase column with subsequent derivatization of the eluting substances. However, this procedure requires use of additional equipment (a postcolumn pump and a reaction coil) and can lead to peak broadening.

The HPLC method described here is suitable for the measurement of TDP in both whole blood and red cells. The method is simple and has adequate sensitivity, selectivity, and precision for use in the routine laboratory assessment of thiamin status in patients suspected of thiamin deficiency. The reference range for erythrocyte TDP is dependent on dietary thiamin intake because there are no known physiological mechanisms that regulate its concentration in blood or tissues. Thus, the erythrocyte TDP concentration in any individual will span the reference range according to his or her dietary intake. The between-batch CV of the proposed method (<8%) is much less than one-half the biological variation and therefore fulfils the current criteria *(33)* for suitability in clinical use.

References

1. Gubler CJ, Thiamin. In: Machlin LJ, ed. Handbook of vitamins. New York: Marcel Dekker, 1991:234–76.
2. Itokawa Y. Thiamin and diseases related to vitamin B deficiencies. J Appl Nutrition 1977;29:5–16.
3. Schrijver J, Speek AJ, Klosse JA, van Rijn HJ, Schtrurs WH. A reliable semi-automated method for the determination of total thiamin in whole blood by the thiochrome method with high performance liquid chromatography. Ann Clin Biochem 1982;19: 52–6.
4. Kimura M, Itokawa YJ. Determination of thiamin and its phosphate esters in human and rat blood by high performance liquid chromatography with post column derivatisation. J Chromatogr 1985; 332:181–8.
5. Bates CJ. Vitamin analysis. Ann Clin Biochem 1997;34:599–626.

6. Vuilleumier JP, Keller HE, Fidanza F, Bitsch R, Schrijver J, Speek AJ, et al. Erythrocyte transketolase activity; total thiamin in whole blood by HPLC; thiamin in urine. In: Fidanza F, ed. Nutritional status assessment—a manual for population studies. London: Chapman & Hall, 1991:228–44.
7. Puxty JAH, Haskew AE, Ratcliffe JG, McMurray J. Changes in erythrocyte transketolase activity and the thiamin pyrophosphate effect during storage of blood. Ann Clin Biochem 1985;22:423–7.
8. Wood B, Breen K, Pennington D. thiamine status in alcoholism. Aust NZ J Med 1997;7:475–84.
9. Kaczmarek MJ, Nixon PF. Variants of transketolase from human erythrocytes. Clin Chim Acta 1983;130:249–56.
10. Friedrich W. Vitamins. Berlin: de Gruyter, 1988:341–94.
11. Fennelly J, Frank O, Baker H, Leevy CM. Red blood cell transketolase activity in malnourished alcoholics with cirrhosis. Am J Clin Nutr 1967;20:946–9.
12. Anderson SH, Nicol A. A fluorometric method for measurement of erythrocyte transketolase activity. Ann Clin Biochem 1986;23: 180–9.
13. Camilo ME, Morgan MY, Sherlock S. Erythrocyte transketolase activity in alcoholic liver disease. Scand J Gastroenterol 1981;16: 273–9.
14. Brin M. Erythrocyte as biopsy tissue for functional evaluation of thiamin adequacy. JAMA 1964;187:762–6.
15. Warnock LG, Prudhomme CR, Wagner C. The determination of thiamin pyrophosphate in blood and other tissues and its correlation with erythrocyte transketolase activity. J Nutr 1978;108: 421–7.
16. Baines M, Davies G. The evaluation of erythrocyte thiamin diphosphate as an indicator of thiamin status in man and its comparison with erythrocyte transketolase activity measurements. Ann Clin Biochem 1988;25:698–705.
17. Baines M. Improved HPLC determination of thiamin diphosphate in erythrocytes. Clin Chim Acta 1985;153:43–8.
18. Bayoumi RA, Rosalki SB. Evaluation of methods of coenzyme activation of erythrocyte enzymes for detection of deficiencies of vitamins B_1, B_2, and B_6. Clin Chem 1976;22:327–35.
19. Mount J, Hedman E, Herd C, Jupp R, Kearney E, Marsh A. Adaptation of coenzyme stimulation assays for the nutritional assessment of vitamins B_1, B_2 and B_6 using the Cobas Bio Centrifugal analyser. Ann Clin Biochem 1987;24:41–6.
20. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307–10.
21. Subcommittee on the 10th Edition of the RDAs, Food and Nutrition Board, Commission of Life Sciences, National Research Committee. Recommended dietary allowances, 10th ed. Washington, DC: National Research Committee, 1989.
22. HMSO. Dietary reference values for food energy and nutrients for the United Kingdom. London: HMSO, 1991.
23. Warnock LG. The measurement of erythrocyte thiamin pyrophosphate by high performance liquid chromatography. Anal Biochem 1982;126:394–7.
24. Floridi A, Pupita M, Palmerini CA, Fini C, Alberti Fidanza A. Thiamin pyrophosphate determination in whole blood and erythrocytes by high performance liquid chromatography. Int J Vitamin Nutr Res 1984;54:165–71.
25. Kimura M, Itokawa Y. Determination of thiamin and thiamin phosphate esters in blood by liquid chromatography with post-column derivatization. Clin Chem 1983;29:2073–5.
26. Eitenmiller RR, Landen WO. Vitamin analysis for the health and food sciences. Boca Raton, FL: CRC Press, 1999:251–69.
27. Mascher HJ, Kikuta C. High performance liquid chromatography determination of total thiamin in human plasma. Methods Enzymol 1997;279:83–90.
28. Gerrits J, Eidhof, Brunnekreeft JW, Hessels J. Determination of thiamin and thiamin phosphates in whole blood by reversed phase liquid chromatography with pre column derivatization. Methods Enzymol 1997;279:74–82.
29. Tallaksen CM, Bohmer T, Karlsen J, Bell H. Determination of thiamin and its phosphate esters in human blood, plasma and urine. Methods Enzymol 1997;279:67–74.
30. Lynch PL, Trimble ER, Young IS. High performance liquid chromatographic determination of thiamin diphosphate in erythrocytes using internal standard methodology. J Chromatogr B 1997;701: 120–3.
31. Herve C, Beyne P, Delacoux E. Determination of thiamin and its phosphate esters in human erythrocytes by high performance liquid chromatography with isocratic elution. J Chromatogr B 1994;653:217–20.
32. Fayol V. High performance liquid chromatography determination of total thiamin in biological and food products. Methods Enzymol 1997;279:57–66.
33. Fraser CG, Petersen PH. Analytical performance characteristics should be judged against objective quality specifications. Clin Chem 1999;45:321–3.