



Fig. 1. Representative derivative melting curves [$(-dF/dT)$ vs T] of the three CD14 genotypes., TT; —, CT; - - - -, CC.

and modified at the 3' end by phosphorylation to block extension. The PCR conditions were as follows: initial denaturation at 95 °C for 120 s, followed by 60 cycles of denaturation (95 °C for 0 s, 20 °C/s), annealing (55 °C for 10 s), and extension (72 °C for 10 s). The melting curve consisted of 1 cycle at 95 °C for 0 s, 45 °C for 10 s, and then increasing the temperature to 95 °C at a slope of 0.2 °C/s.

The fluorescence signal (F) was monitored continuously during the temperature ramp and then plotted against the temperature (T). These curves were transformed to derivative melting curves [$(-dF/dT)$ vs T].

Representative results for the three different genotypes (TT , CT , and CC) are given in Fig. 1. In the 100 patient samples, 27% were TT , 41% were CT , and 32% were CC . The proposed technique and the restriction enzyme technique gave identical results. The assay is rapid and accurate, and seems especially suited for routine laboratories that process large numbers of samples.

We thank Olfert Landt (TIB MOL-BIOL, Tempelhofer Weg 11-12, 10829 Berlin, Germany) for designing the hybridization probes.

References

- Goyert SM, Ferrero E, Rettig WJ, Yenamandra AK, Obata F, LeBeau MM. The CD14 monocyte

differentiation antigen maps to a region encoding growth factors and receptors. *Science* 1988;239:497-500.

- Frey EA, Miller DS, Gullstein Jahr T, Sundan A, Bazil V, Espevik T, et al. Soluble CD14 participates in the response of cells to lipopolysaccharide. *J Exp Med* 1992;176:1665-71.
- Leinonen M, Linnanmäki E, Mattila K, Nieminen MS, Valtonen V, Leirisalo-Repo M, Saikku P. Circulating immune complexes containing chlamydial lipopolysaccharide in acute myocardial infarction. *Microbiol Pathol* 1990;9:67-73.
- Hubacek JA, Pit'ha J, Skodova Z, Stanek V, Poledne R. C(-260)→T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 1999;99:3218-20.
- Unkelbach K, Gardemann A, Kostrzewa M, Philipp M, Tillmanns H, Haberbosch W. A new promoter polymorphism in the gene of lipopolysaccharide receptor CD14 is associated with expired myocardial infarction in patients with low atherosclerotic risk profile. *Arterioscler Thromb Vasc Biol* 1999;19:932-8.
- Lee TH. Reducing cardiac risk in noncardiac surgery. *N Engl J Med* 1999;341:1838-40.

Michael Heesen^{1*}
Dagmar Kunz²
Rolf Rossaint¹
Brunhilde Blömeke³

Departments of ¹Anesthesia,
²Clinical Chemistry and
Pathobiochemistry,
and ³Dermatology
University Hospital RWTH Aachen
Pauwelsstrasse 30
D-52074 Aachen, Germany

*Author for correspondence. Fax 49-241-8888406; e-mail mheesen@post.klinikum.rwth-aachen.de.



Assessment of Vitamin B₁ Status

To the Editor:

The excellent report by Talwar et al. (1) promotes the measurement of thiamin diphosphate (TDP) for the assessment of vitamin B₁ status. My experience with >30 000 people supports this, but only for the investigation of untreated patients.

The TDP assay is more precise than the transketolase activation (ETK) test, and the method described is an important advance for which I thank the authors. In comparing the two methods, Talwar et al. (1) found TDP slightly advantageous in the identification of B₁ deficiency. They and most workers using the ETK test agree that the cutoff point is 25%. I have found it useful to report results in the range 15-25% as borderline. When this is done, there is little to choose between TDP and ETK in terms of clinical usefulness.

Much as I would like to use the more precise TDP assay, there is a problem that surfaces when one wishes to use the laboratory to follow repletion with thiamin. It is very rare for the TDP concentration to remain low after a few days of supplemental B₁, and in many cases, TDP normalizes after a single 100-mg dose. This is not the case for the ETK test. In some cases, several weeks of daily supplementation are needed to normalize the results.

I am in the fortunate position of receiving considerable feedback from the clinicians using our laboratory service and have carefully studied their findings in relation to the laboratory results. In my experience, it is the ETK test that parallels the clinical improvement in supplemented patients.

I support the use of the more precise TDP (HPLC method) in untreated people, but I caution against its use in following supplementation. For this, the ETK test, even with its many limitations, remains the method of choice. A more precise method for measuring this enzyme would be enormously helpful.

I hope this letter will open some further discussion on the use of func-

tional tests in nutrient-related clinical chemistry.

References

1. Talwar D, Davidson H, Cooney J, St. JO'Reilly D. Vitamin B₁ status assessed by direct measurement of thiamin pyrophosphate in erythrocytes or whole blood by HPLC: comparison with erythrocyte transketolase activation test. *Clin Chem* 2000;46:704-10

John McLaren Howard

*Biolab Medical Unit
9 Weymouth St.
London W1 N 3FF, United Kingdom*

Drs. Talwar and St. JO'Reilly respond:

To the Editor:

Dr. McLaren Howard makes an interesting observation relating to the biochemical assessment of thiamin status in people supplemented with the vitamin. In his experience, the indirect measurement of thiamin status using the transketolase (ETK) activation assay is clinically more useful than direct measurement of thiamin diphosphate (TDP) concentrations in red cells in people repleted with thiamin. Unfortunately, we are unable to comment on his observation because no relevant data are presented.

Discrepancies between the ETK activation test and clinical signs of thiamin deficiency have been reported previously, with several studies reporting no relationship between ETK activation results and thiamin intake (1-5). These discrepant findings have raised questions about the usefulness of the ETK activation test as a sole indicator of thiamin status.

Because a valid ETK activation response depends on a kinetically normal enzyme (1,6), certain disease states may affect enzyme cofactor binding and hence the TDP activation effect (6). Because of the potential difficulty in interpretation of ETK activation effect in some disease states and the limitations of enzyme activation tests in general, several authors have suggested the use of more direct measures of thiamin status, such as TDP in whole blood or plasma (4,6,7).

We would agree with Dr. McLaren

Howard that further discussion is required on the merits or otherwise of direct and indirect measures of thiamin status in patients repleted with thiamin. Meanwhile, our experience with the HPLC assay suggests that measurement of TDP in red cells is the single most useful biochemical measurement for assessing thiamin status in patients who are at risk of thiamin deficiency.

References

1. Kerr RA, Clague AE, Morris DJ, Price J. The rapid decline in erythrocyte transketolase on cessation of high dose thiamin administration in Korsakoff patients. *Alcohol* 1986;21:257-62.
2. Iber FL, Blass JP, Brin M, Leevy CM. Thiamin in the elderly: relation to alcoholism and to neurological degenerative disease. *Am J Clin Nutr* 1982;36:1067-82.
3. Nordentoft M, Timm S, Hasselbalch E, Roesen A, Gammeltoft S, Hemmingsen R. Thiamin pyrophosphate effect and erythrocyte transketolase activity during severe alcohol withdrawal syndrome. *Acta Psychiatr Scand* 1993;88:80-4.
4. Gans DA, Harper AE. Thiamin status of incarcerated and nonincarcerated adolescent males: dietary intake and thiamin pyrophosphate response. *Am J Clin Nutr* 1991;53:1471-5.
5. Macias-Matos C, Rodriguez-Ojea A, Chi N, Jimenez S, Zulueta D, Bates CJ. Biochemical evidence of thiamin depletion during the Cuban neuropathy epidemic 1992-1993. *Am J Clin Nutr* 1996;64:347-53.
6. McLaren DS, Docherty MA, Boyd DA. Plasma thiamin pyrophosphate and erythrocyte transketolase in chronic alcoholism. *Am J Clin Nutr* 1981;34:1031-3.
7. Dancy M, Evans G, Gaitonde MK, Maxwell JD. Blood thiamin and thiamin phosphate ester concentrations in alcoholic and non alcoholic liver diseases. *Br Med J* 1984;289:79-82.

Dinesh Talwar*
Denis St. JO'Reilly

*Department of Clinical Biochemistry
Macewen Building
Royal Infirmary
Glasgow G4 0SF, United Kingdom*

*Author for correspondence.

Transient Hyperphosphatasemia of Infancy and Childhood: Study of 194 Cases

To the Editor:

Transient hyperphosphatasemia of infancy and childhood (TH) is a temporary and isolated increase of serum alkaline phosphatase (ALP; EC 3.1.3.1) activity occurring without

obvious cause during the first years of life. Despite several reports about this phenomenon, the origin of TH remains obscure.

Over a period of 8 years (1992-1999), we detected 194 cases of TH in 106 boys and 88 girls. The hyperphosphatasemia was discovered fortuitously during routine investigations in outpatient and inpatient departments of a children's hospital with a capacity of 500 beds. A wide variety of clinical disorders was associated with this condition (gastrointestinal diseases, 24%; respiratory infections, 21%; congenital anomalies and inborn errors of metabolism, 15%; anemia, 10%; malignancies, 7%; neurological disorders, 5%; others, 18%).

We measured total ALP activity using the IFCC-recommended method at 37 °C with Elan (Eppendorf) and Cobas Integra (Roche) analyzers. Our reference interval for children was 0.85-6.80 $\mu\text{kat/L}$ (51-408 U/L). Adult reference intervals are 0.54-1.7 $\mu\text{kat/L}$ (32-104 U/L) for women and 0.76-2.0 $\mu\text{kat/L}$ (45-122 U/L) for men. In each TH case, we saw the characteristic two-band ALP isoenzyme pattern on Cellogel zonal electrophoresis as described by Stein et al. (1) and Behúlová et al. (2).

Although markedly increased ALP activities may occur in TH, frequently only slightly or moderately increased activities are observed, depending on the timing of the blood sample in relation to the natural course of TH. Markedly increased activities, therefore, are not necessary to reach a diagnosis of TH. The peak ALP activity in our series was 2- to 20-fold higher than the pediatric upper reference limit, with the median being a 4-fold increase.

In this series, 49% of cases were detected in the second year of life, and 96% of affected children were younger than 5 years (Fig. 1). We speculate that immaturity of the mechanisms responsible for ALP clearance allows increases of plasma ALP, triggered by an exogenous insult.

We observed a marked seasonal clustering of cases from September to November (43%); the lowest incidence was from January to March