〈Original Article〉

Reference values for whole blood thiamine and thiamine phosphate esters in Japanese adults

Hiroshi Ihara[1)], Akiko Hirano[2)], Linlin Wang[2)],
Mitsumasa Okada[2)] and Naotaka Hashizume[3)]

Summary A reliable estimate of the vitamin B_1 nutritional status is obtainable from the concentrations of B_1 vitamers, i.e., nonphosphorylated free thiamine (T), thiamine monophosphate (TMP), thiamine diphosphate (TDP), and thiamine triphosphate (TTP) in whole blood, but not from the total vitamin B_1 concentration. We established reference values for the B_1 vitamers in whole blood from 969 healthy Japanese (509 men, ages 21-80 and 460 women, ages 18-70) by the NH_2-column HPLC method. The reference values in men (central 95th percentile) for T, TMP, TDP, and TTP, and the ratio of the phosphorylated species expressed as (TMP+TDP+TTP)/T were 2-18 nmol/L for T, 5-47 nmol/L for TMP, 70-229 nmol/L for TDP, and 0-4 nmol/L for TTP, with 9 to 58 for the ratio. In women, the reference values, in the same sequence, were 2-17 nmol/L, 4-60 nmol/L, 63-200 nmol/L, 0-3 nmol/L and 8-58. The reference values for this Japanese population were significantly different from those obtained from Norwegian or Dutch populations. With our reference values, we were able to identify three subjects with vitamin B_1 malnutrition, two of whom were overlooked when based on the measurements of their total B_1 concentration.

Key words: Vitamin B_1, Thiamine, Thiamine diphosphate, Thiamine triphosphate, Phosphorylation ratio, Reference values, Thiamine vitamers

1. Introduction

Thiamine (vitamin B_1) participates in carbohydrate metabolism involving the tricarboxylic acid cycle and the pentose phosphate pathway.[1)] Adequate B_1 concentrations are needed for the normal functioning of these metabolic pathways. In vivo, vitamin B_1 exists as four vitamers: the nonphosphorylated free thiamine (T), and its phosphate esters, thiamine monophosphate (TMP), thiamine diphosphate (TDP), and thiamine triphosphate (TTP). T and TMP are present in plasma, whereas T, TMP, TDP, and TTP are found in erythrocytes and leukocytes.[2, 3)] Since the total vitamin B_1 concentration (i.e., the sum

1) Department of Laboratory Medicine, Ohashi Hospital, Toho University Medical Center, 2-17-6 Ohashi, Meguro, Tokyo 153-8515, Japan
2) Department of Biomolecular Science, Faculty of Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan
3) Department of Health and Nutrition, Wayo Women's University, 2-3-1 Konodai, Ichikawa, Chiba 272-8533, Japan
Correspondence to: Hiroshi Ihara, Department of Laboratory Medicine, Ohashi Hospital, Toho University Medical Center, 2-17-6 Ohashi, Meguro, Tokyo 153-8515, Japan
e-mail, ihara-1@cam.hi-ho.ne.jp
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of free T and its phosphate esters) in whole blood correlates with the concentration of B_1 in tissues,[1] it is possible to estimate the vitamin B_1 nutriture by measuring the total vitamin B_1 concentration in whole blood.[4] Only TDP, however, acts as a coenzyme in cells. T comes from the diet, and is a precursor of TDP. Although TMP exhibits no apparent biological activity, it was reported to be a more sensitive marker of deficiency in whole blood than TDP in patients with Wernicke encephalopathy since the only source of TMP is the dephophorylation of TDP.[5] The role of TTP, if any, is unknown. Our aims in this study were to determine reference values in Japanese adults for the whole blood concentrations of T and its phosphate esters, and to compare these values with those of non-Japanese adults.[6, 7]

2. Materials and Methods

1) Subjects

We collected blood specimens by venipuncture into evacuated collection tubes containing Na_2EDTA from individuals who had fasted at least 12 hours. In our study group were 1,008 healthy Japanese adults (531 men, ages 21-80, mean 54; and 477 women, ages 18-80, mean 49). We had one patient, age 53, who had a diagnosis of spinocerebellar degeneration. Of the 477 women, 78 (ages 18-26, mean 21) provided a three-day dietary history prior to blood collection, none of whom had a history of smoking, regular use of medication(s), use of dietary supplements, or alcohol consumption. Written informed consent was obtained from all subjects, and our study was in compliance with the rules for human experimentation at our institution.

2) HPLC method

We determined the concentrations of T and its phosphate esters in whole blood by a pre-column derivatization HPLC method using the NH_2-column (Asahipack NH_2-504E column, 4.6×250 mm; Shodex, Tokyo, Japan) as previously described.[8] We obtained linear correlations from 1.0 to 250 nmol/L between dilutions of T, TMP, TDP, and TTP and the response of the HPLC. All concentrations in this report are stated in nmol/L of whole blood. The within-day coefficients of variation (CV) of known controls were 2.4, 6.0, 1.9, and 12.4% for T, TMP, TDP, and TTP, respectively. The between-day CV was 1.8, 11.2, 2.0, and 20.5% for T, TMP, TDP, and TTP, respectively. Our recovery of T, TMP, TDP, and TTP added to whole blood was 100-103%.[9] The assay values of the B_1 vitamers were confirmed by checking the absorbance of calibration solutions of T, TMP, TDP, and TTP at 248 nm.[10] The sum of the concentrations of T, TMP, TDP, and TTP by the NH_2-column HPLC method was significantly correlated with the total vitamin B_1 concentration obtained by the gel permeation HPLC method. We found a correlation coefficient of 0.940, with $P < 0.001$. The NH_2-column HPLC method yielded values that were 2% lower, on average, than those obtained from the gel permeation HPLC method.[4] We expressed the phosphorylation ratio in whole blood as: (TMP+TDP+TTP)/T.

3) Statistical analysis of data

Data were analyzed by the Mann-Whitney U test for non-Gaussian variables. Statistical significance was defined as $P<0.05$.

3. Results

1) Concentrations of B_1 vitamers

We assayed the concentrations of T, TMP, TDP, and TTP in 1,008 healthy Japanese adults. We found increased concentrations of T (>30 nmol/L) in 39 of them (22 men and 17 women, ages 33-80, and mean age 69). These 39 healthy adults were excluded from data analysis, since persons having elevated concentrations of T were strongly suspected of taking a supplement containing B_1.[11] The 969 remaining subjects were divided into four age groups used in the Japanese DRIs (dietary reference intakes. See Table 1).[12] We confirmed the findings in previous reports[3, 4, 13] that the frequency distributions of the concentrations of B_1 vitamers and total B_1 were non-Gaussian (histograms not shown). The distribution of values was log-normal. Thus, we obtained a geometric mean (median), with the 95% distribution range defined as

Table 1 Concentrations of thiamine and its phosphate esters in whole blood among different age groups

Age (yrs)	Number of subjects	T (nmol/L)	TMP (nmol/L)	TDP (nmol/L)	TTP (nmol/L)	Total (nmol/L)	Phosphorylation ratio
18-29	Men (n=112)	3 (2-21)	16 (4-35)	99 (50-202) [a]	0 (0-3)	115 (64-226) [a]	29 (7-77)
	Women (n=142)	5 (2-14)	13 (3-52)	103 (63-164) [a]	0 (0-3)	125 (81-191) [a]	22 (9-55)
30-49	Men (n=97)	8 (2-31) [c]	10 (4-30)	124 (61-290)	0 (0-8)	139 (80-304)	16 (6-56) [a]
	Women (n=71)	7 (2-22) [c]	10 (4-50)	106 (50-194) [a, b]	0 (0-4)	123 (64-218) [a, b]	15 (6-40) [a]
50-69	Men (n=89)	8 (2-35) [c]	12 (4-36)	129 (78-212)	0 (0-3)	148 (94-248)	16 (6-35) [a]
	Women (n=72)	12 (3-43) [c]	13 (4-50)	139 (70-263)	0 (0-6)	164 (85-319)	16 (4-56) [a]
70-80	Men (n=211)	7 (3-13)	20 (8-47) [c]	129 (80-208)	0 (0-3)	155 (100-246)	24 (12-53)
	Women (n=175)	6 (3-13)	21 (9-47) [c]	127 (78-202)	0 (0-3)	153 (101-237)	23 (11-57)
All ages	Men (n=509)	7 (2-18)	17 (5-47)	124 (70-229)	0 (0-4)	150 (89-262)	22 (9-58)
	Women (n=460)	6 (2-17)	16 (4-60)	114 (63-200) [b]	0 (0-3)	139 (80-235) [b]	22 (8-58)

Values are median with 95% distribution range in parentheses.
[a] Significantly lower than other age groups ($P < 0.05$).
[b] Significantly lower than men ($P < 0.05$).
[c] Significantly higher than other age groups ($P < 0.05$).

the antilog of [log mean ± (2 log SD)] (Table 1). The whole blood concentrations of total vitamin B_1 and TDP in men in the 18-29 age group and women in the 18-29 and 30-49 age groups were significantly lower than those concentrations in the other gender-matched age groups shown in Table 1 ($P<0.05$ for all; Mann-Whitney U test). For women in the 30-49 age group, the concentrations of total vitamin B_1 and TDP were significantly lower than those of men in the same age group. We summarize the rest of the data in Table 1.

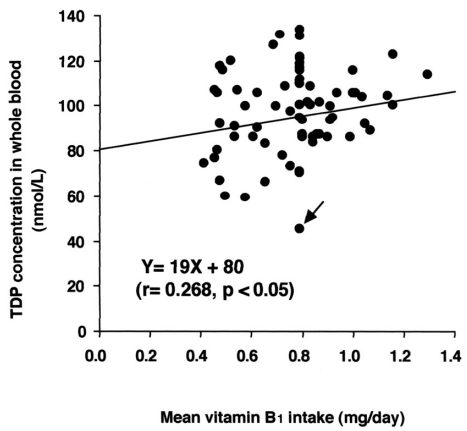

Fig. 1 Correlation between mean oral vitamin B_1 intake and TDP concentration in whole blood from 78 healthy young women. Arrow indicates one TDP-deficient case.

2) Comparison of blood concentrations with B_1 intake levels

Of the 78 young women (ages 18-26) who provided a three-day dietary history prior to blood collection, 57 (73%) stated that their mean vitamin B_1 intake amounts were < than 0.9 mg per day. Here, 0.9 mg is the cutoff value to determine whether a daily intake is inadequate for normal nutrition.[11] In all 78 young women, whole blood concentrations of TDP were only weakly correlated with the mean intake of B_1 ($r= 0.268$, $P <0.05$, Fig. 1). Although the 57 women were considered to have inadequate vitamin B_1 intake based on their dietary history, only one 21-year-old woman had a true vitamin B_1 deficiency based on her concentration of either TDP (see Fig. 1, indicated by arrow) or total vitamin B_1. She took 0.80 mg of vitamin B_1 per day. Her concentrations of T, TMP, TDP, TTP, and total B_1 were 7.2, 23, 45, 0, and 75 nmol/L, respectively, and her phosphorylation ratio was 10. These results indicated that a dietary history was less reliable than an analysis of blood.

3) Case report of a patient with B_1 deficiency

A 53-year-old Japanese man with spinocerebellar degeneration and vitamin B_1 deficiency, especially of TDP and total B_1, was given a 20-mg dose intravenously (Ⅳ) of thiamine hydrochloride (Metabolin, Takeda Chemical Industries, Ltd., Osaka, Japan) (See

Table 2 Case of a B_1-deficient patient[a]

	T (nmol/L)	TMP (nmol/L)	TDP (nmol/L)	TTP (nmol/L)	Total (nmol/L)	Phosphorylation ratio
Before Ⅳ administration	7	4	49	0	60	8
After Ⅳ administration						
30 min	435	5	71	0	511	0.2
60 min	191	7	70	0	268	0.4
95% distribution range from healthy men (50-59 y) [b]	2-35	4-36	78-212	0-3	94-248	6-35

[a] A 53-year-old Japanese male with spinocerebellar degeneration was intravenously (Ⅳ) administered 20 mg of thiamine hydrochloride (Metabolin, Takeda Chemical Industries, Ltd., Osaka, Japan).
[b] 95% distribution range adapted from Table 1.

Table 1). Following the injection, his total B_1 concentrations increased 4-8 fold and were above the reference range for B_1. The increase of TMP and TDP was less than twice that of their original concentrations, and the phosphorylation ratio was 0.4. The concentrations of TDP remained below the lower reference value. Earlier, we measured the concentrations of B_1 vitamers in hundreds of patients before and after intravenous B_1 administration. The usual course in patients showing a normal activity of thiamine pyrophosphokinase (EC 2.7.6.2) in liver involved a dramatic increase of TDP to more than 1,000 nmol/L after B_1 administration. Our patient failed to show a typical response of vitamers to the intravenous injection of thiamine hydrochloride.

4) Cases of B_1 hypervitaminosis expressed as nonphosphorylated free thiamine

Of the 39 healthy seniors with increased concentrations of T>30 nmol/L, 32 also had elevated TDP concentrations. In 23 of the 32 adults, we found a simultaneous increase of TTP (Fig. 2). The concentrations of TMP were significantly correlated with the TDP concentrations ($r= 0.381$, $P<0.01$: Fig. 3). The phosphorylation ratio of 39 volunteers was below the lower reference limit, ranging from 0.2 and 4.5 with a median of 2.3. We had one 60-year-old woman with a phosphorylation ratio of 0.2, which was the lowest value we saw. Her values are shown with arrows in Fig. 2. Her concentrations of B_1 vitamers were 192 for

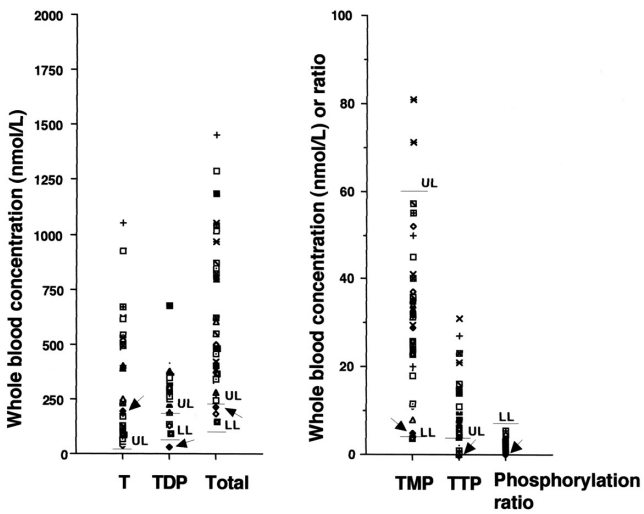

Fig. 2 Concentrations of thiamine and its phosphate esters and phosphorylation ratio in whole blood from 39 healthy volunteers with hypervitaminosis B_1 expressed as nonphosphorylated free thiamine. Each symbol represents one subject. Lower reference limit (LL) and upper reference limit (UL) were those obtained from women of all ages.

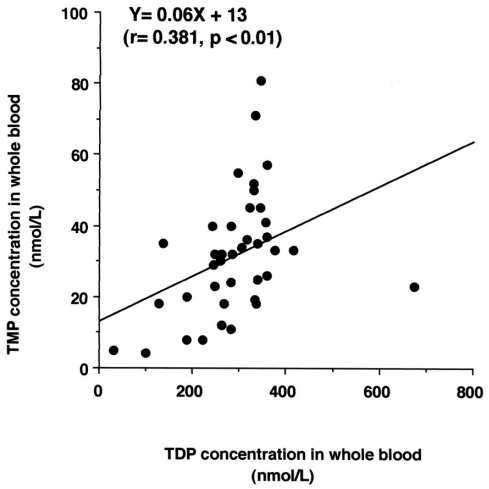

Fig. 3 Relationship between concentrations of TMP and TDP in whole blood from 39 healthy volunteers with hypervitaminosis B_1 expressed as nonphosphorylated free thiamine.

T, 5.0 for TMP, 32 for TDP, and 0 nmol/L (actually undetectable by our method) for TTP. We learned that she took B_1 tablets every day. Her total B_1 was 229 nmol/L (the lower reference limit in our laboratory is 80 nmol/L). Her TDP deficiency was eclipsed by the total B_1 measurement. This woman and two other volunteers among the 39 adults had normal concentrations of total B_1 when compared with the reference values. The remaining 36 had elevated total B_1 concentrations.

4. Discussion

Among the 969 healthy volunteers, we found statistically significant differences in B_1 vitamers for men vs. women in our comparison of certain age groups (See Table 1). We found lower phosphorylation ratios for both men and women aged 30-69, and surmise that this was due to the higher concentrations of T in the 30-69 group compared with the other age groups. Lower amounts of B_1 intake affected the concentrations of TDP (Fig. 1). An important finding in our study was that the concentrations of B_1 vitamers in normal adults changed with age. We found no other reports in the literature that made this observation. Thus, we established reference values for B_1 vitamers for men and women from their central 95th distributions for all ages. Using the lower reference limits for concentrations of each B_1 vitamer, subjects with vitamin B_1 malnutrition could be readily identified in our population of Japanese adults. Our values were significantly different from those obtained in Norway as reported by Tallksen et al..[6] They found concentrations (mean±SD) of 33.4±10.4 nmol/L for T, 10.9±5.1 nmol/L for TMP, 165±40.4 nmol/L for TDP, and <2 nmol/L for TTP in 15 healthy men (median age 43, range 32-54). In 15 healthy women (median age 47, range 23-60), the concentrations were 29.6±10.0 nmol/L, 9.7±2.3 nmol/L, 121±29.6 nmol/L, and <2 nmol/L in the same sequence of values as those above. Their T values in both men and women and for TDP in men were significantly ($P<0.001$) higher than those of our population, whereas their values for TMP in both men and women were significantly lower ($P<0.05$).

Brunnekreeft et al.[7] also reported concentrations of thiamine and its phosphate esters in whole blood. Sixty-five specimens were obtained from healthy Dutch adults; they indicated neither gender nor age in their test group. They found mean concentrations (±SD) for TDP of 120±17.5 nmol/L, 4.1±1.6 nmol/L for TMP, and 4.3±1.9 nmol/L for T. Their TTP concentrations were all <4.0 nmol/L. Since neither the Norwegian nor Dutch investigators used a logarithmic conversion of the assay results prior to calculating the reference values, we could not directly compare our reference values with theirs. However, we concluded that whole blood concentrations of B_1 vitamers in Japanese adults should be evaluated based on reference values derived from healthy Japanese individuals.

Using our reference values, we were able to identify three subjects with vitamin B_1 malnutrition. Two were from healthy female volunteers (21 and 60-years-old), and the third was a patient of ours. The patient and the 60-year-old woman were given B_1 Ⅳ or orally in their diet. There was a reduced enhancement of T to TDP because of the lower phosphorylation ratio. Their TMP concentrations were at or below the lower reference limit, since the only source of TMP was the dephosphorylated TDP.[5]

When vitamin B_1 was given to healthy volunteers, whole blood concentrations of T, TMP, and TDP were elevated at the same time that the phosphorylation ratio was reduced. This was because the T concentrations were increased much more than those of TMP and TDP.

After oral administration of 50 mg thiamine propyl disulfate, Kimura et al.[14] found that the whole blood concentrations of T peaked at 70 nmol/L after 90 minutes, and the elevated concentrations of T (>30 nmol/L) remained high for at least 6 hours. Since many over-the-counter thiamine supplements contain up to 100 mg of thiamine per tablet,[15] increased levels of total vitamin B_1 would often be observed in subjects with underlying vitamin B_1 malnutrition. The reference values described here are required to identify subjects with clinical vitamin B_1 malnutrition. For those given a dietary supplement, including our two cases, vitamin B_1 malnutrition, i.e., a TDP deficiency, may be obscured by measurements of the total vitamin B_1 content.

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