〈Review Article〉

Classification of hypo and hyperthiaminosis

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Summary Hypo and hyperthiaminosis were classified into ten types based on elevated or decreased levels of free thiamine (free T), thiamine monophosphate, thiamine diphosphate (TDP) and thiamine triphosphate, including total vitamin B_1 (total B_1) concentrations in whole blood specimens. Classifications of more than a thousand Japanese subjects revealed that two types (Type Ⅱb and Type Ⅳb) were not observed. Type Ⅲb (decreased levels in TDP) had a clinical problem, because the presence of TDP deficiency was not diagnosed by total B_1 levels. Type Ⅴa (elevated levels in free T) needed further investigaion if this was normal status for the healthy subjects merely observed just after taking higher amounts of vitamin B_1, otherwise, the case included clinical symptoms.

Key words: Vitamin B_1, Thiamine pyrophosphate, Vitamin B_1 deficiency, Nutritional assessment, Hypothiaminosis

Vitamin B_1 (thiamine), an essential nutrient, helps cells produce energy from carbohydrates and is required for the health of the nervous system[1]. In the nutritional assessment of vitamin B_1 status, total vitamin B_1 (total B_1) levels can be determined in whole blood specimens. Whole blood concentrations reflect the body stores of this vitamin, since the total B_1 level in red blood cells is depleted at a rate similar to that in other tissues[2]. Total B_1 consists of free thiamine (free T) and its three phosphate esters, i.e., thiamine monophosphate (TMP), thiamine diphosphate (TDP), also known as thiamine pyrophosphate or cocarboxylase, and thiamine triphosphate (TTP). TDP is known as an enzymatic cofactor and TTP has a specific neurophysiological role. Although free T and TMP play no role in physiological functioning, free T is a precursor of its active metabolites (i.e., TDP and TTP), and TMP is considered to be an intermediate that facilitates the synthesis of free T to TDP and TTP (Fig. 1).

When vitamin B_1 status was assessed in whole blood specimens from self reported healthy volunteers, some volunteers were diagnosed with thiamine deficiency based on total B_1 concentrations lower than the reference value[3]. These deficient subjects had normal or deficient TDP levels. In addition, increased levels of free T with normal or deficient TDP were occasionally observed among these

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subjects. Various changes in thiamine and its phosphate esters make it difficult to understand which changes may have nutritional and clinical significance, and they required precise classification.

These changes in thiamine and its phosphate were classified by comparing subject (or patients) concentration with a reference value (Table 1). Usually, total B_1 concentrations in whole blood specimens were

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

Age (yrs)	Number of subjects	Free T nmol/L	TMP nmol/L	TDP nmol/L	TTP nmol/L	Total B_1 nmol/L
18 - 29	Men 112	3 (2-21)	16 (4-35)	99 (50-202)	0 (0-3)	115 (64-226)
	Women 142	5 (2-14)	13 (3-52)	103 (63-164)	0 (0-3)	125 (82-191)
30 - 49	Men 97	8 (2-31)	10 (4-30)	124 (61-290)	0 (0-8)	139 (80-304)
	Women 71	7 (2-22)	10 (4-50)	106 (50-194)	0 (0-4)	123 (64-218)
50 - 69	Men 89	8 (2-35)	12 (4-36)	129 (78-212)	0 (0-3)	148 (94-248)
	Women 72	12 (3-43)	13 (4-50)	139 (70-263)	0 (0-6)	164 (85-319)
70 - 80	Men 211	7 (3-13)	20 (8-47)	129 (80-208)	0 (0-3)	155 (100-246)
	Women 175	6 (3-13)	21 (9-47)	127 (78-202)	0 (0-3)	153 (101-237)
All ages	Men 509	7 (2-18)	17 (5-47)	124 (70-229)	0 (0-4)	150 (89-263)
	Women 460	6 (2-17)	16 (4-60)	114 (63-200)	0 (0-3)	139 (80-235)

Values are median with 95% distribution range in parentheses.

Table 2 Classification of thiamine status according to blood levels of thiamine and its phosphate esters

	Free T	TMP	TDP	TTP	Total B_1
Type Ⅰa					↑
Type Ⅰb					↓
Type Ⅱa				↑	
Type Ⅱb				↓	
Type Ⅲa			↑		
Type Ⅲb			↓		
Type Ⅳa		↑			
Type Ⅳb		↓			
Type Ⅴa	↑				
Type Ⅴb	↓				

Small "c" is hidden in the table and indicates levels within reference values.

Table 3 Six typical cases of hypo and hyperthiaminosis

	Free T	TMP	TDP	TTP	Total B_1
Type Ⅰb/Ⅲb	7	4	49 ↓	0	60 ↓
Type Ⅱa	2	23	140	7 ↑	172
Type Ⅲb/Ⅴa	192 ↑	5	32 ↓	0	229
Type Ⅳa	4	67 ↑	106	0	178
Type Ⅴa	170 ↑	18	268 ↑	1	457 ↑
(Serum)	185 ↑	22	0	0	207
Type Ⅴb	0 ↓	7	76	0	83

Values are whole blood concentrations except for serum.

obtained by measuring free T after enzymatic hydrolysis of TMP, TDP and TTP by acid phosphatase included as a contaminant in takadiastase. The procedure reported by Kimura and Itokawa[4] is considered to be a candidate reference method of total B_1. However, this procedure could not differentiate thiamine and its phosphate esters. Thiamine esters were measured without the addition of takadiastase[5]. In the latter method, total B_1 concentrations were obtained by calculating the sum of free T, TMP, TDP and TTP. In both methods, assay values were often corrected for red blood cell counts or hemoglobin concentrations in cases of anemia and polycythemia, because hematocrit values changed in those cases[6].

In data from the authors' previous studies, more than a thousand Japanese subjects were classified into ten types according to their thiamine levels in whole blood specimens (Table 2). Small "a" and "b" following the type number meant an elevation and a decrease, respectively. Type Ia is adapted to cases with elevated total B_1 concentrations, in which the differentiation studies of thiamine esters were not conducted. Type Ⅰb is vice versa, but with decreased total B_1 concentrations. From Type Ⅱ to Type Ⅴ, they were exclusively classified by the differentiation studies as an elevation or a decrease of thiamine and its phosphate esters. Type Ⅱa and Ⅱb were defined as an elevation and a decrease of TTP, respectively. Also the same in Type Ⅲ for TDP, Type Ⅳ for TMP and Type Ⅴ for free T. In addition, small "c" is usually hidden in the table, because it means levels within the reference values. Thus, normal subjects represent a type of Ⅰc/Ⅱc/Ⅲc/Ⅳc/Ⅴc. However, cases with Type Ⅱb and Type Ⅳb have not been encountered in our previous studies. Cases with Type Ia and Type Ⅲa were not observed except for those having an unexplained elevation of free T (Type Ⅴa) as will be described later.

Table 3 shows six typical cases of hypo and hyperthiaminosis hitherto observed in healthy subjects. A case with Type Ⅰb/Ⅲb had decreases in both total B_1 and TDP. This case was undoubtedly diagnosed as

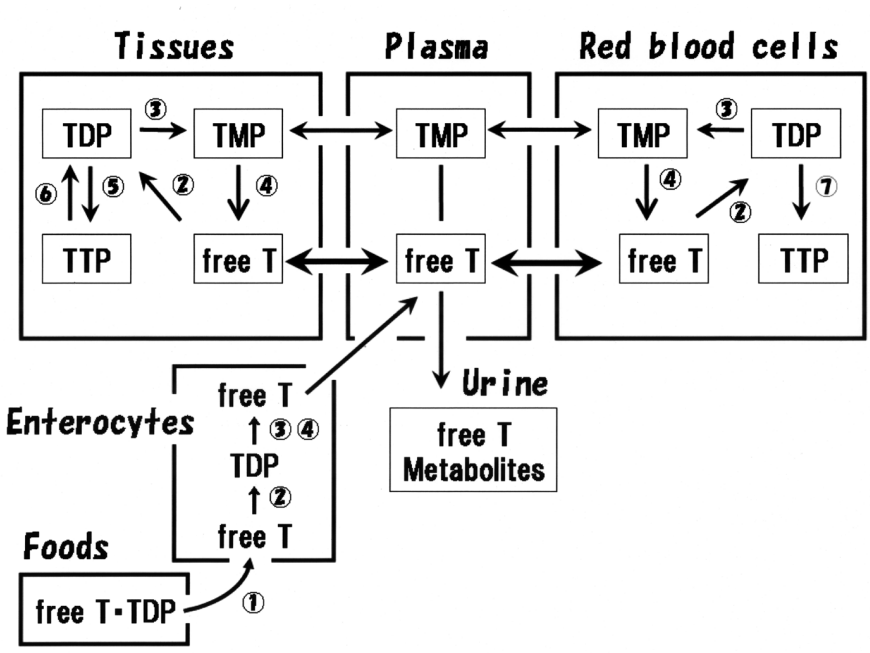

Fig. 1 Distribution of free thiamine from enterocyte to tissue within the body.
Free T: free thiamin, TMP: thiamine monophosphate, TDP: thiamine diphosphate, and TTP: thiamine triphosphate.
① Intestinal alkaline phosphatase (EC 3.1.3.1), ② Thiamine pyrophosphokinase (EC 2.7.6.2), ③ Thiamine pyrophosphatase (EC 3.6.1.15), ④ Thiamine monophosphatase, ⑤ Thiamine-diphosphate kinase (EC 2.7.4.15), ⑥ Thiamine triphosphatase (EC 3.6.1.28), ⑦ Erythrocyte adenylate kinase (EC 2.7.4.3)

hypothiaminosis. A case with Type Ⅱa, which had elevated TTP, was to be further investigate to determine whether any clinical abnormality was present or not, since the TTP present in red blood cells was considered to be formed by adenylate kinase (EC 2.7.4.3, also known as myokinase)[7], not by thiamine-diphosphate kinase (EC 2.7.4.15). A case with Type Ⅲb/Va had decreased TDP and elevated free T. This case was mostly accompanied by normal levels in total B_1. If the case was assessed as Type Ic only by total B_1 measurement, hypothiaminosis was falsely overlooked.

A case with Type Ⅳa had elevated TMP. Although we have almost rarely encountered a case of Ⅳb, Tallaksen et al.[8] reported that TMP was a more sensitive marker of deficiency than TDP and free T. In Type Ⅰb/Ⅲb and Type Ⅲb/Ⅴa, TMP levels were at the lower reference limit, since the only source of TMP was the dephosphorylated TDP (Fig. 1).

A case with Type Ⅴa had elevated free T. This case was accompanied by normal or elevated levels in total B_1. By assay of a serum specimen[9] for the case, free T was revealed to be present one half in serum and the other half in red blood cells; wherein we assumed a hematocrit value of ca. 50%. The authors wondered if the case was the normal status for healthy subjects observed just after taking higher amounts of vitamin B_1; otherwise, the case included clinical symptoms. After a meal, only free T was observed in the intestinal lumen, since its phosphate esters were completely hydrolyzed by intestinal alkaline phosphatase of the gastrointestinal tract. Free T was absorbed from the small intestine by means of two mechanisms: at low intraluminal concentrations less than 2 μmol/L, free T was absorbed via active transport; at higher concentrations, passive transport (diffusion) took place[10]. In the intestinal transport of free T into the enterocytes (Fig. 2), free T was enzymatically esterified to TDP, and then released into blood plasma in circulation as a form of free T. Higher amounts of free T absorbed by passive diffusion would be released into circulation without esterification in the enterocytes. Free T is mainly the transport form of the vitamin. In circulation, free T bound with serum albumin in order to avoid renal excretion, Free T was taken up into red blood cells[11] and also in other tissues followed by successive esterification to TDP in cytoplasm. Free T with higher levels in circulation diffused into these cells without esterification. Free T and its acid metabolites (2-methyl-4-amino-5-pyrimidine carboxylic acid, 4-methyl-thiazole-5-acetic acid and thiamine acetic acid) were excreted principally in the urine[12, 13].

Finally, a case with Type Ⅴb had decreased free

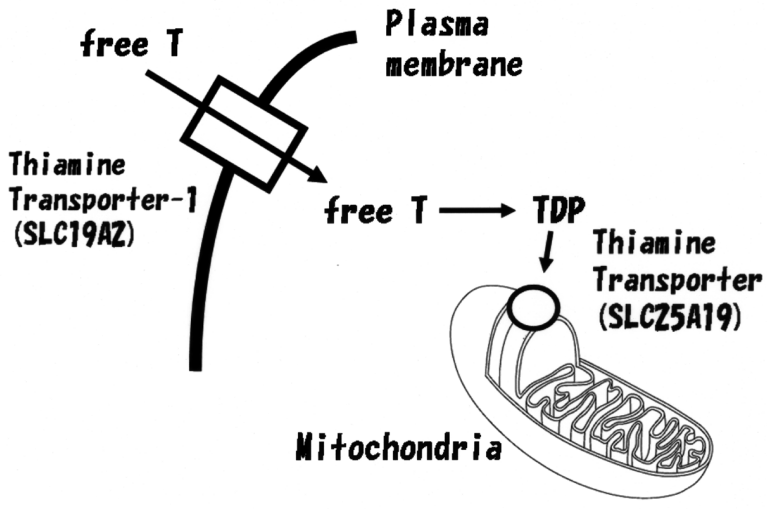

Fig. 2 Thiamine transporters present in plasma membrane of all the cells of the body including red blood cells. Free thiamine was absorbed into cells by transporter-1 via mechanisms of active transport.

T. Since TDP and total B_1 levels were usually close to the lower reference limit, the case needed further investigation as to whether the subject's nutritional status would become poorer thereafter.

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