

Dietary Thiamin Intake and TPP Effect of Elderly Women*

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ABSTRACT

The present study was conducted to assess the dietary and nutritional status of thiamin in forty-two elderly women. Dietary intake was determined by 24 hour recall method and nutritional status of thiamin was determined by measurement of transketolase activity in erythrocytes. Average dietary intake of energy and thiamin were found to be low, and not more than 78% and 63.2% of Recommended dietary allowances(RDA) respectively. Transketolase activity in erythrocytes was distributed in the range of 0.227 - 0.589mU/mgHb and the mean value was 0.434mU/mgHb. The mean value of Thiamin pyrophosphate effect(TPP effect) was 24.0% and the range was from 9.3% to 83.9%. It appeared that 95% of 42 elderly women were severely or marginally deficient in thiamin status, showing 22 persons to be above 20% and 18 persons to be 15 - 20% of TPP effect. Transketolase activity and TPP effect did not show any significant correlation with dietary thiamin intake. (*J Community Nutrition* 2(2) : 141~145, 2000)

KEY WORDS : dietary thiamin · transketolase · TPP effect · the elderly.

Introduction

The importance of nutritional assesment and determination of nutrient requirments in the elderly is emphasized by the steady increase in the size of this population, which appears to be at risk for developing multiple vitamin deficiencies. For a variety of reasons, a percentage of the elderly in both developed and developing nations is at risk of some degree of malnutrition and specific vitamin deficiencies(Darnton-Hill 1992).

It is considered that thiamin is one of the most susceptible vitamin to be deficient marginally or severely. According to National health and nutrition survey report(Shin 2000), daily dietary intake of thiamin in Korea has been known to be enough, showing 126.6% of RDA. However, studies about specific groups have reported a slight difference, showing the distribution from 50 to 131% of RDA(Cho 2000 ; Lee et al. 2000 ; Ro 2000 ; Son et al. 1997 ; Yoon et al. 2000). Also it has been observed that the thiamin nutritional sta-

tus estimated by transketolase assay suggested a high incidence of marginal to deficient status. The results from biochemical assay were not positive as in the survey of dietary intake(Tchai 1977 ; Chang & Kim 1999), although there were just a few studies by biochemical enzymatic assay.

An important issue for older people is the maintenance of cognitive function as well as physiological health. In addition to beri-beri and peripheral neuritis (Iber et al. 1982), thiamin deficiency in men classically induces two types of central nervous system dysfunctions. The first(Wernicke syndrome) is a motor disorder that is associated with clouding of consciousness and typically responds to treatment with large doses of thiamin. The second(Korsakoff psychosis) is associated with defects in new memory and with permanent anatomical lesions. The two conditions tend to merge in the clinical setting and are referred to as the 'Wernicke-Korsakoff syndrome'(Victor et al. 1971). Reductions of thiamin pyrophosphate-dependent enzyme has been observed in thiamin deficiency and in neurodegenerative disorders such as Wernicke-Korsakoff syndrome (Jung et al. 1993) and Alzheimer's disease(Sheu et al. 1996). Thus, a thiamin deficiency has been known to lead to reversible and irreversible brain lesions due to impaired oxidative metabolism. A specific non-cofactor role for thiamin has also been proposed in excitable

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cells, suggesting thiamin triphosphate(TTP) might be involved in the regulation of ion channels(Bettendorff 1996). But recently, scant attention has been paid to thiamin in Korea and its importance has even been underestimated. It is necessary to reconsider the nutritional status of thiamin, especially in the elderly because of thiamin-related neurodegenerative disorders.

Transketolase(EC 2.2.1.1) requires thiamin pyrophosphate and Mg^{2+} for its activity(Jung et al. 1988). The activity of transketolase and TPP effect in hemolysates have been used to determine the nutritional status of thiamin.

In this report, we describe the dietary intake and nutritional status of thiamin in elderly women by 24 hour recall method and transketolase assay in erythrocytes.

Subjects and Methods

Forty-two females, aged over 65 years were recruited through the welfare center for the elderly in Chong-ju area. Height and weight were measured, dietary intake data were obtained through the 24 hour recall method. Subjects who had problems with blood collection were excluded from the study.

The blood was collected using the EDTA treated tube(Vacutainer, Becton Dickinson and Company, NJ). The erythrocytes were separated by centrifugation at $2,000 \times g$ for 10 min, washed three times with saline solution by centrifugation at the same condition as above and kept at $-70^{\circ}C$ until use. To determine the transketolase activity, the hemolysates were prepared with the addition of the same volume of distilled water to the packed red blood cells. The transketolase activity in hemolysates was determined by the formation rate of D-sedoheptulose 7-phosphate by use of ribose 5-phosphate as a substrate(Jung et al. 1991). One hundred microliters of the hemolysates and 80 μ l of 20mM Tris buffer(pH 7.9) were incubated with 20 μ l of 1mM TPP or distilled water at $37^{\circ}C$ for 30min followed by heating at $55^{\circ}C$ for 5min. Then 40 μ l of 36mM ribose 5-phosphate was added and incubated at $37^{\circ}C$ for 30min. The reaction was stopped by the addition of 600 μ l of 7.5% trichloroacetic acid. The following steps were the same as the method of Takeuchi et al.(1984). The values of sam-

ples were obtained from more than duplicate determination. Hemoglobin concentration in hemolysates was determined by the cyan-methemoglobin method with a hemoglobin test kit(Hemo-S reagent, Yeongdong Pharmaceutical Co.).

Thiamin pyrophosphate, D-sedoheptulose 7-phosphate and D-ribose 5-phosphate for transketolase analysis were purchased from Sigma, and all other chemicals were reagent grade materials from commercial sources.

The data analysis was performed using S-plus program. We provided results about mean and standard deviation, frequency and percentages of distribution. Correlation analysis was made by computing Pearson's correlation coefficients to assess the relation of dietary thiamin intake to transketolase activity and TPP effect.

Results and Discussions

General characteristics of the subjects are presented in Table 1. The mean age of subjects was 76.5 years. The mean height and weight were 148.4cm and 49.8 kg, respectively, which are slightly lower than Korean standard values(Recommended Diet Allowances for Korean, 2000). The mean body mass index of the subjects was 22.6, which was in normal range, however two subjects were below 18 and six of subjects were over 27. High and low levels of body mass index have been reported to be associated with poor health among the elderly, even though it is influenced by declining bone mass and changes in hydration of the fat-free body with age(Yoon 2000).

Dietary intake of energy and thiamin based on the 24 hour recall method are presented in Table 2. The RDA for Korean elderly is classified into two age groups, 65-74 years and over 75 years, and in the case of the women, 1,700kcal and 1,600kcal were recommended respectively. Energy intake in this study was found to be very low(78% of RDA), and in 65-74

Table 1. General characteristics

Variables	Mean \pm SD	Standard value
Age(years)	76.5 \pm 5.82	65 -
Height(cm)	148.4 \pm 5.73	152 - 154
Weight(kg)	49.8 \pm 8.30	52 - 54
BMI(kg/m ²)	22.6 \pm 3.28	22.1 - 22.8

Table 2. Dietary intake and percent of RDA for energy and thiamin

Age(years)	N(%)	Calorie(% of RDA)	Thiamin(% of RDA)
66-74	17(40%)	1347.6±343.1(79.3%)	0.638±0.276(63.8%)
75≤	25(60%)	1235.2±220.9(77.2%)	0.627±0.225(62.7%)
Total	42(100%)	1280.7±278.6(78.0%)	0.632±0.244(63.2%)

year-old group showed a little higher intake than over 75 year-old group. There also was a fall in thiamin intake(63% of RDA) but it was found to be satisfiable (98.3%) when compared with RDA of thiamin per each 1000kcal(0.5mg/1,000kcal). The decrease of dietary nutrient intake was accelerated in over 75 year-olds.

Many factors, socio-economic, psychological, ethnic, physiological, and pathological factors, play a role in determining dietary intake(Yoon 2000). In addition to inadequate food intake and malnutrition due to income limitation, other factors including physical disability and inability to chew, may contribute to the risk of inadequate consumption of food in the elderly (Kim et al. 2000). Therefore it is not easy to estimate the nutritional status for the elderly only based on a diet survey. To understand the nutritional status, dietary intake should be informed accurately. However, memory loss and low mental concentration which are common in the elderly, make the interview or survey difficult(Lee et al. 1998).

The assessment of thiamin status improved with the discovery that one of the enzymes of the pentose phosphate pathway, transketolase present in erythrocytes required TPP as a cofactor(Iber et al. 1982). Of all the methods for thiamin measurement, the transketolase assay in erythrocytes has been known as the most practical method to measure thiamin status. The activity of transketolase can be stimulated by adding TPP in vitro to the reaction mixture and that rate of stimulation is designated the 'TPP effect'. The TPP effect is expressed as a percent stimulation of transketolase activity with the in vitro addition of thiamin pyrophosphate, and is supposed to reflect the ratio of apotransketolase. Indeed, low transketolase activity in red blood cells and an unusually large stimulation by adding TPP are used to diagnose thiamin deficiency.

Transketolase activity and TPP effect are shown in Table 3 and the distribution of the TPP effect are shown in Table 4. The activities of transketolase in elderly women were distributed in the range of 0.227

-0.589mU/mgHb and the mean value was 0.434 mU/mgHb. In Korea, there is no available data on transketolase activity to compare with. Chang and Kim(1999) determined the thiamin status in rural area by measurement of transketolase in erythrocytes, however, unfortunately, they showed only TPP effect and not transketolase activity itself. Takeuchi et al.(1988) determined transketolase activity and concentration in hemolysates from young college women and showed the range of 0.4-0.8mU/mgHb, which is slightly higher than in this study. The mean value of TPP effect was 24.0% and the range was broad, from 9.3% to 83.9%. Tchai(1977) showed 14-18% of TPP effect in young college students, and Chang & Kim (1999) showed 49.6% and 30.1% of TPP effect in elderly alcoholics and its control group, respectively.

Usually, above 20 percent of TPP effect has been considered to reflect the state of thiamin deficiency. And from 15 to 20 percent of TPP effect has been considered to reflect marginal deficiency(Tanphaichitr et al. 1970). Forty(95%) of 42 persons were found to be severely or marginally deficient in thiamin status, showing 22 persons(52%) to be above 20 percent and 18 persons(43%) to be from 15 to 20 percent of TPP effect in the present study.

However, this result of biochemical enzymatic assay

Table 3. Transketolase activity and TPP effect in the elderly

Age (years)	Transketolase activity (range)(mU/mg Hb)	TPP Effect(range) (%)
66-74	0.434±0.093	23.8±7.22
75≤	0.434±0.081	24.2±14.65
Total	0.434±0.085 (0.227-0.589)	24.0±12.08 (9.3-83.9)

Table 4. The distribution of TPP effect

TPP effect(%)	n=42(100%)
≤10	1(2.4%)
10 < ≤20	19(45.2%)
20 < ≤30	16(38.1%)
30 < ≤40	4(9.5%)
40 <	2(4.8%)

Table 5. Correlation coefficient among four variables in the elderly

	TKA(- TPP)	TKA(+ TPP)	TPP effect
TKA(+ TPP)	0.8970		
TPP effect	-0.3145	0.1336	
Thiamin intake	-0.0846	0.1054	0.4008

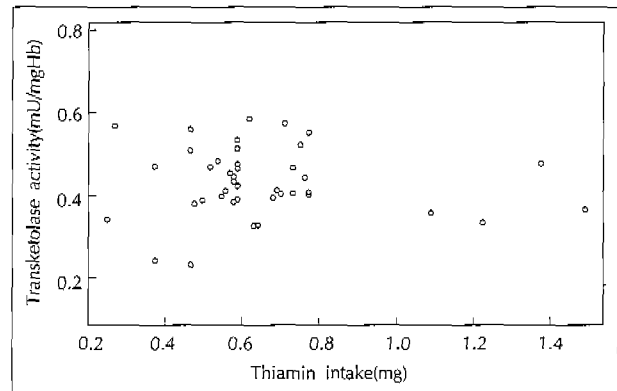
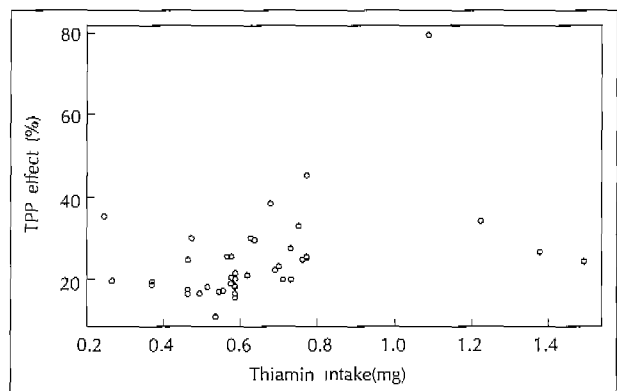
*TKA(- TPP) ; Transketolase activity without in vitro addition of TPP

**TKA(+ TPP) ; Transketolase activity with in vitro addition of TPP

which showed poor thiamin status in most of the subjects, seemed unlikely correlated with the dietary intake of thiamin(Fig. 1, 2). Fig. 1 and Fig. 2 show the distribution of transketolase activity and TPP effect on dietary intake of thiamin respectively.

We tried to compare the thiamin intake with the transketolase activity and TPP effect, since theoretically, transketolase activity and TPP effect are supposed to be decreased when the dietary thiamin intake increased. However, there was marked variation in transketolase activity and TPP effect despite similar intake, and did not show any significant correlation among them(Table 5).

Even though the dietary thiamin intake was much less(0.632mg) than RDA, calculated value of thiamin intake per each 1,000kcal was found to be enough. Therefore, in a sense, it can be considered as an acceptable level. But we found that the thiamin status by transketolase assay was not so desirable as shown in dietary intake data, suggesting either inadequate ingestion or absorption in the elderly. This is in accord with Chang & Kim(1999)'s result which showed that high TPP effect, even when the thiamin intake was high enough level, 1.3mg. This inconsistency can be explained partly by inaccurate information from the survey for the elderly because of memory loss and low mental concentration as mentioned above. And the nutritional status of the elderly is more varied than that of younger adults because of various age-related changes in social, physiological, and pathological status. Thus, the evaluation of nutritional status in the elderly is complicated by many factors that do not significantly affect nutritional status in young adults. It is also plausible that dietary intake of nutrients, which has been estimated mostly by food record or 24 hour recall method for 1 day, may not be able to reflect the chronic nutritional status(Oh & Hong 1998). Fel-

**Fig. 1.** The distribution of transketolase activity on dietary intake of thiamin.**Fig. 2.** The distribution of the TPP effect on dietary intake of thiamin.

dman(1993) also indicated the inadequacy of the current methodology of dietary assessment.

Theoretically, a sample having a low transketolase activity should have a high TPP effect. However some people, especially alcoholics, often showed a low activity with a low TPP effect. This discrepancy has been supposed to be derived from a lack of apoenzyme or inability of apoenzyme to combine with coenzyme. To clarify this, determination of enzyme protein content and mRNA level as well as activity of transketolase will be helpful. Actually, there were several reports on the determination of transketolase protein by western blotting assay(Takeuchi 1988 ; Jung 1991), and mRNA level(Calingasan NY et al. 1995 ; Sheu KFR et al. 1996).

The neurological damage in thiamin deficiency has been intensively studied. It has been suggested that thiamin is related to Alzheimer's disease as well as Wernicke-Korsakoff syndrome(Jung et al. 1993) and interestingly to the performance of a learning task (Terasawa et al. 1999). Therefore, thiamin should be

more emphasized, especially in the elderly, who are susceptible to neurodegenerative disorders.

Conclusions

To evaluate the thiamin status of elderly women, dietary intake and TPP effect were investigated. It appeared that almost every subject(except 3 elderly persons) did not achieve the RDA of energy and thiamin, so that average dietary intakes of energy and thiamin were only 78% and 63.2% of RDA, respectively. Nevertheless, the thiamin intake for each 1,000kcal seemed adequate. However, thiamin status judged by transketolase activity in erythrocytes seemed very poor showing up to 95% of the subjects had marginal or severe deficiency in thiamin status. From these findings, we suggest that there is a need to further emphasis on thiamin especially in the elderly, because of its possible function in age-related neurological diseases. There was no significant correlation between thiamin intake and transketolase activity or TPP effect, indicating 24 hour recall method may not be appropriate enough to reflect chronic nutritional status.

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