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·Original Article·

Correlation of thiamine metabolite levels with cognitive function in the non-demented elderly

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ABSTRACT

Thiamine metabolism is critical for glucose metabolism and also vital for brain function, which is susceptible to decline in the elderly. This study aimed to investigate whether thiamine metabolites correlate with cognitive function in the non-demented elderly and their impact factors. Volunteers >60 years old were recruited and their blood thiamine metabolites and Mini-Mental State Examination (MMSE) scores were measured. The apolipoprotein E (*APOE*) genotype, routine blood parameters, liver and kidney function, and levels of fasting blood glucose and triglycerides were also measured. The results showed that the thiamine diphosphate (TDP) level weakly correlated with MMSE score in the non-demented elderly. Participants with high TDP levels performed better in *Recall* and *Attention and Calculation* than those with low TDP. TDP levels were associated with the *APOE* ε2 allele, body mass index, hemoglobin level, fasting blood glucose, and triglycerides. Our results suggest that TDP, which is easily affected by many factors, impacts cognitive function in the elderly.

Keywords: thiamine diphosphate; cognitive function; non-demented elderly

INTRODUCTION

The human brain accounts for only 2% of body weight but consumes nearly 25% of the total body glucose in the resting awake state[1]. Since glucose is the predominant energy substrate for oxidative processes in the brain[2], appropriate glucose metabolism is vital for sustaining brain functions. The elderly are susceptible to a decline in brain functions[3, 4]. Further, the severity of cognitive impairment, the most common manifestation of declining brain function, is significantly correlated with a reduction in brain glucose metabolism[5–8]. Clarifying the cause and mechanism of reduced brain glucose metabolism in the elderly will help in the search for preventive and therapeutic target(s) against cognitive impairment.

Thiamine metabolism is vital for glucose metabolism. Thiamine diphosphate (TDP), the active form of thiamine, is a critical coenzyme of three key enzymes in glucose metabolism: pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase complex in the Krebs cycle, and transketolase in the pentose phosphate pathway. It has been demonstrated that the elderly tend to suffer from thiamine deficiency with aging itself, rather than co-existent illnesses[9]. Patients with Alzheimer's disease (AD), the most common type of dementia, have reduced TDP content, reduced activity of thiamine-dependent enzymes[10–12], and brain glucose hypometabolism. Therefore, abnormal thiamine metabolism may be a pathogenic factor for the declined brain glucose metabolism in the elderly.

However, studies on the correlation between thiamine metabolism and cognitive function in the non-demented elderly are insufficient and the results are controversial[13–15]. The inconsistent results may be partially attributed to the

small sample sizes in some studies. Also, the methodology for determining the content of thiamine and its derivates needs to be considered. The previous studies mainly evaluated the thiamine metabolites indirectly, using methods such as the erythrocyte transketolase activity assay that records the difference in transketolase activity before and after addition of TDP as a consequence of diminished coenzyme concentration[13, 15]. The precise levels of thiamine and its derivates and the extent of thiamine deficiency are unknown. Recently, high-performance liquid chromatographic (HPLC) fluoroscopy has been used to determine the levels of free thiamine, thiamine monophosphate (TMP), and TDP in whole blood, and has proved to be rapid, accurate, and convenient[16, 17].

So far, little is known about the status of thiamine metabolites in the non-demented elderly in China, which has the largest and most rapidly ageing population in the world. The aim of this study was to investigate the correlation of thiamine metabolites with cognitive function in the non-demented elderly living on Xujiahui Street in the Xuhui District of Shanghai, China. In addition, as multiple factors such as apolipoprotein E *(APOE)* genotype, age, and educational background are involved in cognitive function, we also analyzed the relationship between TDP levels and these factors.

PARTICIPANTS AND METHODS

Participants

Volunteers were recruited from the Physical Examination Department at the Regional Health Service Center of Xujiahui from April 1 to December 31, 2012. Participants were >60 years old and lived on Xujiahui Street, Xuhui District, Shanghai, China. All participants gave informed consent. Information on history of diseases, current medications, and supplementation with vitamins and other health-care products was collected by questionnaire. Participants with major disorders of the gastrointestinal

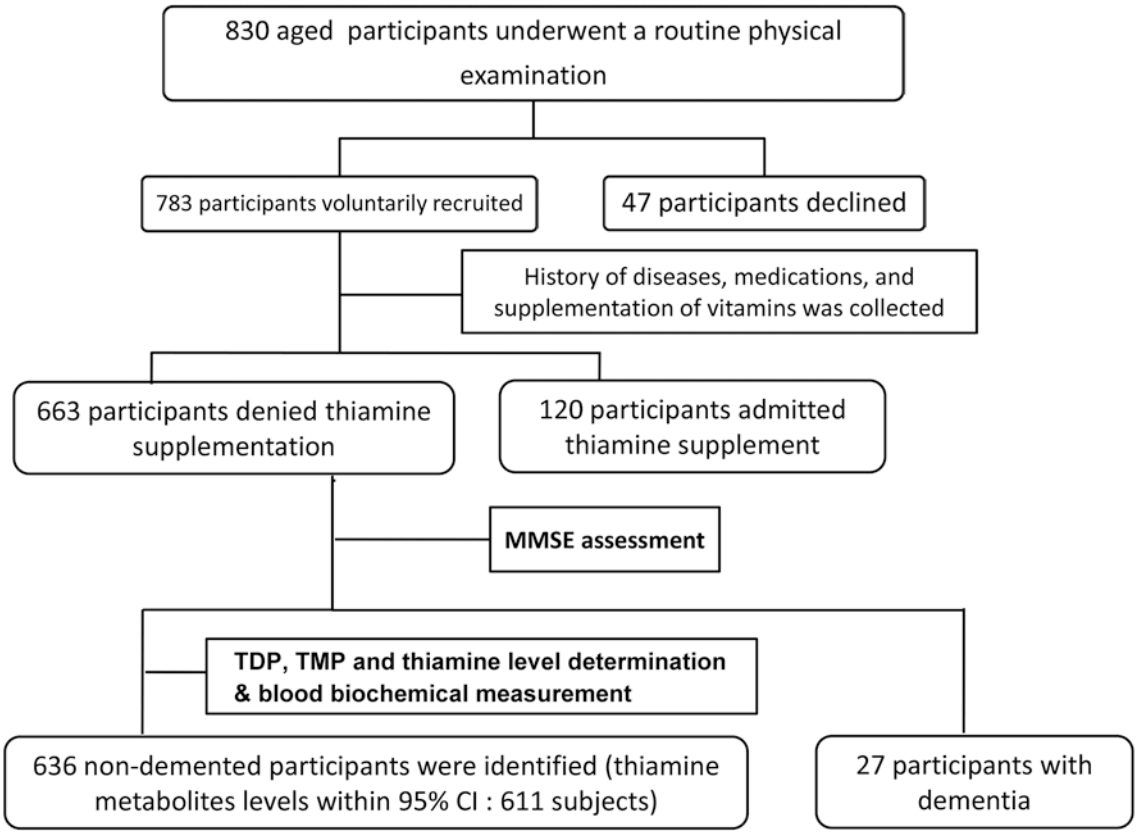

Fig. 1. Flowchart of this study.

Table 1. Demographic and clinical characteristics of participants with normal cognitive function (*n*=611, mean ± SD)

Variables	Value (range)
Age (years)	72.30 ± 5.89 (63–91)
Males (%)	263 (43.04)
MMSE scores	27.83 ± 2.38 (18–30)
Education (years)	9.27 ± 4.96 (0–16)
TDP (nmol/L)	118.60 ± 23.25 (61.75–189.2)
TMP (nmol/L)	3.70 ± 2.16 (0–12.16)
Thiamine (nmol/L)	3.47 ± 2.80 (0–29.65)
Hemoglobin (g/L)	134.1 ± 13.72 (83.0–169.0)
Fasting plasma glucose (mmol/L)	6.04 ± 1.56 (4.01–15.82)
Triglyceride (mmol/L)	1.53 ± 0.98 (0.33–11.32)
Alanine aminotransferase (U/L)	22.19 ± 10.78 (6.7–91.6)
Creatinine (mmol/L)	66.02 ± 21.55 (5.64–307.1)
BMI (kg/m^2)	23.92 ± 3.13 (13.31–34.03)
ApoE genotype (%)	
ε2/ε2	*n* = 20 (3.27)
ε2/ε3	*n* = 66 (10.80)
ε2/ε4	*n* = 17 (2.78)
ε3/ε3	*n* = 414 (67.76)
ε3/ε4	*n* = 88 (14.40)
ε4/ε4	*n* = 6 (0.98)

tract, chronic alcohol abuse, or thiamine supplementation during the previous month were excluded from this study.

A flowchart of the study protocol and demographic information on the participants are shown in Fig.1 and Table 1. The study design and procedures were approved by the Committee on Medical Ethics of Zhongshan Hospital, Fudan University.

Clinical Assessments

All participants completed the Mini-Mental State Examination (MMSE, Chinese version) as a measure of general cognitive function, with scores ranging from 0 (severe impairment) to 30 (no impairment). The non-demented elderly were identified according to the MMSE score adjusted for educational background: illiterate individuals with MMSE scores >17, participants with a preliminary school diploma and scores >21, and participants with >9 years of education and scores >24. Individuals who did not meet the above criteria were excluded. Routine blood parameters, liver and kidney functions, and levels of fasting blood glucose (FBG) and triglycerides were measured. Height and weight were used to calculate body mass index (BMI).

Determination of Thiamine, TMP, and TDP Levels

The levels of thiamine, TMP, and TDP were measured using HPLC as previously described in detail[18]. Briefly, 150 μL of 7.4% perchloric acid was immediately added to 150 μL of fresh blood anti-coagulated with heparin. After mixing by vibration for 30 s for full deproteinization, the mixture was centrifuged at 10 000 rpm for 6 min at 4°C. Then, the supernatant was collected and stored at −20°C until determination. Thiamine and its phosphate esters were derivatized into thiochromes using potassium ferricyanide and separated by gradient elution on a C18 reversed-phase analytical column (250 mm×4.6 mm). The derivatives were measured by HPLC fluoroscopy (Agilent 1100, Santa Clara, CA) at 367 nm excitation and 435 nm emission. Thiamine, TMP, and TDP levels were quantified using standard samples of each compound (Sigma-Aldrich, St. Louis, MO).

Apolipoprotein E Genotype Analysis

APOE alleles were detected using the ABI real-time Taqman SNP genotyping assay (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Human genomic DNA was extracted from blood anti-coagulated with heparin using a genome extraction kit (Tiangen Biotech, Beijing, China). All participants were divided into two subgroups by the *APOE* geneotype: according to the presence of *APOE* ε2 allele, an *APOE* ε2 positive group with the ε2/ε2, ε2/ε3, or ε2/ε4 genotype and a negative group with the ε3/ε3, ε3/ε4, or ε4/ε4 genotype; according to the presence of *APOE* ε4 allele, an *APOE* ε4-positive group with the ε2/ε4, ε3/ε4, or ε4/ε4 genotype and an *APOE* ε4-negative group with the ε2/ε2, ε2/ε3, or ε3/ε3 genotype.

Statistical Analysis

SPSS software (version 18.0; SPSS Inc., Chicago, IL) was used for statistical analyses. Pearson correlation analysis was used to assess the correlation between thiamine metabolite levels and MMSE scores. Two-tailed Student's

t-test or one-way analysis of variance (ANOVA) was used to assess differences in TDP, TMP, and thiamine levels. All data are shown as mean ± SD, with statistical significance set at <0.05.

RESULTS

Participants

Eight hundred and thirty individuals underwent routine physical examination in the Regional Health Service Center of Xujiahuiin, Shanghai and 783 agreed to participate in this study. Among these, 120 had a history of supplementation with thiamine and its analoguesin the last month and were excluded. Six hundred and sixty-three individuals then received MMSE evaluation and 27 with low scores were excluded. Finally, the blood thiamine, TMP, and TDP levels were measured in 636 non-demented individuals. Based on the 95% confidence intervals (CI) of blood thiamine, TMP, and TDP levels, 611 participants were enrolled in our study. Detailed demographic information on the participants is shown in Table 1.

TDP Level Weakly Correlated with MMSE Score

TDP concentration showed a weakly-positive correlation with MMSE score (r = 0.1492, P <0.001, Fig. 2A), while the TMP and thiamine levels did not (TMP: r = 0.0233,

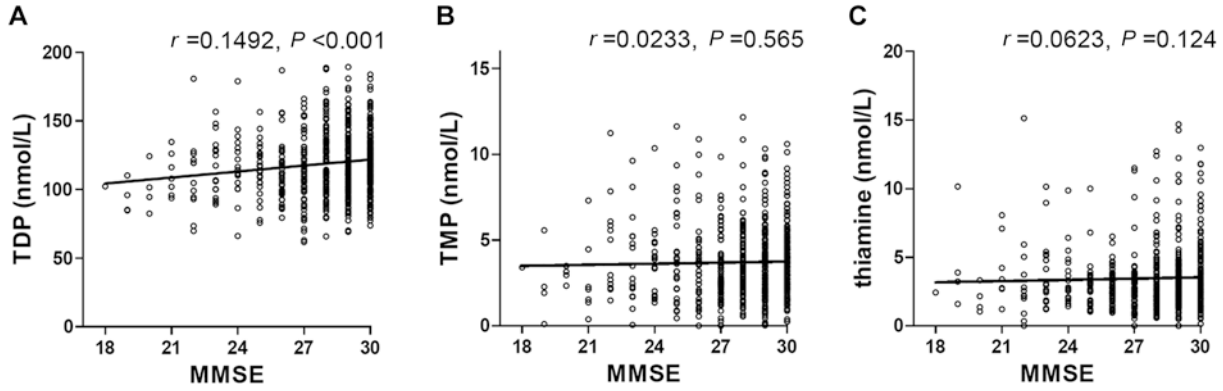

Fig. 2. TDP concentration shows a positive correlation with MMSE. A. TDP concentration showed a weak but significantly positive correlation with MMSE score. B. TMP concentration had no significant correlation with MMSE score. C. Thiamine concentration had no correlation with MMSE score.

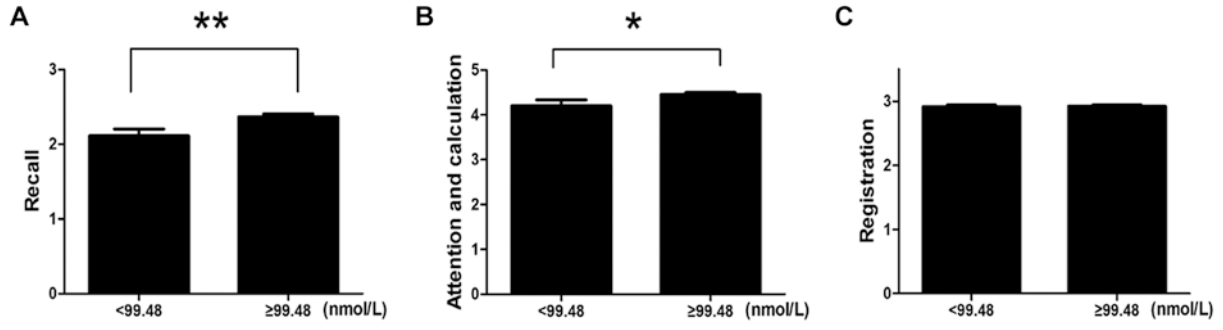

Fig. 3. A high TDP level is associated with better cognitive performance. A. Participants with high TDP levels (≥99.48 nmol/L, *n* = 485) performed better in *Recall* than those with low TDP levels (<99.48 nmol/L, *n* = 126, 2.37 ± 0.04 *vs* 2.11 ± 0.09, *P* <0.01). B. Participants with high TDP levels performed better in *Attention and Calculation* than those with low TDP levels (4.45 ± 0.05 *vs* 4.20 ± 0.14, *P* <0.05). C. There was no significant difference in *Registration* between participants with high TDP levels and low TDP levels (2.93 ± 0.01 *vs* 2.91 ± 0.03, *P* >0.05).

Table 2. Effects of age, gender, BMI, blood hemoglobin, fasting plasma glucose, and triglyceride levels on blood thiamine metabolite values

	TDP (nmol/L)	TMP (nmol/L)	Thiamine (nmol/L)
APOE genotype			
ε2 (ε2/ε2, ε2/ε3, ε2/ε4, *n* = 103)	123.1 ± 23.52	3.93 ± 2.26	3.62 ± 2.79
non-ε2 (ε3/ε3, ε3/ε4, ε4/ε4, *n* = 508)	117.7 ± 23.12	3.66 ± 2.14	3.44 ± 2.80
P	0.0334	ns	ns
ε4 (ε2/ε4, ε3/ε4, ε4/ε4, *n* = 111)	117.9 ± 22.94	3.41 ± 2.01	3.44 ± 2.44
non-ε4(ε2/ε2, ε2/ε3, ε3/ε3, *n* = 500)	118.8 ± 23.34	3.77 ± 2.19	3.47 ± 2.88
P	ns	ns	ns
Age (years)			
≤70 (*n* = 271)	121.9 ±22.81	3.96 ± 2.17	3.47 ± 2.56
71-80 (*n* = 288)	116.8 ± 22.89	3.53 ± 2.14	3.50 ± 3.07
>80 (*n* = 52)	112.0 ± 25.36	3.32 ± 2.10	3.29 ± 2.41
P	0.0033	0.0275	ns
Gender			
Male (*n* = 263)	118.9 ± 23.02	3.19 ± 1.86	2.96 ± 2.28
Female (*n* = 348)	118.4 ± 23.46	4.09 ± 2.28	3.85 ± 3.08
P	ns	<0.001	<0.001
Educational background			
<9 years (*n* = 169)	114.3 ± 23.93	3.68 ± 2.42	3.57 ± 3.60
≥9 years (*n* = 442)	120.3 ± 22.79	3.71 ± 2.05	3.43 ± 2.43
P	0.0042	ns	ns
BMI (kg/m^2)[§]			
<20 (*n* = 57)	110.5 ± 21.64	3.82 ± 2.07	3.91 ± 2.56
20-25 (*n* = 327)	118.8 ± 23.49	3.88 ± 2.20	3.45 ± 2.93
>25 (*n* = 204)	121.5 ± 22.86	3.52 ± 2.14	3.42 ± 2.72
P	<0.0064	ns	ns
Hemoglobin (g/L)			
<120 (*n* = 71)	104.2 ± 22.61	3.45 ± 2.03	3.68 ± 2.51
≥120 (*n* = 540)	120.6 ± 22.68	3.74 ± 2.17	3.44 ± 2.84
P	<0.001	ns	ns
Fasting blood glucose			
<6.1 mmol/L (*n* = 439)	116.7 ± 23.48	3.71 ± 2.18	3.34 ± 2.70
≥6.1 mmol/L (*n* = 172)	123.5 ± 21.99	3.69 ± 2.11	3.78 ± 2.02
P	0.0011	ns	ns
Triglycerides (mmol/L)			
≤1.7 (*n* = 449)	116.4 ± 22.91	3.71 ± 2.10	3.43 ± 2.73
>1.7 (*n* = 162)	124.8 ± 23.13	3.67 ± 2.31	3.56 ± 3.00
P	<0.001	ns	ns

[§]BMI: Height and weight data not available for 23 participants.

P = 0.565; thiamine: *r* = 0.0623, *P* = 0.124, Fig. 2B, C). We further analyzed the MMSE scores on sub-items in different subgroups divided according to TDP level. Based on the TDP cut-off level of 99.48 nmol/L (taken as the lower value to distinguish AD from controls as calculated by ROC analysis, unpublished data), participants with high TDP levels (≥99.48 nmol/L, *n* = 485) performed better in *Recall*, and *Attention and Calculation* than those with low TDP levels (<99.48 nmol/L, *n* = 126; *Recall*: 2.37 ± 0.04 *versus* 2.11 ± 0.09, *P* <0.01; *Attention and Calculation*: 4.45 ± 0.05 *vs* 4.20 ± 0.14, *P* <0.05). There was no significant difference in *Registration* between the two groups (2.93 ± 0.01 *vs* 2.91 ± 0.03, *P* >0.05, Fig. 3A–C).

Effects of *APOE* Genotype, Age, Gender, Educational Background, BMI, Hemoglobin, FBG, and Triglycerides on the Blood Levels of Thiamine Metabolites

To assess the possible metabolic and social factors that affect the status of thiamine metabolism in non-demented elderly people, we investigated the effects of *APOE* genotype, age, gender, educational background, BMI, hemoglobin, FBG, and triglycerides on the levels of blood thiamine metabolites. The frequency of the *APOE* ε2 allele was 16.86% (103/611: ε2/ε2, 20; ε2/ε3, 66; ε2/ε4,17). TDP levels in *APOE* ε2-positive participants were significantly higher than those in *APOE* ε2-negative participants. One hundred and eleven participants were *APOE* ε4 carriers (ε2/ε4,17; ε3/ε4,88; ε4/ε4, 6). There was no significant difference in TDP level between *APOE* ε4-positive and ε4-negative participants. There were no significant differences in TMP and thiamine levels between participants positive or negative for the *APOE* ε2 or ε4 allele (Table 2).

We divided the participants into three subgroups according to age: ≤70 years, *n* = 271; 71–80, *n* = 288; >80, *n* = 52. Blood TDP and TMP concentrations showed a significantly decreased trend with aging (Table 2). TDP levels were not significantly different between male and female participants (Table 2). Surprisingly, both TMP and thiamine concentrations were lower in male than in female participants (both *P* <0.001). Participants with a better educational background tended to have higher TDP levels than those with relatively little education (*P* <0.01, Table 2).

Blood TDP levels in participants with a higher BMI were higher than that in those with a lower BMI (*P* <0.01). Since TDP is mostly present in red blood cells, we further analyzed whether blood hemoglobin level affects the TDP level. We found that TDP levels were significantly higher in participants with hemoglobin levels >120 g/L than in those with <120 g/L (*P* <0.001). In addition, TDP levels were significantly lower in participants with FBG levels <6.1 mmol/L than in those with >6.1 mmol/L (*P* <0.05). However, there were no significant differences in TMP and thiamine levels between the two subgroups. TDP levels in participants with triglyceride levels <1.7 mmol/L were significantly lower than in those with >1.7 mmol/L (*P* <0.001). Also, there were no significant differences in TMP and thiamine levels between participants with triglycerides >1.7 mmol/L and <1.7 mmol/L (Table 2).

DISCUSSION

Cerebral glucose hypometabolism is an early change and correlates with the severity of cognitive decline in individuals with mild cognitive impairment (MCI) and patients with dementia[8, 19-21]. As a key coenzyme in glucose metabolism, TDP has been reported to decrease in AD, the most common type of dementia[22-24]. The activities of TDP-dependent enzymes are also significantly lessened in AD. Studies have revealed that the activity of brain α-ketoglutarate dehydrogenase complex declines >75%, TK activity declines >45%, and pyruvate dehydrogenase complex activity declines up to 41% in AD patients[10, 25]. Further, mild to moderate thiamine deficiency is prevalent in elderly people[26-28]. Hence, it is imperative to clarify the correlation between the levels of thiamine metabolites and cognitive function, which may provide a target to prevent cognitive decline in non-demented elderly.

In this study, we found a significantly positive correlation between TDP levels and MMSE scores in the non-demented elderly (Fig. 2). This result is consistent with that in the studies of La Rue, Ortega, and Requejo[13, 15, 29]. More importantly, further analysis of the sub-items of MMSE showed that participants with high TDP levels performed better in *Recall,* and *Attention and Calculation*, but not in *Registration* (Fig. 3). Previous studies have demonstrated that the test of delayed recall can discriminate individuals with MCI and very mild AD from normal elderly with high accuracy[30-32]. Impaired delayed memory is also an excellent predictor of conversion from MCI to AD[33, 34]. Hence, sustaining normal or even higher TDP levels in the

elderly may help to maintain delayed recall and prevent long-term memory decline.

The *APOE* gene is one of the most common genetic factors associated with dementia[35], especially AD, and the *APOE* ε4 allele is a risk factor for AD[36, 37]. Meanwhile, the *APOE* ε2 allele has a protective effect against cognitive decline compared to the *APOE* ε3 and ε4 alleles[38-41]. In the current study, the TDP levels in *APOE* ε2-positive participants were significantly higher than those in ε2-negative participants, but there was no significant difference in TDP levels between *APOE* ε4-positive and ε4-negative participants (Table 2). However, cognitive status did not differ between *APOE* ε2-positive and ε2-negative participants (Fig. S1), which suggested that the effect of the *APOE* ε2 allele on TDP levels is independent of cognitive function. Further exploration is needed to clarify the relationship between the *APOE* genotype and thiamine metabolism.

Aging appeared to have a negative effect on the levels of thiamine metabolites. We found that the levels of blood TDP and TMP, but not thiamine, decreased with aging in the non-demented elderly (Table 2), consistent with previous studies[26-28]. A more balanced or improved diet pattern with thiamine-enriched food should be advocated for the elderly.

In addition, no significant difference in blood TDP levels was found between male and female participants (Table 2). But surprisingly, blood TMP and thiamine levels were significantly higher in females than in males. The mechanism by which gender impacts the levels of thiamine metabolite needs to be clarified.

Recently, Qizilbash and colleagues found that underweight people (BMI <20 kg/m^2) have a 34% higher risk of dementia than those with a healthy weight[42]. In our study, BMI had a substantially positive effect on blood TDP concentration, which was positively correlated with cognitive function. In addition, we found that hemoglobin levels were correlated with TDP concentrations. TDP levels were higher in the subgroup with hemoglobin levels >120 g/L than in the subgroup with hemoglobin levels <120 g/L (Table 2). These results reflect the fact that blood TDP mainly exists in red blood cells.

In summary, we found a positive correlation between blood TDP levels and cognitive function in non-demented elderly Chinese, especially in *Recall* and *Attention and Calculation*. We also investigated the effects of the *APOE* ε2 allele, BMI, and other factors on the levels of thiamine metabolites. Considering its critical role in cerebral glucose metabolism, we conclude that the levels of TDP, the active form of thiamine that is easily affected by multiple factors, correlate with cognitive function and provide a potential target against cognitive decline in the elderly. However, the status of thiamine metabolism *in vivo* is complicated and easily affected by many biological and social factors. When we used a multiple linear regression model to analyze the impact factors for TDP levels, the results had some minor differences from those deduced from simple regression (Table S1), such as gender. This may be partly due to the differences in age and educational level between male and female participants. In future studies, analysis by multiple linear regression should be conducted in a larger population to explore in more detail the relationships between TDP level and its impact factors.

ELECTRONIC SUPPLEMENTARY MATERIAL

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s12264-015-1563-3.

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