行政院國家科學委員會專題研究計畫　成果報告

探討維生素 B6 營養狀況及補充劑的介入對血漿同半胱胺酸及冠狀動脈疾病相關危險因子的影響(2/2)

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行政院國家科學委員會補助專題研究計畫期末報告

探討維生素 B6 營養狀況及補充劑的介入對血漿同胱胺酸及冠狀動脈疾病相關危險因子的影響 (2/2)

The effect of vitamin B6 status and supplementation on plasma homocysteine

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□**國際合作研究計畫國外研究報告書一份**

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中文摘要

高同半胱胺酸血症已被證實為心血管疾病的獨立危險因子，目前許多研究已指出補充維生素 B_{12} **或葉酸有助於降低血漿同半胱胺酸的濃度，但維生素** B_6 **與血漿同半胱胺酸濃度的相關性卻未獲得證實。本研究目的為探討冠狀動脈心臟病的病人，給予維生素** B_6 **補充劑的介入是否能降低血漿同半胱胺酸濃度。本實驗的受試者為患有冠狀動脈心臟病的病人，以隨機的方式分為五組。最後五組人數分別為**(1) placebo (n=8)，(2) **維生素** B_6 5 mg/d (n=8)，(3) **維生素** B_6 10 mg/d (n=8)，(4) **維生素** B_6 50 mg/d (n=9)，(5) **維生素** B_{12} 0.25 mg/d **及葉酸** 5 mg/d(n=9)，**介入** 12 **週。研究結果顯示介入** 12 **週後，任一劑量維生素** B_6 **補充劑的介入，對降低血漿同半胱胺酸的濃度並無作用。值得注意的是，補充** placebo、**維生素** B_6 10 mg/d **及維生素** B_6 50 mg/d **的組別，雖未達統計上的差異，但其** 12 **週後的血漿同半胱胺酸濃度分別有增加** 25.5%、16.2%、18.3%**的傾向。相反的，維生素** B_{12} **及葉酸的介入，則可顯著地降低同半胱胺酸的濃度約** 32% (p<0.001)**。本研究結果顯示當單獨使用維生素** B_6 **補充劑的介入而無維生素** B_{12} **及葉酸時，對於冠狀動脈心臟病的病人降低血漿同半胱胺酸的濃度並無影響。**

關鍵詞：同半胱胺酸、磷酸比哆醛，維生素 B_{12}**，葉酸，補充劑，冠狀動脈疾病**

英文摘要

The purpose of this study was to investigate whether vitamin B-6 supplementation had a beneficial effect on lowering fasting plasma homocysteine concentrations in coronary artery disease (CAD) patients. A single-blind intervention study. The study was performed at the Taichung Veterans General Hospital, the central part of Taiwan. Fifty subjects were identified by cardiac catheterization to have at least 70% stenosis of one major coronary artery. Forty-two patients successfully completed this study. Patients were randomly assigned to 1 of 5 groups

and treated with a daily dose of placebo (n = 8), 5 mg vitamin B-6 (n = 8), 10 mg vitamin B-6 (n = 8), 50 mg vitamin B-6 (n = 9), or 5 mg folic acid combined with 0.25 mg vitamin B-12 (n = 9) for 12 weeks. Nutrient intakes were recorded by using 24-hour diet recalls when patients returned to the cardiology clinic before the intervention (week 0) and at week 12. Vitamin B-6 status was assessed by direct measures (plasma pyridoxal 5'-phosdphate) and indirect measures (erythrocyte alanine and aspartate aminotransaminase activity coefficient). Fasting plasma homocysteine, serum folic acid and vitamin B-12 were measured.
Fasting plasma homocysteine concentration did not respond to high or low doses of vitamin B-6 when compared with a placebo treatment after 12 weeks of supplementation. Mean fasting plasma homocysteine concentration, however, decreased significantly after 12 weeks of folic acid combined with vitamin B-12 supplementation ($p = 0.047$). Further, within group, mean fasting plasma homocysteine concentration was nonsignificantly increased by 25.5%, 16.2%, and 18.3% in placebo, 10 mg/d and 50 mg/d vitamin B-6 supplemented groups, respectively; whereas folic acid combined with vitamin B-12 supplementation significantly reduced fasting plasma homocysteine concentration by 32% ($p < 0.001$). Our results indicate that vitamin B-6 supplementation alone is less effective than folic acid combined with vitamin B-12 in lowering plasma homocysteine concentrations in CAD patients.

Introduction

Studies have shown that hyperhomocysteinemia is an independent risk factor for coronary heart disease (CAD) (Stampfer *et al*, 1992; Boushey *et al*, 1995; Rydlewicz *et al*, 2002; Ford *et al*, 2002, Bautista *et al*, 2002). Many factors are associated with elevated homocysteine concentration. Of the most interests are nutritional deficiencies in the vitamin cofactors that are required for homocysteine metabolism; namely folic acid, vitamin B-12 and B-6. Most cross-sectional, retrospective, case-controlled, and prospective cohort studies (Lindenbaum *et al*, 1990; Boushey *et al*, 1995; Morrison *et al*, 1996; Verhoef *et al*, 1996; Bailey *et al*, 2002) have shown that folic acid and vitamin B-12 intake and/or biochemical status have a strong negative correlation with fasting plasma homocysteine concentration. A recent meta-analysis indicated that dietary supplementation in the range of 0.5 to 5 mg/d folic acid reduced homocysteine concentrations by 25% (95% CI: 23 – 28%); mean 0.5 mg/d vitamin B-12 intake produced an additional 7% (95% CI: 3 – 10%) reduction in homocysteine levels (Clarke & Armitage, 2000). Thus, it is suggested that folic acid combined with vitamin B-12 supplementation may be protective via lowering fasting plasma

homocysteine concentration.

There was, however, no consistent evidence to show the effect of vitamin B-6 on lowering fasting plasma homocysteine concentration. In the Physicians' Health Study, plasma pyridoxal 5'-phosphate (PLP) was inversely correlated with homocysteine (r=-0.29, $p < 0.001$) (Chasan-Taber *et al*, 1996). In a recent randomized, double-blind, placebo-controlled trial, vitamin B-6 (1.6 mg/d) was given for 12 weeks to 11 healthy elderly subjects after repletion with folic acid and riboflavin. Results showed that vitamin B-6 supplementation significantly reduced fasting plasma homocysteine concentration by 7.5% (McKinley *et al*, 2001). In contrast, some studies showed vitamin B-6 had no effect on fasting plasma homocysteine concentration. Miller *et al* (1992) indicated that total fasting plasma homocysteine concentrations were not initially elevated in vitamin B-6 deficient humans and rats. Brattström *et al* (1990) studied homocysteine metabolism in 72 patients with occlusive arterial disease. Authors found plasma PLP was decreased in most patients but there was no correlation between plasma PLP and homocysteine concentration. In addition, 20 patients receiving pyridoxine hydrochloride (240 mg/d) alone for 2 weeks did not show a decrease in basal fasting plasma homocysteine concentration (Brattström *et al*, 1990). In a placebo-controlled study, pyridoxine dose (10 mg) did not significantly reduce fasting plasma homocysteine concentrations (Ubbink *et al*, 1994). A recent study reported that patients with cardiovascular disease had significantly lower plasma PLP than those of controls; however, fasting plasma homocysteine concentrations did not correlate with PLP levels (r = -0.03, $p > 0.05$) (Chan *et al*, 2002). It seems that the requirement for vitamin B-6 on lowering homocysteine needs further investigation.

Although the effect of vitamin B-6 supplementation on homocysteine is unclear at present, low vitamin B-6 status has been demonstrated to be an independent risk factor for cardiovascular disease (Serfontein *et al*, 1985; Robinson *et al*, 1995; Chan *et al*, 2002). The present study was undertaken to ascertain whether various doses of vitamin B-6 supplementation would have beneficial effects on lowering fasting plasma homocysteine concentrations in CAD patients.

Subjects and methods

Patients

A single-blind study was conducted from January 2002 to December 2002. Patients were recruited from the cardiology clinic of the Taichung Veteran General Hospital, which is a 1359-bed teaching hospital in the central part of Taiwan. Study patients were identified by cardiac catheterization to have at least 70% stenosis of one

major coronary artery. All subjects with diabetes (defined by history of antidiabetic drugs use, or fasting plasma glucose concentration > 140 mg/dL), liver or renal diseases (identified by serum creatinine, and aspartate aminotransferase analyses) were excluded to minimize the influence of other cardiovascular risk factors. Subjects currently taking B-vitamin supplements were also excluded. The use of medications was recorded. None of our subjects had acute myocardial infarction within the past six months. Fifty patients (46 men and 4 women) with the mean age of 71.6 ± 9.7 y enrolled after informed consent was obtained. The study was approved by the Committee for Ethics of Chung Shan Medical University.

Experimental protocol

Patients were randomly assigned to 1 of 5 groups: placebo (n = 10), 5 mg vitamin B-6 (pyridoxine HCl) (n = 10), 10 mg vitamin B-6 (n = 10), 50 mg vitamin B-6 (n = 10), or 5 mg folic acid combined with 0.25 mg vitamin B-12 (hydroxocobalamin) (n = 10). The vitamin tablets were commercially available preparations (Chin-Teng Pharmaceutical Industrial Co., Ltd., Taichung, Taiwan). Intervention was administered for 3 months. Patients were instructed to take one tablet daily after dinner or before going to bed and to refrain from using any other vitamin supplements during the study period. To monitor the compliance, investigators called every other week to remind patients to take the tablet. Patients returned to the cardiology clinic to get additional supplements, at which time any unused tablets from the previous 4 weeks were returned and counted. Subjects were taught to maintain their usual diets and activities during the study period. To ensure that patients were keeping their usual dietary intake, they completed 24-hour diet recalls when they returned to the cardiology clinic before the intervention (week 0) and at week 12.

Fasting venous blood samples were collected in Vacutainer tubes (Becton Dickinson, Rutherford, NJ) containing an EDTA as an anticoagulant to determine homocysteine and vitamin B-6, and no anticoagulant to determine serum glucose, serum creatinine, total serum cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triacylglycerol, alanine aminotransferase and serum alkaline phosphatase, vitamin B-12 and folate status. During the intervention period, blood samples were taken on the 4^{th} and 12^{th} week after the subjects had fasted overnight. Blood samples were transported on ice and separated into plasma (or serum) and red blood cells within 1 h by low speed centrifugation (2500 rpm, 15 min). Samples were then stored frozen (- 80° C) until analysis.

Biochemical measurements

Fasting plasma homocysteine was measured by using high performance liquid chromatography (HPLC) according to the method of Araki and Sako (1987). The intraassay and interassay of fasting plasma homocysteine variabilities were 5.1% (n=5) and 1.2% (n=8), respectively. Plasma PLP was determined by HPLC as previously described (Bates *et al*, 1999). The intraassay and interassay of plasma PLP variabilities were 2.0% (n=6) and 2.9% (n=3), respectively. Erythrocyte alanine aminotransaminase (EALT) and erythrocyte aspartate aminotransaminase (EAST) with and without PLP stimulation in vitro were measured by the method of Woodring and Storvick (1970). All EALT and EAST activity measurements were performed by using fresh erythrocyte samples collected on the day of analysis. The intra- and inter-assay of EALT and EAST activity coefficient (EALT-AC and EAST-AC) variabilities were 1.9% (n=5), 5.2% (n = 14) and 2.0% (n=5), 4.1% (n=14), respectively. Plasma homocysteine and PLP concentrations and transaminase activity measurements were carried out under yellow light to prevent photodestruction. Serum folate and vitamin B-12 were analyzed by using standard competitive immunochemiluminometric methods on a Chiron Diagnostics ACS:180 Automated Chemiluminescence Systems (Chiron Diagnostics Corporation, USA). All analyses were performed in duplicate.

Statistical analyses

Data were analyzed using the SAS software package (version 6.12; The SAS Institute Inc., Cary, NC). Kruskal-Wallis one-way analysis of variance on ranks was used to compare the differences in age, BMI, and hematological measurements among groups at baseline. Within each group, mean measurements of plasma concentration of homocysteine and PLP, serum folate and vitamin B-12, and erythrocyte vitamin B-6 metabolites at week 4 and week 12 were compared for significant differences with values at week 0 by using Friedman repeated measures analysis of variance on ranks. Pearson correlation coefficients were used to assess the relationship between fasting plasma homocysteine and B-vitamins. Statistical results were considered to be significant at $p < 0.05$. Values presented in the text are means ± standard deviation (SD) with the median value in parentheses.

Results

Characteristics of subjects

Characteristics of subjects in each group are shown in Table 1. A total of 42 CAD patients successfully completed the intervention study. Patients' ages ranged

from 39 to 86 y, with a mean age of 71.4 y. There were no significant differences among groups with respect to age, BMI, and serum creatinine. The compliance of all was excellent.

Dietary intakes

Nutrient intake from the 24-h diet recall kept by the subjects at baseline and week 12 was calculated (data not shown). The mean intake of macronutrients, vitamin B-6, folic acid, vitamin B-12, and riboflavin between the baseline and week 12 was not significantly different in each group. Ninety point five percent and 64.3% of subjects had a vitamin B-6 and folic acid intakes below the current Taiwan dietary reference intakes (Taiwan DRI; Department of Health, Taiwan, 2002) (vitamin B-6: 1.6 mg/d; folic acid: 400 μg/d for both adult men and women > 51 y), respectively. Sixty-nine percent of subjects, however, had mean vitamin B-12 intake higher than the current Taiwan DRI (2.4 μg/d for both adult men and women > 51 y). No significant correlation was found between fasting plasma homocysteine and the intake of vitamin B-6, folic acid, vitamin B-12, or riboflavin ($p > 0.05$).

B-vitamin intervention

At baseline (week 0), there were no significant differences with respect to fasting plasma homocysteine, plasma PLP, EALT-AC, EAST-AC, folic acid, and vitamin B-12 among the five groups. Baseline fasting plasma homocysteine concentrations ranged from 7.1 to 29.1 μmol/L, with a mean concentration of 14.4 μmol/L. The mean concentrations of plasma PLP, serum folic acid and vitamin B-12 at baseline in each group were > 20 nmol/L, > 6.8 nmol/L, and > 125.0 pmol/L, respectively (Table 2), which are the suggested values for adequate vitamin B-6, folic acid and vitamin B-12 status (Food and Nutrition Board – Institute of Medicine, 1998).

Table 2 shows responses of fasting plasma homocysteine and B-vitamins to 12-week supplementation with 3 different dose of vitamin B-6 or folic acid combined with vitamin B-12 in CAD patients. After 4-week of vitamin supplementation, supplementations of vitamin B-6 did not appear to affect fasting plasma homocysteine concentration when compared with the placebo treatment ($p > 0.05$). Similar results were found after 12-week supplementation of vitamin B-6. Mean fasting plasma homocysteine concentration, however, significantly decreased after 12-week ($p = 0.047$) of folic acid combined with vitamin B-12 supplementation when compared with mean concentration of the placebo-supplemented group.

A within-group comparison shows that only folic acid combined with vitamin B-12 supplement decreased significantly fasting plasma homocysteine concentration by 32.0% ($p < 0.001$) after 12-week of vitamin supplementation when compared with

the basal concentration (week 0) (Table 2). However, 5 mg/d vitamin B-6 for 12-week supplementation only caused 2.1% nonsignificant reduction in fasting plasma homocysteine concentration (Table 2). It was surprising to find that supplementations of placebo, 10 mg/d and 50 mg/d vitamin B-6 for 12 weeks slightly, although not significantly, increased the mean fasting plasma homocysteine concentration by 25.5%, 16.2%, and 18.3% in CAD patients, respectively (Table 2).

Only in the group receiving the folic acid combined with vitamin B-12 did all patients respond with a reduction in fasting plasma homocysteine concentrations. In the other groups, the response of fasting plasma homocysteine concentrations to the vitamin supplements was not consistent (data not shown).

Pearson correlation coefficients were performed to understand the relation between B-vitamins and fasting plasma homocysteine. Fasting plasma homocysteine concentration was strongly and inversely associated with serum folate (r = -0.261, p = 0.0034) and vitamin B-12 (r = -0.261, p = 0.0032). There was no significant association between fasting plasma homocysteine and plasma PLP, EALT-AC, and EAST-AC ($p > 0.05$).

Discussion

Our previous study (Lee *et al*, 2003) and others (Brattström *et al*, 1990; Genest *et al*, 1990) have shown that hyperhomocysteinemia is prevalent in patients having CAD. If fasting plasma homocysteine concentration could be effectively reduced by vitamin supplementation, it might lessen the morbidity and/or mortality of CAD. Our results were in agreement with previous studies (Brattström *et al*, 1990; Miller *et al*, 1992; Ubbink *et al*, 1994), showing that vitamin B-6 supplementation did not affect homocysteine concentration. In addition, an unexpected finding was that fasting homocysteine levels slightly although not significantly increased in the placebo group during the study. The increase in homocysteine levels in the placebo group was not clear. This was a single-blind study; therefore, the expectation from the subject could be eliminated. Potential causes might be due to the nature of the coronary event, stress, or medication uses.

Our results, however, showed that the homocysteine lowering effect (≈ 32% reduction) is significant by supplementation with 5 mg folic acid combined with 250 μg vitamin B-12 for 12 weeks in CAD patients. McKinley *et al* (2001) indicated that low-dose vitamin B-6 (1.6 mg/d) for 12 weeks effectively reduced fasting plasma homocysteine concentration in healthy elderly subjects only when subjects were both folate and riboflavin replete. Studies (Ubbink *et al*, 1994; Verhoef *et al*, 1996; Brouwer *et al*, 1999) have shown that folate is a very powerful homocysteine-lowering agent. A possible explanation has been attributed to the role

of *S*-adenosylmethionine in the regulation of homocysteine metabolism. *S*-adenosylmethionine (an activator for the enzyme cystathionine β-synthase) favors folate and vitamin B-12-dependent homocysteine remethylation to methionine in the fasting state; vitamin B-6 deficiency does not affect *S*-adenosylmethionine (Selhub & Miller, 1992; Ubbink *et al*, 1996). Homocysteine is only directed to the transsulphuration pathway when methionine is in excess. Brattström *et al* (1990) indicated that supplementation with 240 mg of vitamin B-6 for 2-week decreased the mean post methionine load increase in the homocysteine concentration by 26% ($p < 0.001$) in 20 very early-onset vascular disease patients. Other studies also showed similar results with a pyridoxine supplement at dose of 20 ~ 100 mg (Dudman *et al*, 1993; Ubbink *et al*, 1996). Vitamin B-6, therefore, mainly affects homocysteine only in the postprandial state.

Moreover, we observed that high doses of vitamin B-6 supplementation slightly although not significantly decreased serum folate concentration after 4-week of vitamin B-6 supplementation (Table 2). Since plasma folate concentrations fluctuate rapidly with recent changes in folate intakes, we assessed subjects' dietary intakes. Subjects' mean folate intake remained nonsignificantly changed during the intervention period. The decrease in folate status, thus, might be due to the vitamin B-6 supplementation. Ubbink *et al* (1996) indicated that plasma folate concentration significantly declined during 20 mg pyridoxine supplementation in both asthma patients and healthy controls. A similar finding was seen in the study of Mansoor *et al* (1999), the mean serum folate concentration decreased 13% after 120 mg vitamin B-6 supplementation for 5-week in healthy subjects. In a recent study, Bosy-Westphal *et al* (2001) observed the 27% reduction ($p < 0.01$) of plasma folate concentration after 25 mg of pyridoxine supplementation for 10 days in healthy subjects. However, the underlying mechanism of serum folate reduction by high dose of vitamin B-6 remains unclear at present. Further study is warranted to study the interrelationship between vitamin B-6 and folate status.

Although serum folate concentration declined after vitamin B-6 supplementation, fasting plasma homocysteine concentration remained significantly unchanged in some previous studies (Ubbink *et al*, 1996; Mansoor *et al*, 1999; Bosy-Westphal *et al*, 2001). It may be possible that homocysteine is directed to the transsulfuration pathway when the homocysteine remethylation is impaired by low folate status due to a temporary (short period) vitamin B-6 supplementation. However, supplementation of vitamin B-6 without folate repletion for a prolonged period might have negative effects on fasting plasma homocysteine concentrations since *S*-adenosylmethionine prefers the folate and vitamin B-12-dependent homocysteine remethylation pathway. Arnadottir *et al* (1993) found that fasting plasma homocysteine concentrations increased

significantly after a high dose of pyridoxine treatment (300 mg) for 4 months in dialysis patients. In the present study, there was a slight increase in fasting plasma homocysteine concentration during 12-week of 10 mg and 50 mg vitamin B-6 supplementation in CAD patients. We, therefore, speculated that if the intervention period of vitamin B-6 supplementation lengthens, serum folate status might probably be compromised. In the current study, the decrease in serum folate concentration after vitamin B-6 supplementation might be reason to increase fasting plasma homocysteine concentrations.

Most of the studies were done the vitamin supplementation in lowering homocysteine in subjects without CAD, or in those with high homocysteine levels. Although the small number of subjects in each subgroup was the major limitation of this study, our results could provide more information to what has been known in CAD patients. In conclusion, our study demonstrates that vitamin B-6 supplementation alone is less effective than folic acid combined with vitamin B-12 in lowering plasma homocysteine concentrations in CAD patients. It is possible that vitamin B-6 supplementation for a prolonged period might impair serum folate status and further cause hyperhomocysteinemia.

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計劃成果自評

本篇研究內容與原計畫相評目前已達成 100%**。受試者的取得比原先預期的順利，原因為參與本研究的心臟科醫師的全力支持及協助。此研究計劃內容已經獲得** European Journal of Clinical Nutrition **期刊的接受並即將發表於該其期刊。**

Table 1 Demographic and health characteristics of subjects[1]

	Placebo *(n = 8)*	*Vitamin B-6 – 5 mg* *(n = 8)*	*Vitamin B-6 – 10 mg* *(n = 8)*	*Vitamin B-6 – 50 mg* *(n = 9)*	*Folic acid – 5 mg + Vitamin B-12 – 250 μg* *(n = 9)*
Sex (Male / Female)	8 / 0	7 / 1	6 / 2	9 / 0	9 / 0
Age (y)	73.3 ± 12.5 (73.5)	70.1 ± 4.6 (71.0)	70.3 ± 13.9 (75.0)	67.8 ± 11.2 (71.0)	75.4 ± 3.5 (75.0)
Body mass index (kg/m^2)	23.9 ± 1.8 (23.3)	25.4 ± 2.7 (25.7)	26.0 ± 1.8 (25.6)	26.7 ± 2.9 (26.5)	26.0 ± 3.6 (26.4)
Serum creatinine (mg/dL)	1.2 ± 0.2 (1.2)	1.3 ± 0.2 (1.2)	1.3 ± 0.4 (1.2)	1.2 ± 0.2 (1.2)	1.3 ± 0.3 (1.3)

[1] Values are means ± SD with the median value in the parentheses.

Table 2 Responses of plasma homocysteine, vitamin B-6, folic acid, and vitamin B-12 to different vitamin supplements at week 0, week 4 and week 12[1]

Biochemical measurements	*Placebo (n = 8)*			*Vitamin B-6 – 5 mg (n = 8)*			*Vitamin B-6 – 10 mg (n = 8)*			*Vitamin B-6 – 50 mg (n = 9)*			*Folic acid – 5 mg + Vitamin B-12 – 250 μg (n = 9)*		
	week 0	*week 4*	*week 12*	*week 0*	*week 4*	*week 12*	*week 0*	*week 4*	*week 12*	*week 0*	*week 4*	*week 12*	*week 0*	*week 4*	*week 12*
Homocysteine	14.5±4.8	13.0±6.2	18.2±5.3	13.9±2.6	13.7±3.8	13.6±3.9	15.4±6.8	16.2±7.3	17.9±7.7	14.2±6.4	14.5±6.3	16.8 ± 9.1	14.7± 2.3	11.4±2.3*	10.6±2.5*†
(μmol/L)	(14.0)	(16.5)	(17.4)	(13.8)	(14.0)	(13.9)	(13.5)	(14.5)	(14.7)	(13.4)	(14.0)	(13.4)	(14.6)	(10.9)	(10.1)
Vitamin B-6															
PLP (nmol/L)	51.5±12.8	48.8±37.6	58.5± 48.4	55.2± 21.3	158.4± 94.7*	163.4±78.1*	57.6 ± 44.7	185.8±109.9***	165.0 ± 70.8*	75.9 ± 48.0	227.8±139.5***	298.8±136.2*†	65.0±47.4	34.5± 9.8	57.6± 30.7
	(58.6)	(37.5)	(41.8)	(51.9)	(157.0)	(165.4)	(38.4)	(160.2)	(165.1)	(63.6)	(216.6)	(243.8)	(75.7)	(41.5)	(63.2)
EALT-AC	1.2 ± 0.3	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	1.3 ± 0.2	1.1±0.2	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	1.2 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.2±0.3	1.1± 0.1	1.2±0.2
	(1.2)	(1.2)	(1.2)	(1.1)	(1.1)	(1.2)	(1.1)	(1.0)	(1.0)	(1.2)	(1.0)	(1.0)	(1.2)	(1.1)	(1.1)
EAST-AC	1.9 ± 0.7	1.5 ± 0.3	1.5 ± 0.3	1.7 ± 0.5	1.2 ± 0.3*	1.1± 0.4*†	1.4± 0.4	1.5 ± 0.2	1.1 ± 0.2	1.6 ± 0.4	1.2 ± 0.2*	1.0± 0.1*†	1.5±0.3	1.3± 0.3	1.4±0.3
	(1.8)	(1.5)	(1.5)	(1.7)	(1.2)	(1.2)	(1.4)	(1.3)	(1.2)	(1.5)	(1.1)	(0.8)	(1.5)	(1.6)	(1.4)
Folic acid	34.0 ± 37.6	20.2 ± 11.3	23.1 ± 15.8	23.9 ± 13.7	19.7 ± 4.5	20.2 ± 4.8	20.8 ± 7.0	17.9 ± 5.3	24.7 ± 11.1	25.8 ± 28.1	19.0 ± 12.9	23.3 ± 15.0	27.9± 9.3	173.1±134.1	225.2±138.3
(nmol/L)	(21.1)	(18.6)	(16.8)	(19.7)	(19.3)	(18.1)	(19.3)	(17.4)	(22.7)	(14.0)	(16.1)	(18.6)	(10.9)	***	-†
														(152.6)	(213.2)
Vitamin B-12	278.2±66.0	310.6±149.6	350.4±96.1	308.3±132.7	286.9±77.1	351.0±154.9	353.6±133.3	349.2±114.8	360.4±117.0	317.8±83.2	321.2±74.1	342.1±83.3	318.7±127.9	379.4±133.9	466.1±85.5*
(pmol/L)	(270.8)	(269.7)	(327.6)	(269.7)	(262.7)	(306.7)	(276.7)	(325.8)	(357.5)	(315.0)	(340.1)	(313.6)	(267.4)	(335.7)	(479.8)

[1] Values are means ± SD with the median value in the parentheses. PLP, pyridoxal 5'-phosphate; EALT-AC, erythrocyte alanine aminotransferase activity coefficient; EAST-AC, erythrocyte aspartate aminotransferase activity coefficient.

* Values are significantly different from week 0 within the group ($p < 0.05$).

** Values are significantly different from the placebo group at week 4 ($p < 0.05$).

† Values are significantly different from the placebo group at week 12 ($p < 0.05$)