Original Article

Effects of consuming red furu (fermented bean curd) on serum vitamin B-12, homocysteine and other cardiometabolic risk factors in young healthy volunteers: A randomized controlled trial

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Background and Objectives: The health benefits of red furu in young, healthy volunteers had not been adequately investigated. The aim of this study was to determine the effect of a single meal containing red furu on serum vitamin B-12 (B-12), homocysteine and other cardiometabolic risk factors compared with that of tofu. **Methods and Study Design:** Twenty-three healthy volunteers from Zhejiang University, China, were randomly assigned to two groups of consumption, either red furu (n=11, 5 women and 6 men) or tofu (n=12, 6 women and 6 men). Volunteers consumed one breakfast meal composed of either 50 g of red furu (intervention group) or 50 g of tofu (non-active comparison group) with two slices of bread. Fasting blood was collected at 0 h, 24 h, and 72 h. Standard methods were used to measure the volunteers' biochemical parameters. **Results:** The consumption of 50 g of red furu a day did not significantly affect serum B-12 and showed a non-significant trend to reduce serum homocysteine. In the red furu group, but not in tofu group, serum concentrations of B-12 and folate were negatively associated with homocysteine, and B-12 was positively associated with folate. **Conclusions:** A breakfast meal with 50 g of red furu containing 0.096 μg of B-12 did not increase serum B-12 in healthy volunteers. These results suggested that one meal containing B-12 could be sufficient to reduce serum Hcy.

Key Words: red furu, vitamin B-12, homocysteine, folate

INTRODUCTION

Vitamin B-12 (B-12) in fermented soybean products is considered beneficial to human health.[1] B-12 benefits include the prevention of megaloblastic anemia, the promotion of blood maturation, and the maintenance of a healthy nervous system.[2] Recent research has shown considerable interest in B-12, particularly in the association between homocysteine (Hcy) and chronic diseases, especially vascular diseases.[3] Folate and B-12 are influential in the regulation of Hcy concentration.[4] Blood Hcy is directly influenced by folic acid and B-12 status, with lower levels being associated with elevated Hcy.[5]

The health benefits of soybean products have been documented extensively. However, in Asian countries, fermented soybean is the preferred form because it contains B-12. Red furu is used as a cooking ingredient in Chinese cuisine and as a condiment when consuming noodles, Chinese bun or dumplings.[6] A literature review revealed that no reports of human trials on the effect of red furu consumption on serum B-12, Hcy, and other cardiometabolic risk factors currently exist.

The aim of the present study was to compare the effects of a commonly consumed Chinese fermented soybean product with high B-12 content with those of a similar soybean product containing no B-12. Tofu was used as the non-active comparator. The effects of red furu on the serum levels of B-12 and Hcy were measured at 0 h, 24 h and 72 h as primary outcomes, and the serum levels of lipids and other biochemical parameters were also measured as secondary outcomes in the present study.

METHODS

Blood specimen collections

For serum biochemical analysis, fasting blood samples were collected by venepuncture from fasted volunteers in the mornings. Visit 1 (0 h) – a baseline fasted venous blood sample (10 mL) was collected and instructions on what to eat and not eat during the trial period were elaborated in the questionnaire and also further explained during the blood withdrawal sessions. Visit 2 (24 h) and visit 3 (72 h) - fasted venous blood samples (10 mL and 5 mL,

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respectively) were collected. Serum was used for all biochemical parameters. Serum was isolated by centrifugation at 1000 g for 10 min. Serum B-12 levels, iron, folate, ferritin and Hcy were measured at the clinical testing laboratory using commercial kits with the following normal ranges, folate was 7.0-46.4 nmol/L, ferritin; 20-500 ng/mL (men): 20-200 ng/mL (women), B-12; 138-652 pmol/L, iron; 5.8-34.5 μmol/L, Hcy; 5-9 μmol/L. There was insufficient serum to measure all lipids levels for some volunteers.

Study design and meals

The study was a randomized controlled trial. The study protocol was approved by the Ethics Committee of College of Biosystem Engineering and Food Science at Zhejiang University (ZJU-BEFS-2017001). The exclusion criteria were no history of gastrointestinal, liver, or kidney diseases, not taking antibiotics or B-12 supplements within one month prior to enrolment. Thirty volunteers were recruited through social media from Zhejiang University, China. Six volunteers withdrew for personal reasons and one volunteer was excluded for a medical condition. Twenty-three volunteers were subsequently randomized by a computer-generated random numbering program retrieved from the website randomizer.org (Table 1 and Figure 1). The volunteers signed a consent form before participating. The volunteers were advised to continue with their usual diet, and to avoid the consumption of fermented soybean products during the three days trial period. The volunteers were requested to record their dietary intake in the 72 h trial period. The study was conducted in order to investigate the serum changes of lipids and other biochemical parameters of the volunteers 1 day before, 1 day during and 1 day after the intervention (0 h, 24 h, and 72 h). The breakfast test meal was administered by the researcher in the same location in the Department of Food Science and Nutrition. The red furu used for the study contained 0.192 μg of B-12 for 100 g of product while the tofu contained 0 ug B-12 per 100 g measured using standard microbiological assay, which uses Lactobacillus leichmannii ATCC 7830. Red furu is usually consumed as a cooking ingredient or condiment. Hence, in the present study, the two groups were allocated to consume a breakfast test meal containing either 50 g of either 50 g red furu (n=11) or of 50 g tofu (n=12) with two slices of plain bread at the start of the trial. Subjects maintained their usual diets for the next 72 hours. Twenty-three healthy young volunteers aged 19-39 years completed the trial (Table 1). Thereafter, comparisons between groups were performed.

A questionnaire including socio-demographics, health history and diet pattern was completed by volunteers with the researcher's assistance. Volunteers were asked what motivated their dietary pattern choice and whether they normally consumed any foods and beverage items or supplements to boost their B-12 concentrations.

The volunteers were guided by food props (number of portions; cups, pieces, slices, grams and milligrams), for measurement accuracy. Volunteers' diet history was used to evaluate B-12 intake, assess energy, nutritional composition micronutrient and macronutrient intake adequacy of the diet in order to ensure that volunteers' daily diet habits were captured. The volunteers' normal diet habits were monitored to detect what B-12-containing foods they consumed apart from fermented soybean products. Food consumption was transformed into energy and nutrient intakes (B-12, folate, iron, calcium, vitamin C, vitamin D, carbohydrate, fat and protein) using Chinese nutrition software program, calculated on the basis of the China Food Composition (2nd ed.) provided by the Chinese Nutrition Society, which was compiled using Chinese value for the nutrient composition of foods calculated by Nutrition calculator adapted from Yang et al.[7]

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism 7, San Diego, CA, USA). For group comparison, a one-way ANOVA test was used for comparing the changes of tested biomarkers effects of time and the interaction of intervention and time points between groups. When a significant time X group interaction was observed, a post hoc test was applied to examine the group X time interaction between the two groups. Variables were assessed

Table 1. Baseline demographics of volunteers in the red furu and tofu groups

	Tofu group (*n*=12)	Red furu group (*n*=11)	% Overall
Age			
Women (20-39 y)	29.17±6.41	23.67±3.86	49.0
Men (20-37 y)	25.67±4.50	27±7.35	51.0
Gender, n			
Women	6	5	49.0
Men	6	6	51.0
BMI (kg/m²)	22.86±4.05	23.88±2.81	
Dietary category, n			
Non-vegetarian	11	8	83.3
Lacto-ovo-vegetarian	1	3	16.7
Race, n			
African	8	7	66.7
Asian	4	1	20.8
European	0	2	8.3
Indian	0	1	4.2
Subjects	12	11	23

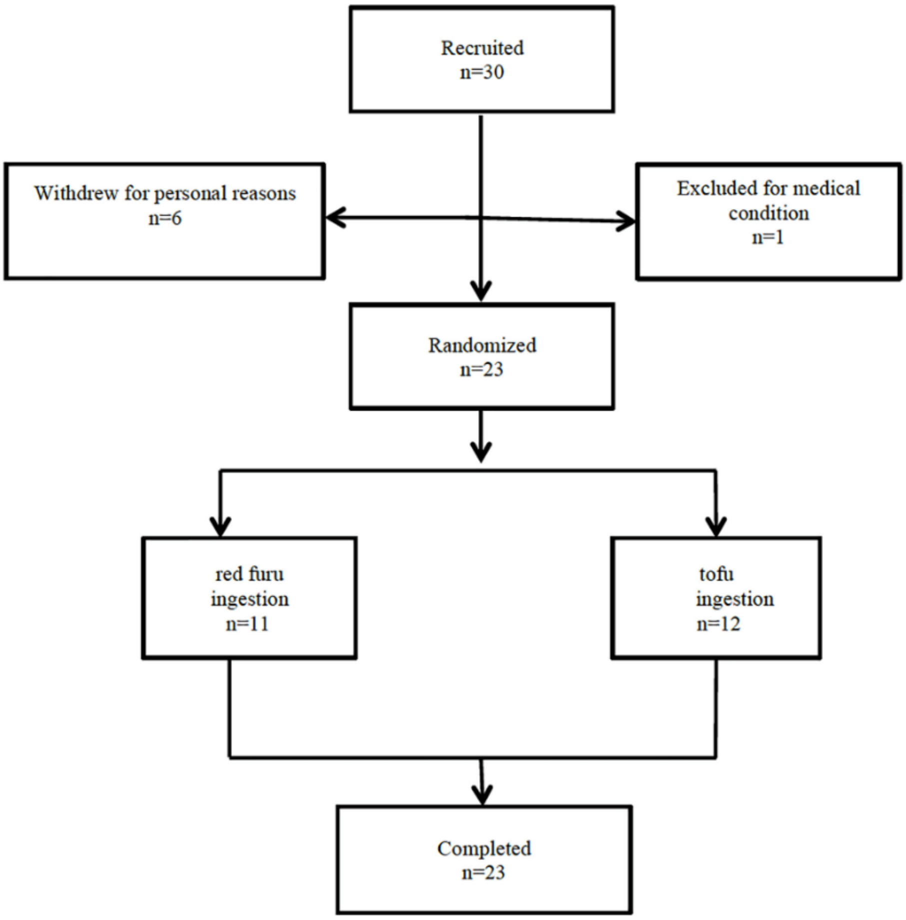

Figure 1. Diagram of the study design. Fermented bean curd was used as intervention (red furu group) and traditional non-fermented white tofu was used as a non-active comparator (tofu group).

with Pearson's correlation. The values were reported as mean ± SD in all the results tables and mean ± SEM for all the graphs. *p*-value less than 0.05 were considered to be significant.

RESULTS

Characteristics of volunteers

A total of 23 volunteers completed the study; (11 women and 12 men), (19 omnivorous and 4 lacto-ovo-vegetarians), red furu group n =11 (6 men, 5 women) and tofu group n=12 (6 men and 6 women). The study included 15 (66.7 %) Africans, 5 (20.8 %) Asians, 2 (8.3 %) Europeans and 1 (4.2 %) Indian (Table 1).

Flow diagram of volunteers

The flow diagram summarizes study assignment according to randomization group, (red furu; n=11) and tofu; n=12). Thirty subjects were recruited, 7 withdrew, 6 for personal reasons and 1due to a medical condition (Figure 1).

Figure 2. Study test meal sample; red furu (0.192 µg per 100 g of B-12). The meal consisted of 50 g of red furu with two slices of bread consumed for 1 day

Dietary intakes

The commercial red furu, a fermented bean curd that was used for the intervention in the present study, is illustrated in (Figure 2). The dietary nutrient intakes of volunteers are presented in (Table 2). The volunteers' intake of carbohydrates, fibre, folate, calcium, and vitamin D significantly increased compared with the baseline for both groups over the 72 h. In total, 62.5 % of the volunteers consumed fermented soybean products either as a condiment or as cooking ingredient (once to twice a week), whereas 37.5 % did not consume fermented soybean products in their usual diets. In their usual diets, 78 % of the volunteers obtained their B-12 by consuming red meat, fish, eggs, seafood, poultry and dairy. On the other hand 13 % of the volunteers supplemented their diets with B-

Table 2. Dietary intake of nutrients for red furu and tofu groups

Dietary intakes	0 h	24 h	72 h	*p*-value		
				Group	Time	Time x Group
Energy(Kcal)				0.99	0.98	0.99
Tofu	2000±170	2005±165	2009±154			
Red furu	2000±397	2007±397	2009±398			
Protein (g)				0.32	0.10	0.90
Tofu	49±8	54±10	57±12			
Red furu	48 ±15	54±17	57±16			
Carbohydrate (g)				0.002*	0.02*	0.69
Tofu	179±60	213±60	239±76			
Red furu	159±57	192±57	205. ±74			
Fat (g)				0.74	0.67	0.20
Tofu	61±21	62±27	57±21			
Red furu	56±18	58±22	60±21			
Cholesterol (mg)				0.28	0.71	0.46
Tofu	208±76	141±72	207±113			
Red furu	196±115	181±177	220±144			
Fiber(g)				0.23	0.001*	0.58
Tofu	5±2	9±3	7±3			
Red furu	5±2	8±3	6±2			
Total iron (mg)				0.31	0.71	0.04
Tofu	10±2.	14±4	13±2			
Red furu	12±5	13±4	12±3			
Folate (μg)				0.14	0.01*	0.37
Tofu	30±19	26±17	43±42			
Red furu	28. ±16	34±22	50±37			
Calcium (mg)				0.61	0.03*	0.64
Tofu	271±81	356±283	366±208			
Red furu	251±94	385±219	371±203			
Vitamin D (μg)				0.03*	0.03*	0.55
Tofu	0±0	2±3	1±1			
Red furu	0±1	2±3	1±1			
vitamin B-12 (g)				ND	ND	ND
Tofu	ND	ND	ND			
Red furu	0.09±0.11	ND	ND			
Vitamin C (μg)				0.64	0.74	0.07
Tofu	60±45	51±32	75±46			
Red furu	62±50	68±61	61±40			
Vitamin A (μg)				0.36	0.66	0.97
Tofu	436±351	580±501	556±392			
Red furu	480±381	611±465	584±385			

ND: not detected.
†One-way ANOVA was performed to test differences of continuous variables between groups and time points. Interaction test was conducted by including group, time and the interactions terms (group multiplying time) simultaneously.
*p<0.05 was regarded as statistically significant.

12 and 9 % of the volunteers consumed fortified foods and beverages. One Rastafarian lacto-ovo- vegetarian volunteer did not consume any dairy or fish. Volunteers' dietary records noted the consumption of protein-rich foods and fried and boiled tofu. Volunteers' intake of iron was from both heme and non-heme sources of iron, which encompassed fish, egg, poultry, fruits, vegetables, legumes and grains.

The high dietary folate intake observed in the present study was attributed to the high intake of folic acid from fortified food, fruits, vegetables and legumes. Folate intakes increased for both groups over the 72 h period. During the trial, red furu was the only food item identified as a source of B-12 by the Chinese nutritional software program used (Table 2). This outcome was likely influenced by the limited data available on B-12 content in Chinese Food Composition Data Base.

Changes in serum biochemical parameters

Changes of serum concentrations of B-12, Hcy, folate, ferritin and iron after one meal of red furu or tofu between groups and time points are shown in (Table 3). There were no significant changes in any of the serum parameters measured, and particularly for B-12, Hcy or folate over the 72 hours of this study (Table 3).

Although there was no significant change in B-12 over the 72 h for either group, Figure 3 shows that B-12 levels for red furu group were reduced in 6 of 10 of eleven volunteers at 72 h compared with 0 h and there was an increase in one of the eleven volunteers at 72 h. In the tofu group, the B-12 levels declined in six of twelve of the volunteers for tofu group at 72 h (Figure 4).

Although there was no significant change in Hcy over the 72 h for either group, there was a non-significant trend for serum Hcy to decrease in the red furu group (Figure 5) shows that Hcy levels decreased in 9 of 11 volunteers at 72 h in the red furu group (Figure 5). In the

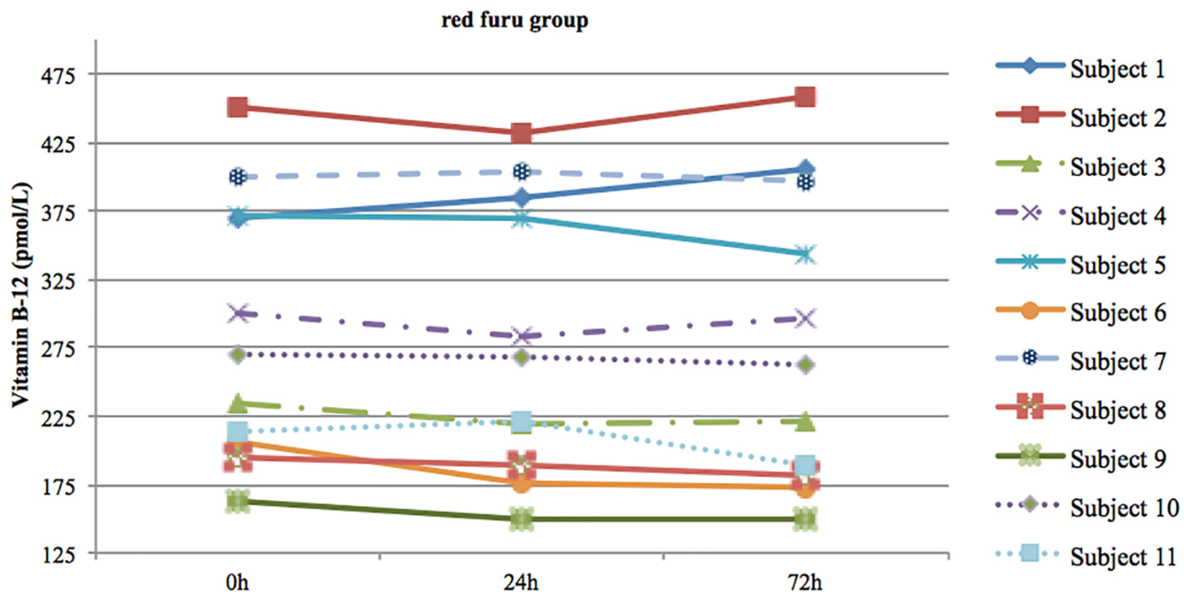

Figure 3. Pattern of change in individual serum B-12 in the red furu group during the 72 h trial period.

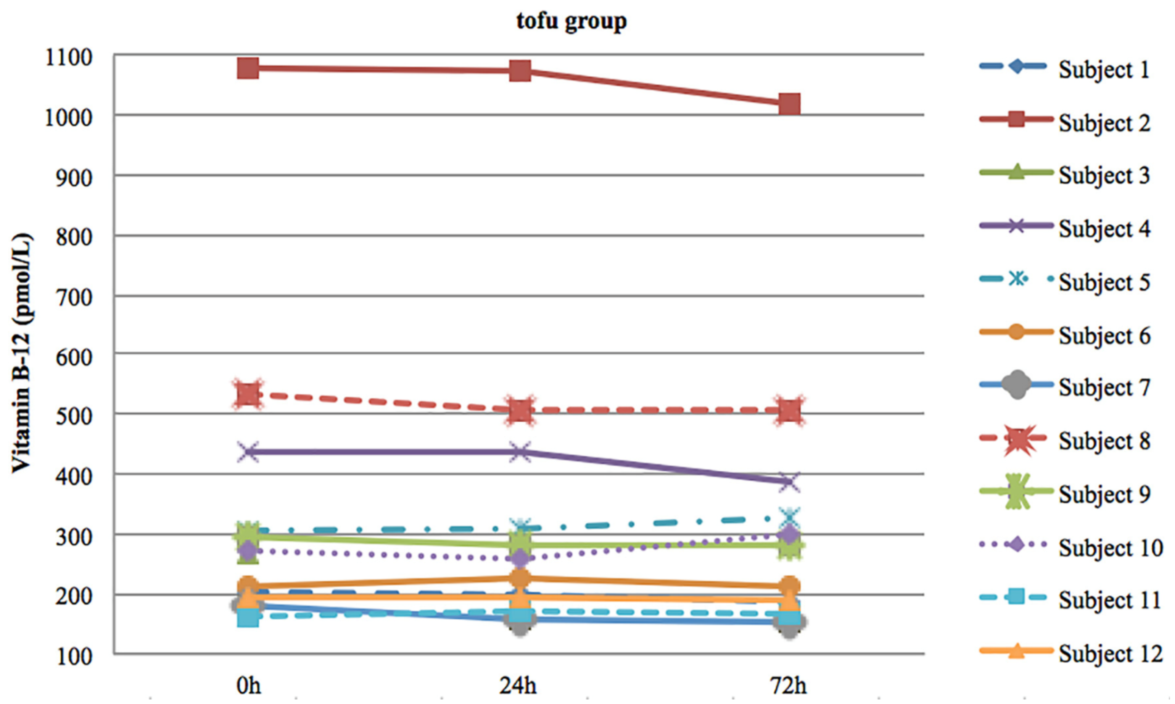

Figure 4. Pattern of change in individual serum B-12 in the tofu group during the 72 h trial period.

tofu group, three of twelve showed an increase at 72 h (Figure 6). Changes of glucose (GL), total cholesterol (TC), triglyceride (TG), high density lipoproteins-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) after one meal containing either red furu or tofu are presented in (Table 3). There were no significant changes in TC, TG, HDL-C and LDL-C observed in either the red furu or the tofu group.

The relationship between serum concentrations of B-12, folate with Hcy, and the relationship between B-12 with folate for red furu and tofu groups are reported in Figures 7 to 9. There were highly significant negative correlations between serum Hcy and B-12 at each time point of the study in the red furu group, but not in the tofu group. There were also significant negative correlations between Hcy and folate at each time point of the study in the red furu group, but not in the tofu group. Finally, there were significant positive correlations between serum Hcy and B-12 at each time point of the study in the red furu group, but not in the tofu group.

DISCUSSION

The present study revealed that one meal of 50 g red furu containing 0.096 ug B-12 did not significantly increase the serum B-12 in the 72 h following the meal in young, healthy volunteers. Whether the short intervention period or the adequate B-12 status was responsible for this outcome could not be determined. In this trial, red furu which is a good source of B-12 did not significantly alter serum B-12, Hcy or folate, however it was observed that there was a non-significant trend for Hcy to decrease over the 72 h of the trial in the red furu group. We found some

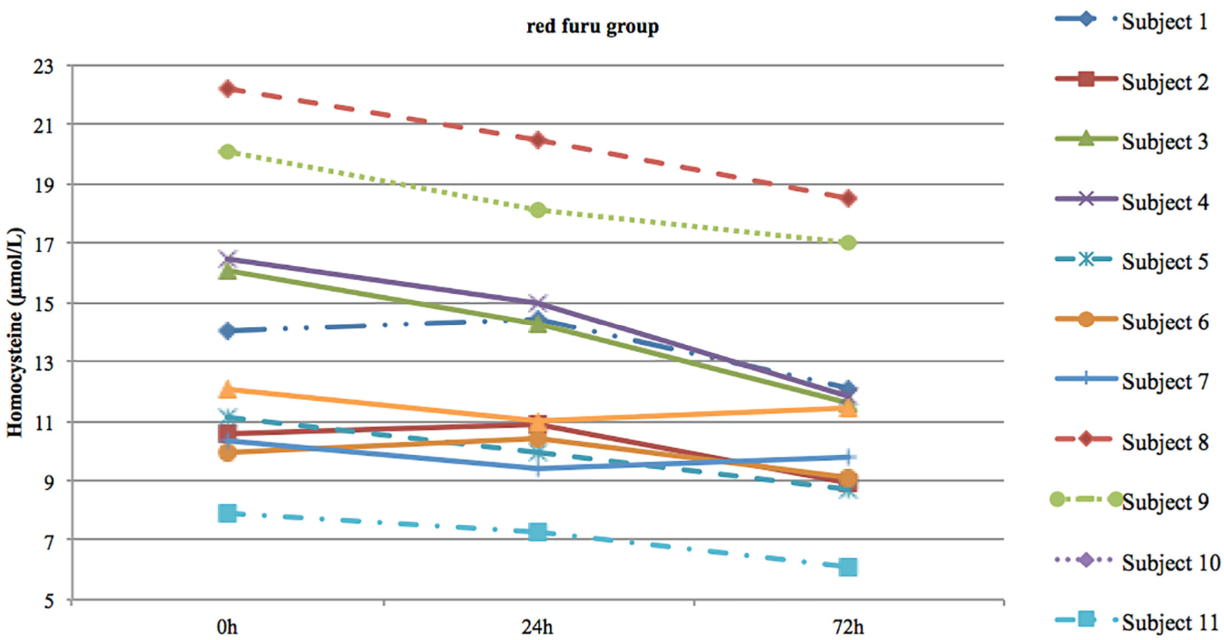

Figure 5. Pattern of change in individual serum Hcy in the red furu group during the 72 h trial period

Table 3. Biomarkers for red furu and tofu groups among young, healthy volunteers

Variables	0 h	24 h	72 h	*p*-value		
				Group	Time	Time x Group
B-12 (pmol/L)				0.23	0.99	0. 99
Tofu	344.9±255.6	338.0±254.3	329.5 ±239.7			
Red furu	288.6±96.1	281.6 ±100.3	279.6±107.8			
Hcy (μmol/L)				0.59	0.97	0.26
Tofu	12.7±3.7	13.2±4.7	14.5 ±6.1			
Red furu	13.7±4.5	12.8±4.0	12.6 ±5.0			
Ferritin (ng/ml)				0.75	0.99	0.99
Tofu	60.1 ±57.9	63.8 ±62.1	62.8±58.0			
Red furu	68.5 ±80.6	68.4 ±78.1	65.9. ±77.1			
Iron (μmol/L)				0.79	0.93	0.77
Tofu	13.7±8.5	14.6 ±12.0	15.7±8.4			
Red furu	16.3±7.3	14.3±7.2	15.0 ±4.51			
Folate (nml/L)				0.27	0.63	0.93
Tofu	11.8 ±3.0	10.4±3.9	11.1 ±3.3			
Red furu	10.5±3.5	9.9±3.3	10.0±3.3			
Glucose (mmol/L)				0.64	0.82	0.36
Tofu	3.8 ±0.7	4.2 ±1.0	3.3±1.5			
Red furu	4.4 ±0.3	3.7±0.8	4.7±0.3			
TC (mmol/L)				0.38	0.42	0.26
Tofu	3.5±0.6	4.0 ±1.82	4.8±2.1			
Red furu	4.1 ±0.5	3.2 ±1.3	3.2 ±1.3			
TG (mmol/L)				0.80	0.70	0.52
Tofu	0.8±0.3	0.7±0.2	0.6 ±0.2			
Red furu	0.7±0.2	0.6±0.1	0.6 ±0.2			
HDL-C (mmol/L)				0.77	0.48	0.21
Tofu	0.9 ±0.3	1.2 ±0.0	1.4 ±0.1			
Red furu	1.1 ±0.2	0.9 ±0.3	1.1±0.1			
LDL-C (mmol/L)				0.69	0.40	0.29
Tofu	1.3±0.3	1.4±1.1	1.5 ±1.0			
Red furu	1.6±0.4	1.1±0.6	1.1±0.5			

†One-way ANOVA was performed to test differences of continuous variables between groups and time points. Interaction test was conducted by including group, time and the interactions terms (group multiplying time) simultaneously.
**p*<0.05 was regarded as statistically significant.

interesting correlations between these three serum parameters in the red furu group but not in the tofu group. First, it was established that there was a significant negative correlation between serum B-12 and Hcy and between serum folate and Hcy in the serum in the red furu group. Thirdly, we found that there was a significant positive correlation between serum B-12 and folate in the red furu group. These correlations might be explained by the interrelationships between these parameters as shown in (Figure 10). A study by McKully[8] found supplementation of

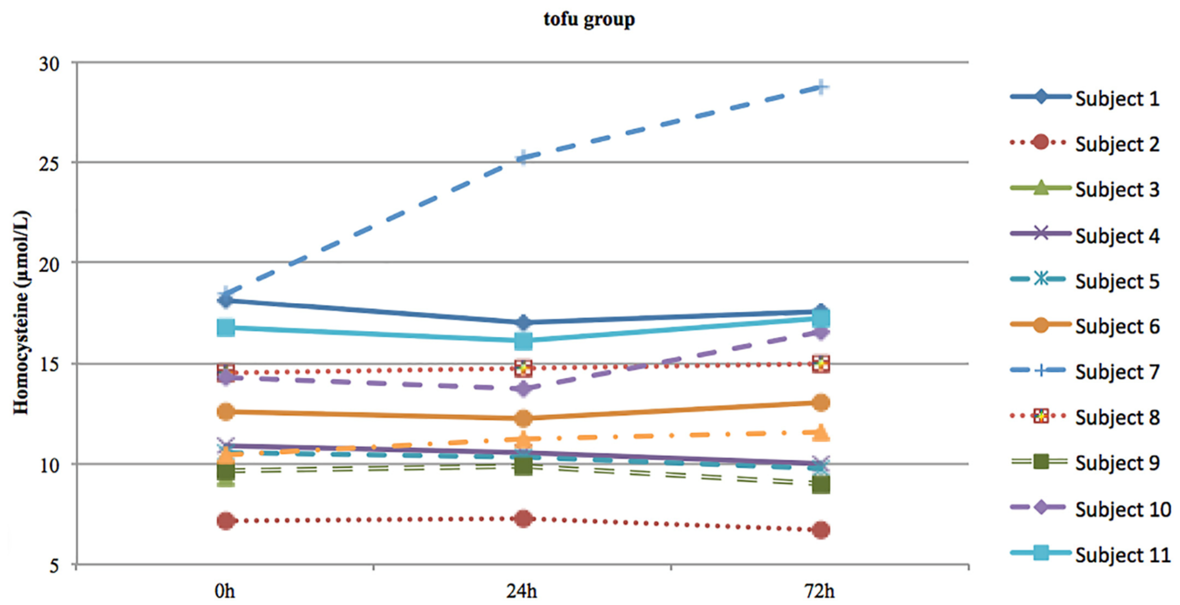

Figure 6. Pattern of change in individual serum Hcy in the tofu group during the 72 h trial period.

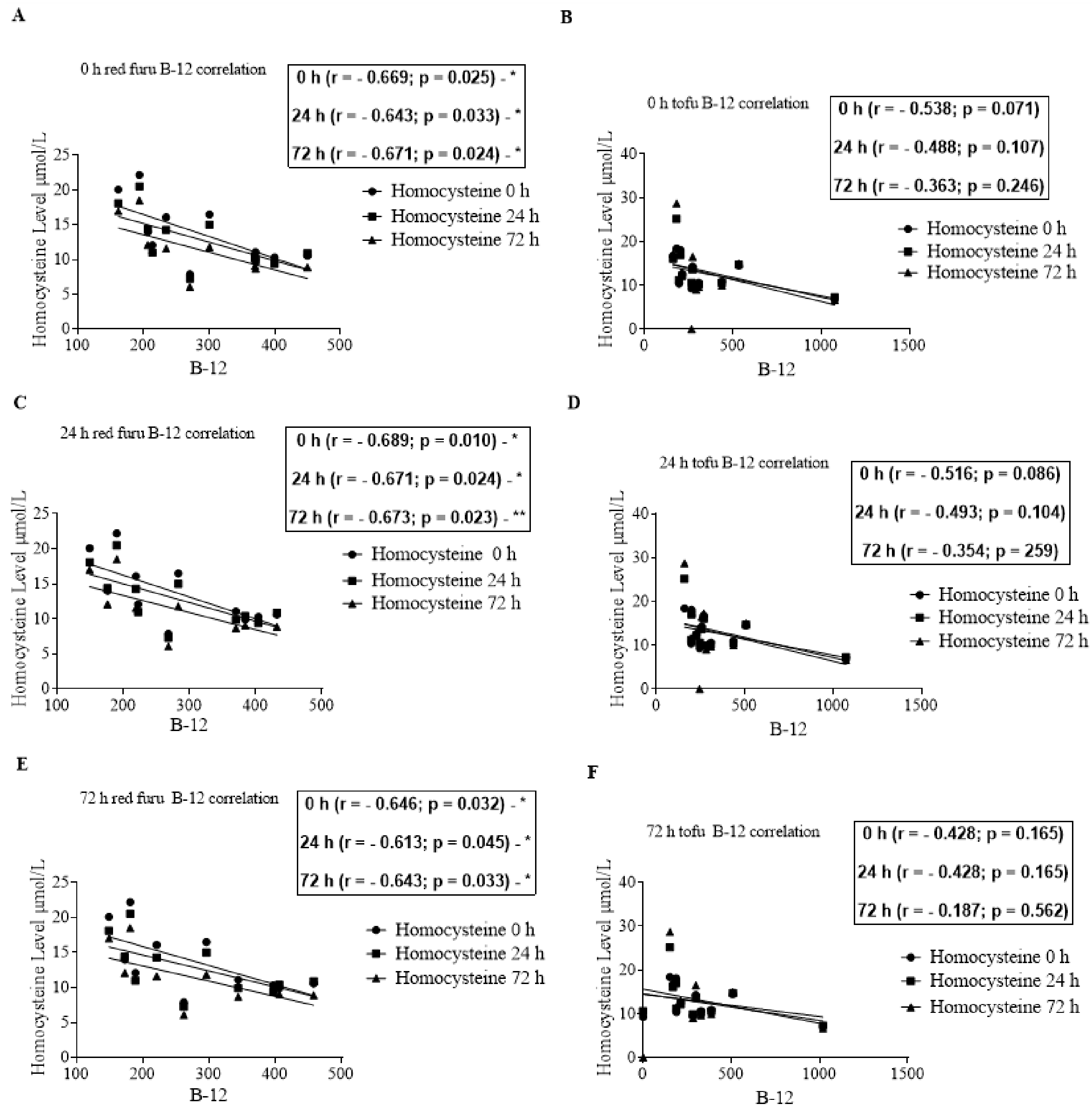

Figure 7. Correlation between B-12 and Hcy at (0 h- 24 h- 72 h) three-time points in red furu (A, C, E) and tofu (B, D, F) groups. Mean ± SEM were used when comparing the measured values statistically. Red furu and tofu results were tested by Person's correlation coefficient.

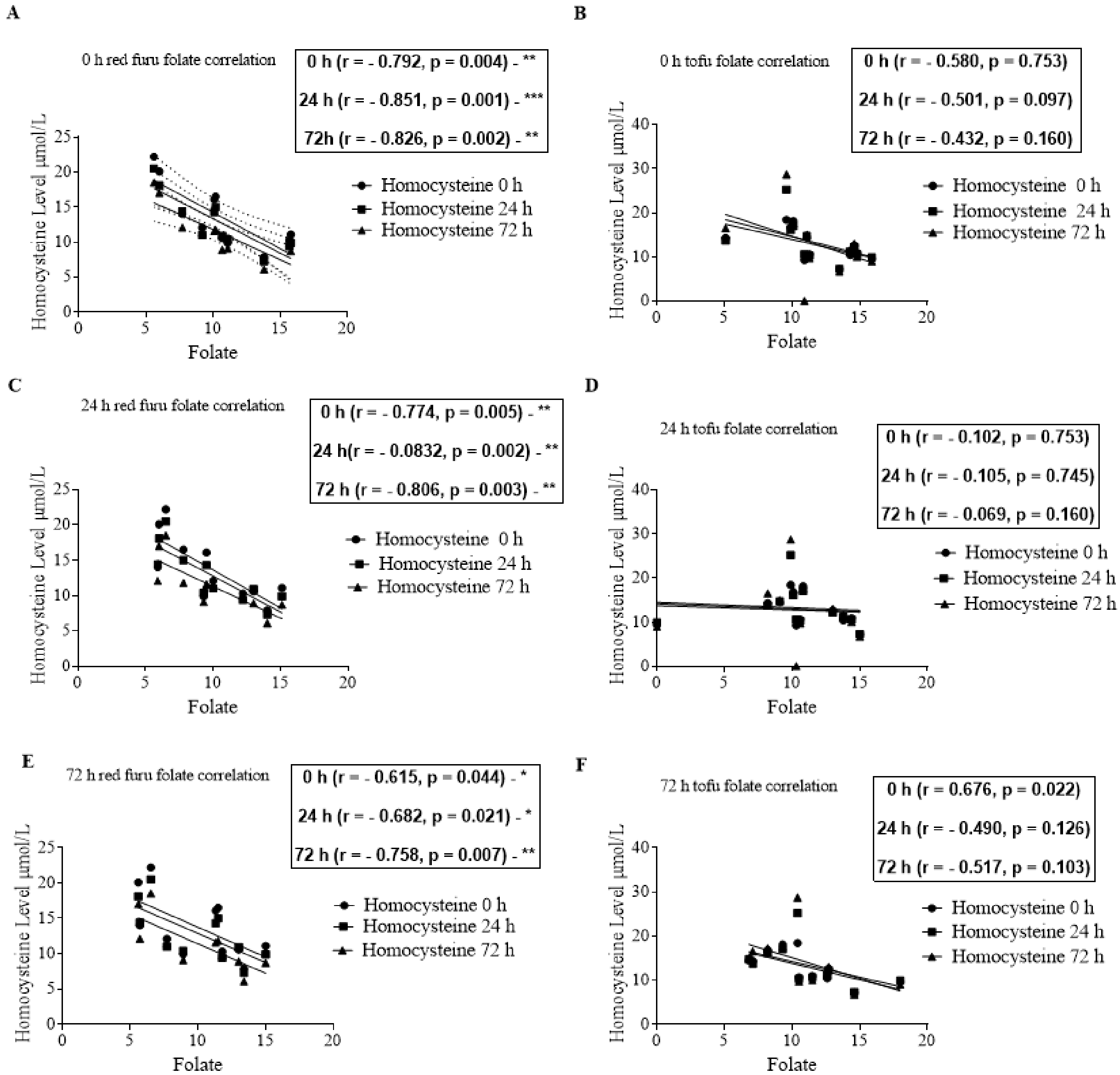

Figure 8. Correlation between folate and Hcy at (0 h- 24 h- 72 h) three-time point in red furu (A, C, E) and tofu (B, D, F). Mean ± SEM were used when comparing the measured values statistically. Red furu and tofu results were tested by Person's correlation coefficient.

B-12 and folate were effective in reducing plasma Hcy concentrations. A study in B vitamin supplementation comparing the effects in patients with Hcy related disease with those in healthy volunteers found that B-12 supplementation effectively reduced Hcy levels in patients and in healthy volunteers.[9] Although, the strongest Hcy-lowering effect was noticed in volunteers with high initial Hcy and also in those with low initial B-12 concentrations. This study concluded that combined vitamin supplementation reduces Hcy levels effectively in patients and in healthy volunteers. However, the researchers observed a moderate reduction in Hcy levels in volunteers with Hcy and B-12 levels within the normal range; from this observation the researchers raised the question that B-12 supplementation does not only reduce Hcy levels in patients but also in volunteers with Hcy and B-12 levels within normal ranges.[9] On the other hand another study conducted largely in younger individuals, showed that B vitamins lowered plasma Hcy by substantial amounts and that this effect was greater in people with higher Hcy and lower folate levels.[10] This study was confirmed in older men, and showed that the Hcy-lowering effect was highest in those who had lower serum concentration of B-12.[10]

Therefore, in the present study in healthy young adults, while there was no significant reduction in plasma Hcy, there was an obvious trend to decreasing levels in the red furu group, and the plasma Hcy was strongly negatively correlated with serum B-12. Folic acid was not supplemented in this study, but it was found that the serum Hcy was also strongly negatively correlated with serum folate.

Hcy is an intermediary sulphur containing product of the methionine metabolism. In the normal metabolism, Hcy is converted to methionine by 5-methyltetrahydrofolate Hcy methyltransferase via trans-methylation reaction in the presence of B-12 and folate.[11] Therefore the proportions of vitamin B-12 and folate to Hcy level are inversely associated and the supplementation of B-12 and folate reduces plasma homocysteine level.[12] We propose that the following explanations might account for the correlations observed. The red furu meal containing B-12 assisted in the lowering of serum Hcy which could account for the negative correlation observed

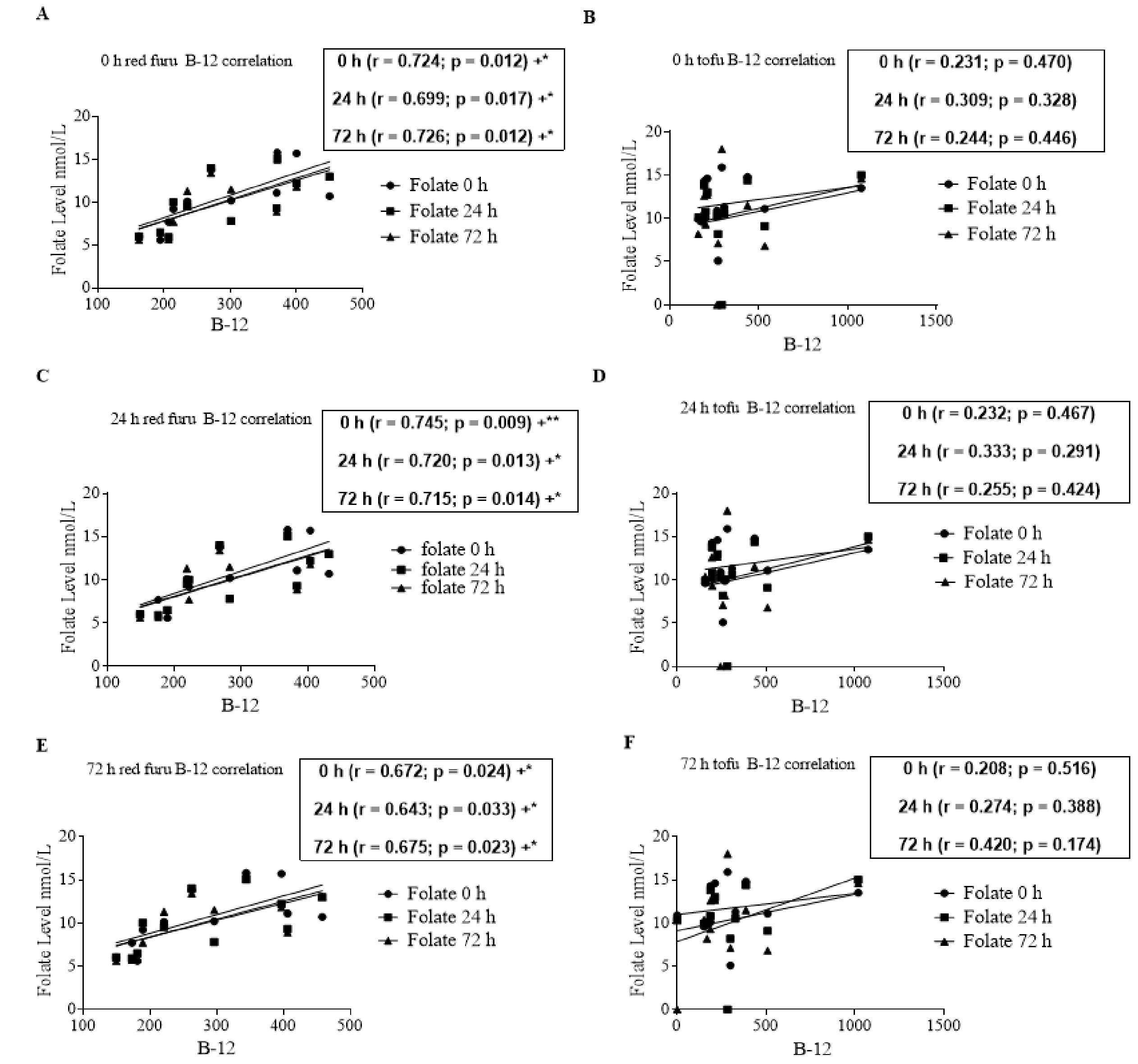

Figure 9. Correlation between B-12 and folate at (0 h- 24 h- 72 h) three-time point in red furu (A, C, E) and tofu (B, D, F). Mean ± SEM were used when comparing the measured values statistically. Red furu and tofu results were tested by Person's correlation coefficient.

between these two parameters. The foods consumed by the volunteers in the 72 h following the meal contained adequate folate sources (as evidenced by serum folate levels). Therefore the folate together with the supply of B-12 from the red furu worked together to reduce the Hcy; this could account for the negative correlation observed between folate and Hcy. In the tofu group, there was no additional B-12 provided so we propose there was no stimulus to the re-methylation cycle to lower Hcy.

Previous studies have reported the importance of reduction of Hcy levels by investigating the Hcy-lowering effects of B-12, or folate alone or in combination.[13-15] A prospective, community-based study found plasma Hcy to be strongly inversely associated with plasma folate level and only weakly associated with plasma levels of B-12.[16] Closely associated to the folate status are B-12, and Hcy, whose concentration greatly depends on the folate status. Serum folate concentrations are directly associated with serum vitamin B-12 and both are inversely associated with Hcy.[17]

Given that red furu exhibited effect although non-significant, on the reduction of Hcy levels in the young, healthy volunteers of various ethnicities who participated in our study. These results suggested that red furu could be efficient in reducing levels of Hcy in young, healthy individuals in general. Our study demonstrated that the factors influencing dietary intake trial outcomes are complex and require further clarification. To determine the reasons underlying the decreased, as opposed to an increase in the serum levels of B-12 observed in young, healthy volunteers after the consumption of red furu in the present study, additional studies should be conducted.

Several limitations must be considered when interpreting our results. Firstly, the low number of healthy subjects from different ethnic groups with dissimilar dietary habits, who consumed a single meal containing 50 g of red furu for a short period of time. Secondly, the subjects were not deficient in B-12 or folate. The above factors may have resulted in insufficient power when comparing the two groups. Thirdly, while we recommended that volunteers maintain their usual diet, except for the additional

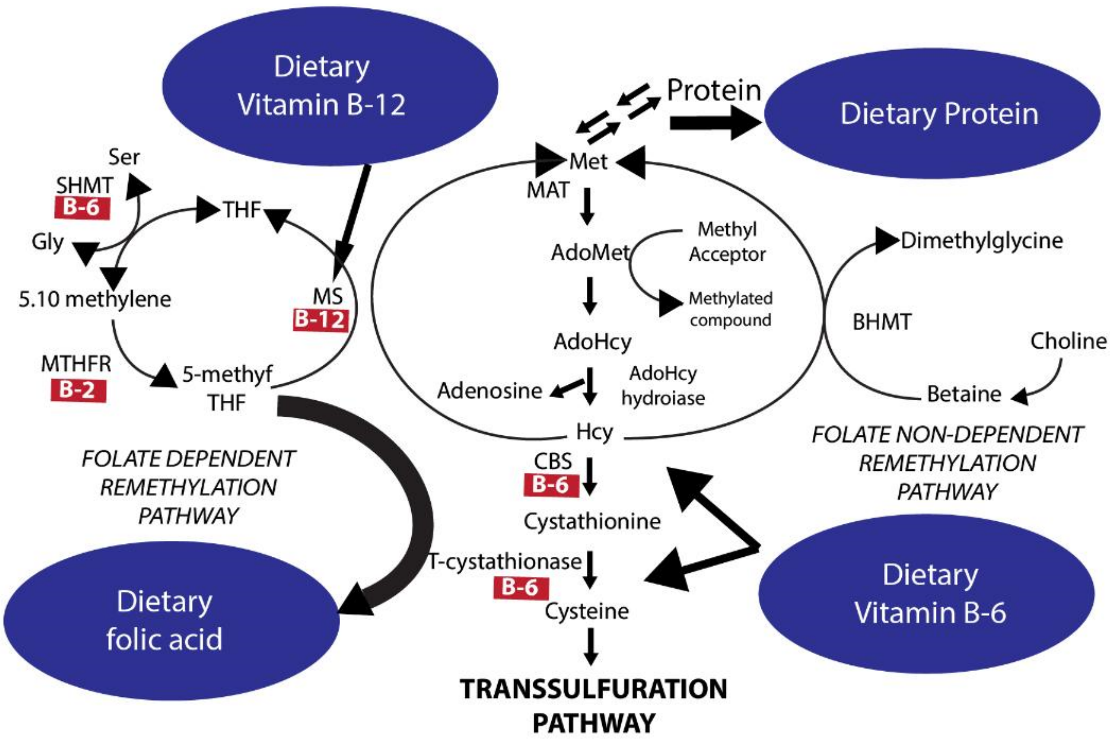

Figure 10. Schematic representation of homocysteine metabolism by Castro et al, 2006 with modification adapted from Verhoef & Stampfer et al, 1996. Metabolism of methionine to cysteine and remethylation of homocysteine to methionine, indicating the pivotal role played by the B-vitamins: folate, vitamin B-6 and vitamin B-12. The remethylation pathway requires vitamin B-12, folate, and the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR). 5-MTHF works together with vitamin B-12 as a methyl-group donor in the conversion of homocysteine back to methionine. Abbreviations: Met, methionine; MAT, ATP-L-methionine S-adenosyltransferase; AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; Hcy, homocysteine; THF, tetrahydrofolate; Ser, serine; Gly, glycine; MS, methionine synthase; BHMT, betaine-homocysteine methyltransferase; MTHFR, 5,10-methylenetetrahydrofolate reductase; CBS, cystathionine β-synthase.

consumption of fermented soybean products, but we did not monitor their intakes during the 72 h trial. Finally, the Chinese nutrition software program used could not detect B-12 in the foods and beverages consumed by volunteers during the study period, apart from the red furu.

Conclusions

The present study results showed that a meal containing 50 g of red furu did not increase the serum concentrations of B-12 of healthy volunteers over 24 or 72 h, compared with a meal containing 50 g of tofu. However, the findings revealed a significant negative correlation between serum B-12 and Hcy in the red furu group suggesting the potential of red furu to lower serum Hcy. This has relevance in reducing the risk of developing cardiovascular diseases. Therefore, studies with a larger sample size, longer duration, different study design, larger dose of B-12, and volunteers who are deficient in B-12 should be conducted in the future to examine red furu's effect on the serum B-12 and Hcy.

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AUTHOR DISCLOSURES

The authors declare no conflict of interest.

The commercial products used and documented in this paper were gifted to the project by the producer. The authors do not endorse them or receive any financial benefit from the manufacturers.

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