Original Article

Effect of vitamin B_{12} and n-3 polyunsaturated fatty acids on plasma homocysteine, ferritin, C-reaction protein, and other cardiovascular risk factors: a randomized controlled trial

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Objectives: Vitamin B_{12} and n-3 polyunsaturated fatty acids (PUFA) decrease blood homocysteine (Hcy) concentrations. However, the combined effect of these nutrients on Hcy and ferritin, and C-reactive protein is limited and inconclusive. The objective was to examine the synergistic effect of vitamin B_{12} in combination of n-3 PUFA on plasma Hcy, ferritin, and other biochemical markers. **Methods:** In a randomized controlled trial, thirty eligible subjects were randomly divided into three groups, and assigned to receive 1000 μg of vitamin B_{12}, 2 g fish oil, or 1000 μg vitamin B_{12} and 2 g fish oil, respectively, for 8 weeks. Plasma phospholipids (PL) fatty acids and biochemical markers were determined. This study was registered under ClinicalTrials.gov Identifier: NCT01762072. **Results:** Plasma PL 20:5n-3, 22:6n-3 and n-3 PUFA was increased after 4 and 8 week supplementation of fish oil, and vitamin B_{12}+fish oil. Plasma concentrations of triacylglycerol, uric acid, C-reactive protein, and ferritin were significantly decreased after 4 and 8 week supplementation of fish oil, and vitamin B_{12}+fish oil. In all groups, significant changes in plasma Hcy were observed during the study period. Vitamin B_{12}, fish oil, and vitamin B_{12}+fish oil supplementation lowered plasma Hcy concentrations by 22%, 19%, and 39%, respectively. **Conclusions**: The combination of vitamin B_{12} and fish oil has a synergistic effect on lowering plasma concentrations of Hcy.

Key Words: homocysteine, vitamin B_{12}, fish oil, ferritin, C-reaction protein

INTRODUCTION

Homocysteine (Hcy) is a thiol-containing amino acid derived from methionine metabolism. Elevated plasma Hcy concentration has been demonstrated to be an independent risk factor for cardiovascular diseases (CVD).[1] Plasma Hcy can be lowered with B vitamin supplementation.[2] In 1988, Brattström et al showed that healthy subjects responded to a high dose of folic acid with a marked reduction in their Hcy levels.[3] Since then, several studies have demonstrated that daily supplementation with folic acid, vitamin B_6, vitamin B_{12}, or a combination reduces Hcy levels to varying degrees in intervention studies.[4] Previous meta-analysis of randomized controlled trials (RCT) determined that B vitamin supplementation decreased plasma Hcy concentration.[5]

Modification of dietary fat composition has been demonstrated to improve the lipid and carbohydrate metabolism, thus decreasing cardiovascular risk.[6] Previous studies reported that platelet/plasma phospholipids (PL) n-3 polyunsaturated fatty acids (PUFA) were negatively associated with plasma Hcy in middle aged and geriatric hyperlipaemia patients[7] and in healthy Australian male subjects.[8] Over the past two decades, several intervention studies of small sample size and short duration have demonstrated that n-3 PUFA supplementation decreases plasma Hcy in patients with diabetic dyslipidemia,[9] patients with acute myocardial infarction,[10] and men with hyperlipidemia.[11] However, the efficacy of n-3 PUFAs on plasma Hcy level in humans has not been consistently demonstrated. A meta-analysis reported that n-3 PUFA decrease plasma Hcy levels.[12]

Furthermore, it has also been suggested that erythrocyte PUFA, particularly n-6 PUFA, are related to circulating C-reaction protein (CRP) which is an independent risk factor for CVD.[13] Serum n-3 PUFA and especially the long chain n-3 PUFA concentration are inversely associated with serum CRP in men.[13] Numerous intervention studies have demonstrated that n-3 PUFA decreases blood CRP levels.[14] Ferritin, one of the key proteins regulating iron homeostasis, is associated with higher risk of type 2 diabetes and metabolic syndrome.[15] Previous studies explored the

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effects of n-3 PUFA eight-week's supplementation (20:5n-3 and 22:6n-3, 2.4 g/d) on ferritin in 35 patients with chronic renal failure. A significant decrease of the ferritin levels was observed after dietary intervention regime.[14]

Supplementation with vitamin B_{12} and n-3 fatty acids corrects hyperhomocysteinemia and reduces platelet reactivity in vegetarians.[16] However, no study has reported the effect of vitamin B_{12} in combination with fish oil on plasma Hcy, ferritin, CRP and other CVD risk factors in Chinese. The present intervention study was performed to assess the synergetic effects of fish oil and vitamin B_{12} on these biochemical markers in Chinese healthy subjects.

SUBJECTS AND METHODS

Recruitment and eligibility of participants

Thirty apparently healthy subjects, aged 23±3 years, were recruited for an 8-week study at Zhejiang University, Hangzhou, China. The study was conducted in Zhejiang University Hospital, Hangzhou, China. Selection criteria included regular eating habits, normal weight (18.5 kg/m^2≤BMI≤23.9 kg/m^2), non-drinking (never drink and past drink), and non-smoking status (never smoke and past smoke). None of the selected subjects used any vitamins or dietary supplements or had taken any medication for at least 8 weeks before the start of and during the entire experimental period. Participants were encouraged to maintain constant dietary habits and pursue their normal activities throughout the study period. The study was conducted between October and December in 2011. All subjects gave their informed consent, and the protocols were approved by the Ethics Committee of Biosystems Engineering & Food Science, Zhejiang University.

Study design and protocol

Eligible participants were randomly divided into the following three groups: VitB_{12} group (VitB_{12}, n=10), Fish oil group (FO, n=10), and VitB_{12}+fish oil group (VitB_{12}+FO, n=10). Each group received 8 weeks of treatment with daily oral doses of 1) 1000 μg of vitamin B_{12} (one capsule) (General Nutrition Center, USA), 2) 2 g of fish oil in the form of two capsules of fish oil) (Nepstar Chain Drugstore Ltd, Shenzhen, China) (each 1 g capsule provided 490 mg of 22:6n-3, and 98 mg of 20:5n-3), or 3) a combination of 1000 μg of vitamin B_{12} and 2 g of fish oil, respectively (Figure 1).[10,12] The capsules given to the separate treatment groups were identical in appearance, smell, and taste. The participants were asked to maintain their regular diet and to record their daily intake of capsules during the trial. Compliance was checked by counting the number of unused capsules remaining in capsule dispensers and by verifying pill counts in the participants' diaries. Researchers were asked to monitor the daily capsule intakes of the participants.

Blood collection

Subjects attended the Zhejiang University Hospital in the morning following an overnight fast. Subjects were allowed to sit relaxed for 10 min, the subject's weight, height were measured. Then venous blood was taken in plain and EDTA vacuum tubes with 21-gauge needles (Longhe, Nanchang China). Within one hour after blood collection, this blood sample was placed on ice water and centrifuged at 2000×*g* for 10 min at a temperature of 4°C within 30 mins of collection. All plasma samples were stored at −80°C before laboratory analysis.

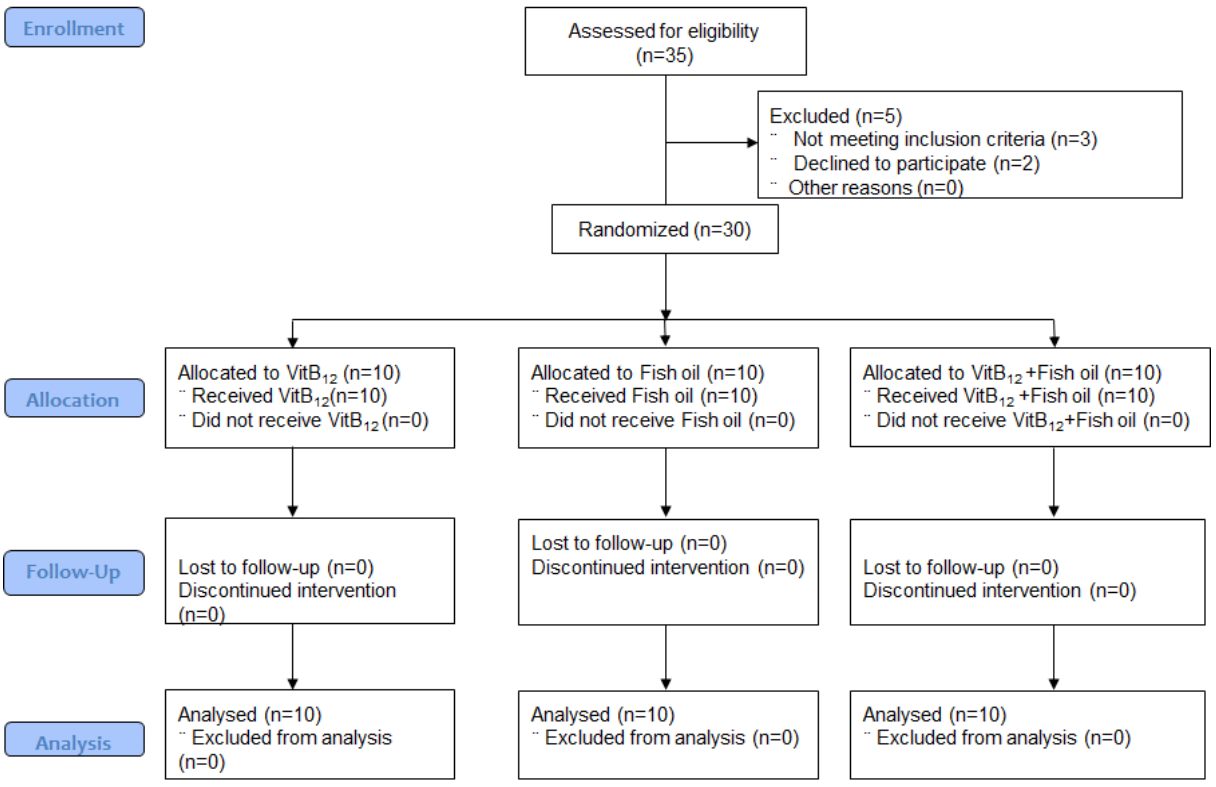

Figure 1. Flow diagram of the study

Laboratory measurements

Plasma total Hcy was determined by polarized fluorescence immunoassay in an AXSYM system. Plasma folate and vitamin B_{12} were measured using immulitechemiluminescent kits according to the manufacturer's instructions (Diagnostic Products Corporation/Siemens, Los Angeles, CA). Plasma lipids, uric acid, and insulin were determined on an auto-analyzer (Olympus AU2700, Tokyo, Japan), via commercially available kits (Olympus, Tokyo, Japan). CRP was determined via the immunoturbidimetric method with Denka Seiken, Japan reagents (Hitachi 7600 automated analyzer, Hitachi Inc., Tokyo, Japan). Glucose was measured with a Hitachi 7600 analyzer using a glucose oxidase phenol 4-aminoantipyrine peroxidase kit (GOD-PAP; Randox, Crumlin, UK). Serum ferritin was determined by sandwich immunoassay method with fluorescence detection in a final phase (ELFA) (Shenggong Inc., Shanghai, China). Total lipid content of plasma was extracted with solvents, the PL fraction was separated by thin lay chromatography (TLC) and the fatty acid methyl esters were prepared and separated by gas-liquid chromatography.

Statistical analyses

All dependent variables were checked for normal distribution. TG values were log-transformed before analysis. Baseline characteristics between treatment groups were compared by one-way analysis of variance (ANOVA) for continuous variables. The average concentrations of the biochemical variables at the screening and randomization visits were calculated for each participant and defined as "baseline" values. Differences in concentrations of blood variables at baseline and at follow-up were assessed with 2-factor measures ANOVA (3 measurements×3 treatment groups) with treatment and period as fixed factors, participants as random factors and baseline values as covariates. Further fixed terms corresponding to treatment-period and treatment-baseline value interactions were included. Tukey's post hoc tests were used to assess differences between intervention groups. These analyses were performed with mixed models (SAS PROC MIXED procedure), an extension from the linear regression model that includes random effects. All analysis were conducted by using SAS statistical software (version 9.1; SAS Institute Inc, Cary, NC), The two-sided p value ≤ 0.05 was considered statistically significant.

RESULTS

Characteristics of subjects at baseline

A summary of the demographic characteristics of the participants at baseline is shown in Table 1. These characteristics were not significantly different between the treatment groups.

Plasma phospholipid fatty acids

Plasma PL proportions of 20:5n-3, 22:6n-3and total n-3 PUFA were significantly increased after 4 and 8 weeks supplementation of fish oil, and vitamin B_{12}+fish oil. Interestingly, plasma PL proportions of total saturated fatty acid (SFA) were also significantly increased, whereas the proportions of the n-6 PUFA were decreased after 4 and 8 weeks supplementation of fish oil (Table 2).

Blood biochemical markers

The concentrations of plasma biochemical markers (plasma lipids, glucose, insulin, uric acid, C-reactive protein, and ferritin) at baseline and at 4 and 8 weeks of supplementation are presented in Table 3. There was a significant time × treatment interaction for total triacylglycerol (TG), high density lipoprotein cholesterol (HDL-C), uric acid, and ferritin ($p<0.01$). Plasma mean HDL-C concentration was significantly increased, whereas TG, uric acid, and ferritin concentrations were significantly decreased after 4 and 8 weeks of supplementation with fish oil and vitamin B_{12}+fish oil (Table 3). Plasma glucose concentrations in FO and VitB_{12}+FO groups were decreased after 8 weeks of supplementation. In VitB_{12} group, no significant changes were observed for any of the blood biochemical markers.

Plasma ferritin concentration was significantly lowered from 73.5±10.6 pmol/L at baseline to 51.2±10.3 at the end in FO group, and decreased from 82.0±18.6 pmol/L at baseline to 52.9±7.11 at the end in VitB_{12}+FO group. Plasma ferritin concentration was significant lower in VitB_{12}+FO group than that in VitB_{12} group (Table 3 and Figure 2).

Plasma concentrations of homocysteine, vitamin B_{12} and folate

There was a significant time × treatment interaction for plasma concentrations of vitamin B_{12} and Hcy ($p<0.001$). In the VitB_{12} and VitB_{12}+FO groups, significant changes in plasma vitamin B_{12} and Hcy were observed during the study period. Plasma Hcy concentration was reduced from 12.3±1.65 to 9.57±1.05, 12.9±1.45 to 10.4±1.76 and 11.8±1.19 to 7.18±0.61 μmol/L after 8 weeks of supplementation with vitamin B_{12}, fish oil and vitamin B_{12}+fish oil, respectively (Table 4). Vitamin B_{12}, fish oil, and vitamin B_{12}+fish oil supplementation significantly lowered mean plasma Hcy concentrations by 22%, 19%, and 39%, respectively (Figure 2).

Table 1. Characteristics of subjects at baseline

	VitB_{12} (n=10)	FO (n=10)	VitB_{12}+FO(n=10)	p
Age, year	24.5±0.7	24±0.5	23.4±0.7	0.73
Male, %	6 (60)	5 (50)	6 (60)	0.87
Weight, kg	62.9±7.0	53.2±2.5	54.2±1.9	0.08
Height, cm	173±2.2	166±1.8	168±2.5	0.18
BMI, kg/m^2	21.6±1.2	20.9±1.4	19.5±1.0	0.32

VitB_{12}: Vitamin B_{12} group; FO: Fish oil group

Table 2. Fatty acid compositions of plasma phospholipids at the beginning of the study (T0), after 4 (T4) and 8 (T8) weeks of intervention[†]

% of total fatty acids	$VitB_{12}$			FO			$VitB_{12}$+FO		
	Baseline (T0) n=10	4 weeks (T4) n=10	8 weeks (T8) n=10	Baseline (T0) n=10	4 weeks (T4) n=10	8 weeks (T8) n=10	Baseline (T0) n=10	4 weeks (T4) n=10	8 weeks (T8) n=10
14:00	0.19±0.02	0.22±0.02	0.40±0.18	0.21±0.01	0.25±0.02	0.40±0.05	0.21±0.01	0.25±0.03	0.31±0.04
15:00	1.31±0.05	1.19±0.14	1.46±0.16	1.21±0.10	1.50±0.20	1.09±0.08	1.27±0.10	1.12±0.14	1.34±0.14
16:00	20.8±0.38	20.6±0.31	21.6±0.33	21.4±0.56	20.0±0.71	22.6±0.31	21.4±0.37	20.8±0.51	22.1±0.45
18:00	12.2±0.29	12.3±0.47	12.6±0.32	12.3±0.26	11.1±0.42	13.3±0.36	12.0±0.30	12.4±0.49	12.7±0.34
20:00	0.26±0.02	0.23±0.02	0.22±0.04	0.33±0.07	0.31±0.04	0.43±0.16	0.25±0.03	0.29±0.03	0.27±0.03
SFA	34.7±0.51	34.6±0.65	36.3±0.42	35.4±0.69	36.1±0.94	37.8±0.46*	35.1±0.65	34.9±0.73	36.7±0.64*
14:1n-5	0.11±0.01	0.14±0.01	0.36±0.12	0.12±0.01	0.15±0.01	0.65±0.20	0.12±0.02	0.17±0.02	0.25±0.03
16:1n-7	0.36±0.03	0.49±0.04	0.60±0.11	0.51±0.04	0.64±0.06	0.92±0.16	0.34±0.03	0.50±0.06	0.74±0.11
18:1n-7	1.20±0.04	1.06±0.12	1.17±0.06	1.14±0.04	1.20±0.04	1.01±0.03	1.13±0.03	0.97±0.11	1.07±0.03
18:1n-9	14.6±0.74	13.4±0.40	12.3±0.23	15.2±0.63	13.0±0.48	12.9±0.43	15.0±1.52	13.4±0.30	12.4±0.18
20:1n-9	0.22±0.01	0.24±0.05	0.21±0.03	0.32±0.09	0.34±0.12	0.44±0.19	0.36±0.11	0.22±0.02	0.21±0.01
22:1n-9	0.34±0.04	0.82±0.29	0.63±0.23	0.36±0.02	1.07±0.26	0.64±0.16	0.90±0.25	0.75±0.16	0.74±0.09
MUFA	16.8±0.74	16.2±0.28	15.3±0.37	17.6±0.66	16.4±0.29	16.6±0.37	17.8±1.59	16.0±0.41	15.4±0.26
18:2n-6	20.0±0.88	23.4±1.09	19.5±0.47	19.5±1.00	23.1±1.37	18.2±0.84	19.6±0.50	23.6±1.46	20.0±0.47
18:3n-6	0.20±0.05	0.23±0.06	0.30±0.10	0.42±0.10	0.37±0.08	0.59±0.24	0.32±0.08	0.24±0.08	0.23±0.05
20:2n-6	0.36±0.02	0.34±0.02	0.40±0.05	0.41±0.11	0.45±0.10	0.38±0.08	0.43±0.05	0.34±0.02	0.37±0.01
20:3n-6	1.17±0.08	1.13±0.05	1.13±0.07	1.21±0.09	1.08±0.09	1.07±0.08	1.14±0.07	1.10±0.06	1.05±0.06
20:4n-6	14.1±0.58	13.3±0.45	14.0±0.24	13.0±0.59	11.5±0.56	11.2±0.47	13.0±0.52	12.0±0.99	12.3±0.39
22:2n-6	2.63±0.14	2.21±0.14	2.37±0.24	2.19±0.15	1.80±0.14	1.85±0.10	1.87±0.25	1.75±0.22	1.95±0.10
22:4n-6	0.72±0.03	0.87±0.18	1.01±0.21	0.85±0.16	0.64±0.13	1.35±0.37	1.07±0.20	0.78±0.19	1.15±0.26
22:5n-6	1.94±0.12	1.41±0.17	2.11±0.15	1.89±0.14	1.64±0.25	1.94±0.26	1.65±0.16	1.67±0.22	1.90±0.15
n-6 PUFA	41.1±0.51	42.8±0.79	40.8±0.63	39.4±0.44	40.6±1.34	36.6±0.61*	39.1±0.95	41.5±1.03	39.0±0.50
18:3n-3	0.34±0.05	0.30±0.03	0.26±0.01	0.36±0.12	0.34±0.04	0.35±0.03	0.29±0.02	0.39±0.06*	0.35±0.03*
20:5n-3	1.06±0.04	0.90±0.11	1.19±0.06	1.11±0.05	1.34±0.10*	1.43±0.22*	1.02±0.04	1.38±0.20*	1.41±0.07*
22:5n-3	2.10±0.09	1.50±0.17	2.25±0.07	2.06±0.16	1.68±0.24	2.12±0.10	2.30±0.20	1.77±0.19	2.39±0.07
22:6n-3	3.79±0.15	3.79±0.42	3.91±0.16	3.63±0.14	4.67±0.29*	4.98±0.14*	3.77±0.34	4.13±0.30*	5.14±0.28*
n-3 PUFA	7.29±0.19	6.49±0.53*	7.61±0.15*	7.17±0.21	7.84±0.33*	7.85±0.23*	7.57±0.52	7.97±0.31*	8.99±0.27*
n-3:n-6	0.18±0.00	0.15±0.01*	0.19±0.01	0.18±0.01	0.20±0.01*	0.22±0.01*	0.19±0.01	0.19±0.01	0.23±0.01*

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.
[†]All values are Mean±SD.
*Significantly different from baseline, $p<0.05$.

Table 3. Plasma biochemical markers at the beginning of the study (T0), after 4 weeks (T4), and after 8 weeks (T8) of intervention[§]

Plasma biochemical markers	Duration	$VitB_{12}$ (n=10)	FO (n=10)	$VitB_{12}$+FO (n=10)
LDL-C, mmol/L	Baseline (T0)	2.54±0.10	2.60±0.11	2.51±0.15
	4 weeks (T4)	2.54±0.18	2.83±0.22	2.58±0.17
	8 weeks (T8)	2.35±0.11	2.50±0.15	2.68±0.14
HDL-C, mmol/L	Baseline (T0)	1.35±0.05	1.49±0.08	1.44±0.14
	4 weeks (T4)	1.34±0.05	1.64±0.05[†,‡]	1.66±0.13[†,‡]
	8 weeks (T8)	1.45±0.06	1.60±0.06[†,‡]	1.66±0.13[†,‡]
TC, mmol/L	Baseline (T0)	3.80±0.26	4.14±0.13	4.16±0.22
	4 weeks (T4)	3.75±0.22	4.17±0.22	4.02±0.22
	8 weeks (T8)	3.72±0.13	3.60±0.22[†]	4.07±0.22
TG, mmol/L	Baseline (T0)	0.85±0.22	0.75±0.07	0.85±0.08
	4 weeks (T4)	0.60±0.08	0.60±0.08[†]	0.68±0.06[†]
	8 weeks (T8)	0.70±0.09	0.67±0.13	0.63±0.10[†]
Glucose, mmol/L	Baseline (T0)	5.33±0.06	5.54±0.14	5.36±0.07
	4 weeks (T4)	5.15±0.10	5.19±0.07	5.40±0.18
	8 weeks (T8)	5.27±0.07	5.31±0.12[†]	5.19±0.08[†]
Insulin, pmol/L	Baseline (T0)	4.90±0.51	6.60±0.93	5.40±0.70
	4 weeks (T4)	5.34±0.54	5.36±0.57	5.34±0.73
	8 weeks (T8)	5.10±0.67	5.01±0.43[†]	5.09±0.49
CRP, mg/L	Baseline (T0)	0.55±0.05	0.65±0.15	0.56±0.11
	4 weeks (T4)	0.75±0.07	0.66±0.10	0.66±0.13
	8 weeks (T8)	0.46±0.08	0.52±0.09[†]	0.38±0.05[†]
Uric acid, μmol/L	Baseline (T0)	319± 6.6	347±25.2	369±15.7
	4 weeks (T4)	307±11.4	260±16.6[†]	237±20.1[†]
	8 weeks (T8)	308±13.2	308±17.9[†]	255±22.3[†]
Ferritin, pmol/L	Baseline (T0)	80.7±14.9	73.5±10.6	82.0±18.6
	4 weeks (T4)	83.2±13.4	59.6±11.3[†,‡]	64.6±21.8[†,‡]
	8 weeks (T8)	87.5±13.5	51.2±10.3[†,‡]	52.9±7.11[†,‡]

TC: total cholesterol; TG: total triglyceride; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; CRP: C-reactive protein,

[§]All values are Mean±SD

No significant differences between the 3 treatment groups were observed at baseline for all biochemical markers, $p>0.05$ (ANOVA with Tukey's post hoc tests).

[†]Significantly different from baseline, $p<0.05$.

[‡]Significantly different from $VitB_{12}$ group, $p<0.05$ (ANOVA with Tukey's post hoc tests).

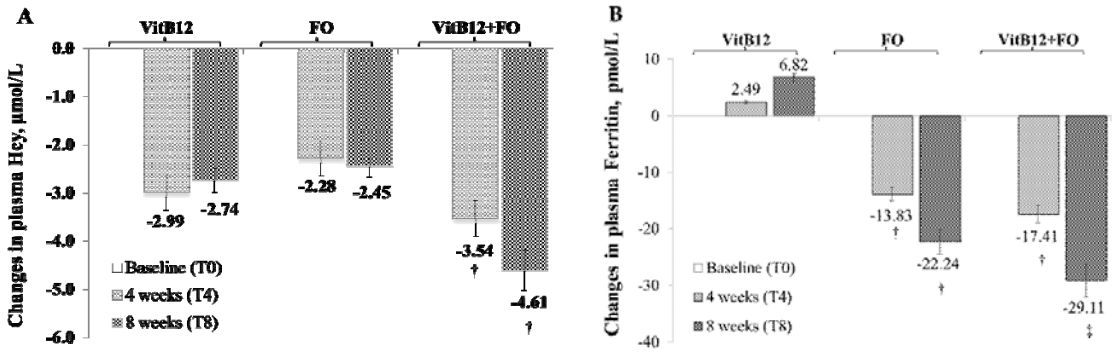

Figure 2. Mean changes in plasma homocysteine and ferritin concentrations at the beginning of the study (T0), after 4 weeks (T4), and after 8 weeks (T8) of intervention.

Number of subjects in each group was 10.

No significant differences between the 3 treatment groups were observed at baseline for all biochemical markers.

The changes in plasma Hcy and ferritin concentrations were calculated compared with baseline data.

Figure 2A: [†]Significantly different from $VitB_{12}$ group or FO group, $p<0.05$.

Figure 2B: [†]Significantly different from $VitB_{12}$ group, $p< 0.05$. [‡]Significantly different from FO group, $p<0.05$.

Table 4. Plasma homocysteine, vitamin B_{12}, folate concentrations in participants by treatment group at baseline, 4 weeks, and 8 weeks or supplementation[††]

Hcy and vitamin B group	Duration	$VitB_{12}$ n=10	FO n=10	$VitB_{12}$+FO n=10
Vitamin B_{12}, ng/mL	Baseline (T0)	271±22.5	289±40.6	264±32.0
	4 weeks (T4)	570±51.3[†]	256±33.2[‡]	662±64[†]
	8 weeks (T8)	668±57.0[†]	273±29.9[‡]	561±54.5[†]
Folate, pmol/L	Baseline (T0)	3.82±0.39	4.20±0.71	5.84±0.67
	4 weeks (T4)	3.60±0.28	4.41±0.85	5.94±0.58
	8 weeks (T8)	3.45±0.36	4.29±0.70	6.27±0.56
Hcy, μmol/L	Baseline (T0)	12.3±1.65	12.9±1.45	11.8±1.19
	4 weeks (T4)	9.32±0.76[†]	10.6±1.24[†]	8.25±1.46[†,§]
	8 weeks (T8)	9.57±1.05[†]	10.4±1.76[†]	7.18±0.61[†,§,¶]

[††]All values are Mean±SD; A significant time × treatment interaction was observed for Vitamin B_{12} and Hcy, $p<0.001$ (ANOVA). No significant differences between the 3 treatment groups were observed at baseline for all biochemical markers, $p>0.05$ (ANOVA with Tukey's post hoc tests).
[†]Significantly different from baseline, $p<0.05$.
[‡]Significantly different from the $VitB_{12}$ and $VitB_{12}$+FO groups, $p<0.05$ (ANOVA with Tukey's post hoc tests).
[§]Significantly different from FO group, $p<0.05$ (ANOVA with Tukey's post hoc tests).
[¶]Significantly different from VitB12 group, $p<0.05$ (ANOVA with Tukey's post hoc tests).

DISCUSSION

Our main findings were that high supplementation of fish oil alone or in combination with vitamin B_{12} decreased plasma Hcy, ferritin, and CRP concentrations. Vitamin B_{12} in combination with fish oil had synergistic effects on plasma Hcy concentrations.

Previous RCTs documented a plasma Hcy lowering effect following n-3 PUFA supplementation[6,17-22,16-21] whereas in contrast, supplementation with n-3 PUFA was also associated with a significantly greater increase in plasma Hcy compared with control subjects.[23,24] However, some studies did not show a significant decrease in plasma Hcy.[23-26] The conflicting results may be due to different durations of intervention and non-comparable populations. For example, the median level of Hcy in Beavers's study population is 2-3 times higher than other studies.[26] Characteristics of the sample population used in their study, specifically, renal disease, may also help to explain why fish oil supplementation had no effect on Hcy levels. End-stage renal disease (ESRD) is associated with significant morbidity, and Hcy levels were shown to be four times higher in ESRD patients compared with an apparently healthy population.[26] There was no significant effect on plasma Hcy concentration in a 3 months of supplementation with fish oil in continuous ambulatory peritoneal dialysis patients, and it was hypothesized that longer exposure to fish oil might control Hcy level more effectively.[27] Moreover, daily administration of 6 capsules of n-3 fatty acids (160 mg of 20:5n-3 and 100 mg of 22:6n-3 per capsule) had no effect on Hcy levels even when the researchers extended the fish oil supplementation protocol to 6 months.[26] In a meta-analysis, it was estimated that plasma Hcy levels were significantly decreased by high-dose n-3 PUFA supplementation.[12] A very recent cohort study showed that elevated Hcy (>14.5 μmol/L) was associated with 80% higher mortality than low Hcy level (<9.3 μmol/L).[28] Therefore, the present findings further suggest that the management of Hcy is of practical significance.

The potential mechanisms by which n-3 PUFA decrease plasma Hcy have been investigated in animal and population studies.[29-31] In animal studies, we found that plasma Hcy was significantly decreased by tuna oil rich in 22:6n-3. Methionine adenosyl transferase (MAT) activity was significantly increased and *MAT* mRNA expression was significantly upregulated by 22:6n-3; cystathionine-gamma-lyase mRNA expression was significantly upregulated by 22:6n-3; we suggested that 22:6n-3 decreased the concentration of plasma Hcy by increasing MAT activity and upregulating mRNA expression of *MAT* and cystathionine-gamma-lyase (*CSE*) gene, both of which are involved in Hcy metabolism.[31] However, hyperhomocysteinemia has multifactorial determinants; it reflects genetic and environmental factors or their interactions. Therefore, genetic variants involved in Hcy metabolic pathways may modify the effects of dietary fatty acids on plasma Hcy in humans. The previous population studies have shown that two functional *MTHFR* variants, 1298A>C and 677C>T, which are not in linkage disequilibrium in Boston Puerto Rican adults, are significantly associated with hypertension. Importantly, these variants exhibited significant interactions with intakes of total and n-6 PUFA and with the n-3:n-6 PUFA ratio of the diet in determining plasma Hcy concentration. Participants with combined genotypes of both single nuclear polymorphisms (SNP) (677 TT with 1298 AC or CC) who consumed high levels of n-3 PUFA (>0.66% energy) had lower plasma Hcy compared with those who had the same genotype and consumed low levels of n-3 PUFA (≤0.66% energy).[30] Therefore, it is suggested that dietary PUFA intake modulates the effect of MTHFR variants on plasma Hcy.[30] Moreover, genetic variant MAT1A 3U1510 displayed a significant interaction with the dietary n-3:n-6 PUFA ratio in determining plasma Hcy. Homozygotes for 3U1510G have significantly lower plasma Hcy concentrations than those who are major allele homozygotes and heterozygotes (AA+AG) and when the n-3:n-6 ratio is >0.09. Two other *MAT1A* variants (d18777 and i15752),

also show significant interactions with different constituents of dietary fat in influencing Hcy concentration. Furthermore, haplotypes consisting of three variants display a strong interaction with n-3:n-6 ratio influencing Hcy concentrations.[29]

Elevated CRP is an independent risk factor for cardiovascular disease. Over the past twenty years, several intervention studies have documented the effect of n-3 PUFAs on blood CRP level.[32] In the present study, we confirmed that fish oil alone or in combination with vitamin B_{12} significantly decreased plasma CRP concentration. The n-3 PUFA have a biological basis in regulating CRP level. Both nuclear factor κB (NF-κB) and peroxisome proliferator agonist receptors (PPARs) contribute to the potential mechanism responsible for the observed blood CRP-lowering effect of n-3 PUFA.[33] NF-κB can initiate the expression of genes encoding for inflammatory-related proteins which regulate hepatic synthesis of CRP.[33] In addition, PPARs are lipid-activated transcription factors that reduce inflammatory responses, possibly by stimulating the breakdown of inflammatory eicosanoids or by interfering with the activation of NF-κB.[33] Therefore, the interaction of NF-κB and PPARs may help explain the mechanism of n-3 PUFAs in reducing blood CRP level.

Ferritin is a widely recognized clinical biomarker to evaluate iron status and especially important for detecting iron deficiency.[15] Elevated circulating ferritin concentrations were associated with higher risk of type 2 diabetes and metabolic syndrome in middle-aged and elderly Chinese.[15] A previous RCT demonstrated that n-3 PUFA eight-week's supplementation (20:5n-3+22:6n-3, 2.4 g/d) decreased ferritin in 35 patients with chronic renal failure.[14] In the present study, supplementation of fish oil alone or in combination with vitamin B_{12} significantly lowered the plasma ferritin concentration in healthy subjects. The potential mechanism by which fish oil decreases plasma ferritin level is not understood. However, we hypothesize that n-3 PUFA from fish oil may regulate the gene/protein expression of ferritin or affect the enzyme activity in ferritin metabolic pathway, however further explorations are warranted. Several limitations need to be considered when interpreting our findings. Although the capsules given to each group were identical in appearance, smell, and taste, the number of total capsules to take was different among groups. In this case, potential bias may exist. In addition, the sample size in the present study was small. Therefore, a larger sample size intervention study is required to confirm these findings.

In summary, supplementation of fish oil alone or in combination with vitamin B_{12} decreased plasma concentrations of Hcy, ferritin and CRP. Oral supplementation with vitamin B_{12} in combination with fish oil had a synergistic effect on lowering plasma concentrations of Hcy.

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AUTHOR DISCLOSURES

The authors have no financial/commercial conflicts of interest in this work.

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Original Article

Effect of vitamin B_{12} and n-3 polyunsaturated fatty acids on plasma homocysteine, ferritin, C-reaction protein, and other cardiovascular risk factors: a randomized controlled trial

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维生素 B_{12} 和欧米伽 3 多不饱和脂肪酸对血浆同型半胱氨酸、铁蛋白、C 反应蛋白和其它心血管危险因素的影响：一项随机对照临床试验

目的：维生素 B_{12} 和欧米伽 3 多不饱和脂肪酸能够降低血液同型半胱氨酸浓度。但是，这两种营养元素是否对同型半胱氨酸、铁蛋白、C 反应蛋白有协同效应仍不清楚。**方法**：为进一步解决该问题，我们开展了一项随机对照实验，38 位参与者随机分为三组，每天分别食用 1000 μg 维生素 B_{12}、2 g 鱼油、1000 μg 维生素 B_{12}+2 g 鱼油。八周以后，收集受试者血样，测定血浆磷脂脂肪酸组成，生物标志物等。**结果**：四周或者八周干预以后，鱼油组和鱼油+维生素 B_{12} 组的血浆磷脂 20:5n-3、22:6n-3 和总欧米伽 3 脂肪酸显著升高，然而，血浆甘油三酯、尿酸、C 反应蛋白以及铁蛋白显著降低。维生素 B_{12} 组、鱼油组、维生素 B_{12}+鱼油组，血浆同型半胱氨酸分别降低 22%、19% 和 39%。**结论**：维生素 B_{12} 和鱼油在调节同型半胱氨酸代谢过程中存在协同效应。

关键词：同型半胱氨酸、维生素 B_{12}、鱼油、铁蛋白、C 反应蛋白