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Associations of plasma phospholipid fatty acids with plasma homocysteine in Chinese vegetarians

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Abstract

The association of plasma phospholipid (PL) fatty acid composition with plasma homocysteine (Hcy) in Chinese vegetarians is not understood. The main aim of the present study was to investigate the plasma PL fatty acid status, and its association with plasma Hcy in Chinese vegetarians and omnivores. A total of 103 male vegetarians and 128 male omnivores were recruited in Linyin Temple, Hangzhou. Plasma Hcy and PL fatty acid concentrations were determined by standard methods. Compared with omnivores, plasma PL *n*-3 PUFA ($P<0·001$), 22 : 6*n*-3 ($P<0·001$), 22 : 5*n*-6 ($P=0·021$), 22 : 2*n*-6 ($P<0·001$) and SFA ($P=0·017$) were significantly lower, while plasma PL *n*-6 PUFA ($P=0·007$) and total PUFA ($P<0·001$) were significantly higher in vegetarians. The prevalence of hyperhomocysteinaemia (HHcy) in vegetarians (26·47 %) was significantly higher than that in omnivores (13·28 %). In vegetarians, plasma PL 22 : 6*n*-3 ($r -0·257$, $P=0·046$) was significantly negatively associated with plasma Hcy. In omnivores, plasma PL 18 : 1*n*-7 ($r 0·237$, $P=0·030$) was significantly positively associated with plasma Hcy. Plasma PL 22 : 6*n*-3 ($r -0·217$, $P=0·048$) was negatively associated with plasma Hcy in omnivores. Plasma PL SFA were positively associated with the prevalence of HHcy. It would seem appropriate for vegetarians to increase their dietary *n*-3 PUFA and decrease dietary SFA, and thus reduce the risk of HHcy.

Key words: Homocysteine: PUFA: Vegetarians: Hyperhomocysteinaemia: Omnivores

It is widely recognised that overall morbidity and mortality are lower in vegetarians compared with omnivores[1]. The dietary patterns of vegetarians as well as their healthful lifestyle practices are thought to at least partly explain these differences. One notable difference relates to the type and amount of fat in the diet. Vegetarian diets are slightly lower in total fat than omnivorous diets[2]. However, vegetarians eat about one-third less saturated fat (vegans about one-half) and about one-half as much cholesterol (vegans consume none) as omnivores[3]. In addition, vegetarian diets are rich in fibre, Mg, Fe^{3+}, folic acid, vitamins C and E, *n*-6 PUFA, phytochemicals and antioxidants[4].

However, vegetarian diets are low in Na, Zn, Fe^{2+}, vitamins A, B_{12} and D, and especially *n*-3 PUFA[4]. A low intake of total fat, SFA and Na and a high intake of fibre, phytochemicals and antioxidants in vegetarians are associated with low blood pressure and BMI, which are known to reduce the risk of CVD[4]. Vitamin B_{12}, which is mainly from seafood, animal meats, eggs and liver and not found in plant foods, plays an important role in homocysteine (Hcy) metabolism. Vitamin B_{12} deficiency directly leads to high plasma Hcy, which is an independent risk factor for CVD[5].

With respect to essential fatty acid intake and balance, vegetarian diets appear to offer no advantages over omnivorous dietary patterns. Some have suggested that vegetarians could be at a significant disadvantage, as consumption of α-linolenic acid (18 : 3*n*-3) is low relative to linoleic acid (18 : 2*n*-6), resulting in the limited conversion of α-linolenic acid to EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3)[6]. In addition, vegetarians consume very little EPA and DHA[2].

Our previous studies reported that platelet/plasma phospholipid (PL) *n*-3 PUFA were negatively associated with plasma Hcy in middle-aged and geriatric hyperlipaemia patients[7] and in healthy male subjects[8]. Our animal study

Abbreviations: Hcy, homocysteine; HHcy, hyperhomocysteinaemia; MAT, methionine adenosyl transferase; MAT1A, methionine adenosyltransferase I, α; MTHFR, methylenetetrahydrofolate reductase; PL, phospholipid.

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suggested that DHA decreases plasma Hcy concentration by regulating critical gene expression and enzyme activity[9]. Our population studies found that dietary fatty acids interact with methylenetetrahydrofolate reductase (MTHFR) and methionine adenosyltransferase I, α (MAT1A) genetic variants in determining plasma Hcy concentration[10,11].

To date, limited research comparing plasma PL fatty acid composition of omnivores and vegetarians suggests that vegetarians have lower serum and/or platelet levels of DHA[12]. We previously also reported that *n*-3 PUFA were lower in vegetarians than in omnivores[13]. However, no study has reported the associations of plasma PL fatty acids with plasma Hcy concentration in Chinese vegetarians.

The purpose of the present study is to investigate the status of plasma PL fatty acids and to examine the potential relationship between PL fatty acids and plasma Hcy levels in Chinese vegetarians and omnivores.

Materials and methods

Subjects

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Ethics Committee, College of Biosystem Engineering and Food Science, Zhejiang University, China. Written informed consent was obtained from all subjects/patients.

A total of 103 male vegetarians (aged 40 (SD 10) years) were recruited in Linyin Temple, Hangzhou. A vegetarian was defined as someone who ate no red meat, consumed fish or chicken less than once per week, and had been following this diet for at least 6 months before the study. A total of 128 male omnivores (aged 44 (SD 8) years) were recruited through a health check programme during the period of October 2010 to March 2011 in the Zhejiang Hospital. An omnivore was defined as someone who ate meat at least five times per week.

Blood collection

Subjects visited the Zhejiang Hospital in the morning following an overnight fast. Subjects were allowed to sit relaxed for 10 min, and the subject's weight, height, waist:hip ratio and blood pressure were then measured. Subsequently, venous blood was taken in plain and EDTA vacuum tubes with a twenty-one-gauge needle (Longhe). Within 1 h after blood collection, plasma samples were prepared quickly by using a refrigerated centrifuge, and aliquoted into separate tubes and stored at −20°C until analysis.

Laboratory measurements

Plasma total Hcy was determined by polarised fluorescence immunoassay in an AxSYM system (Abbott Laboratories)[14]. Hyperhomocysteinaemia (HHcy) was defined as a Hcy level above the 95th percentile (14 μmol/l)[15]. Plasma folate and vitamin B_{12} were measured using immulite chemiluminescent kits according to the manufacturer's instructions (Diagnostic Products Corporation/Siemens). Plasma lipids were determined using an autoanalyser (Olympus AU2700; Olympus France), via commercially available kits (Olympus). Fasting glucose was measured by standard methods as described previously[16]. Total lipid content of plasma was extracted with solvents, the PL fraction was separated by TLC, and fatty acid methyl esters were prepared and separated by GLC as described previously[17].

Statistical analysis

Data analyses were performed using SAS for Windows, version 9.1 (SAS Institute). All continuous variables were

Table 1. Demographic and biochemical measurements in vegetarians and omnivores (Mean values and standard deviations)

	Vegetarians (*n* 103)		Omnivores (*n* 128)		
	Mean	SD	Mean	SD	*P**
Age (years)	40	10	44	8	0·019
BMI (kg/m^2)	23·7	3·1	24·3	2·7	0·149
Weight (kg)	66·4	10·3	69·5	11·3	0·044
Years of vegetarianism	12	11	0		
SBP (mmHg)	110·4	11·5	126·1	17·4	0·001
DBP (mmHg)	81·3	7·5	76·9	13·1	0·005
Total TAG (mmol/l)	1·53	0·84	1·98	1·10	0·002
TC (mmol/l)	4·27	0·94	5·55	0·98	<0·001
HDL-C (mmol/l)	1·18	0·26	1·29	0·30	0·005
LDL-C (mmol/l)	2·12	0·57	2·71	0·69	<0·001
ALB (g/l)	47·25	3·07	47·29	2·69	0·931
Glucose (mmol/l)	4·92	1·21	5·36	0·89	0·009
Uric acid (μmol/l)	307·15	72·08	308·65	80·54	0·721
Hcy (μmol/l)	13·99	4·63	10·85	5·06	0·002
Vitamin B_{12} (pg/ml)	168·38	76·61	376·54	162·66	<0·001
Folate (ng/ml)	6·98	3·11	5·23	2·28	<0·001

SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; ALB, albumin; Hcy, homocysteine.
* $P<0·05$ indicates significant difference between the groups (*t* test).

examined for a normal distribution. We categorised plasma PL fatty acids into quantiles (median) using the SAS program (PROC RANK). Differences between the two groups for each outcome were analysed using ANOVA. The associations between plasma PL fatty acid composition and Hcy were determined by partial correlation, controlling for potential confounding factors (age, sex, vitamin B_{12} and folate). Population medians for plasma PL fatty acids such as total SFA, MUFA and PUFA, and *n*-3:*n*-6 were used as cut-offs to dichotomise these variables. All data are expressed as means and standard deviations. Differences between the groups were considered to be statistically significant at $P<0{\cdot}05$.

Results

Compared with omnivores, body weight was significantly lower in vegetarians ($P=0{\cdot}044$). Systolic blood pressure was significantly lower in vegetarians ($P=0{\cdot}001$), while diastolic blood pressure was significantly higher in vegetarians ($P=0{\cdot}005$). In addition, plasma total cholesterol, total TAG, HDL-cholesterol, LDL-cholesterol, glucose and vitamin B_{12} were also significantly lower in vegetarians. However, plasma Hcy and folate were significantly higher in vegetarians than those in omnivores (Table 1).

Plasma PL fatty acid composition was significantly different between vegetarians and omnivores. Compared with omnivores, plasma PL *n*-3 PUFA ($P<0{\cdot}001$), 22 : 6*n*-3 ($P<0{\cdot}001$), 22 : 5*n*-6 ($P=0{\cdot}021$), 22 : 2*n*-6 ($P<0{\cdot}001$) and SFA ($P=0{\cdot}017$) were significantly lower in vegetarians, while plasma PL *n*-6 PUFA ($P=0{\cdot}007$) and total PUFA ($P<0{\cdot}001$) were significantly higher in vegetarians. Plasma PL MUFA ($P=0{\cdot}094$) and *n*-3:*n*-6 PUFA ($P=0{\cdot}064$) were not significantly different between the two groups (Table 2).

In vegetarians, plasma PL 22 : 6*n*-3 ($r-0{\cdot}257$, $P=0{\cdot}046$) was significantly negatively associated with plasma Hcy. In addition, plasma PL *n*-6 PUFA (r 0·249, $P=0{\cdot}045$) were significantly positively associated with plasma Hcy. In omnivores, plasma PL 18 : 1*n*-7 (r 0·237, $P=0{\cdot}030$) was significantly positively associated with plasma Hcy in omnivores. Plasma PL 22 : 1*n*-9 (r −0·228, $P=0{\cdot}037$) and 22 : 6*n*-3 (r −0·217, $P=0{\cdot}048$) were negatively associated with plasma Hcy in omnivores (Table 3). There were no further partial correlations between any fatty acid and Hcy in either vegetarians or omnivores.

Multiple regression analysis adjusted for age, sex, BMI, vitamin B_{12}, folate, glucose, lipids, insulin, vegetarian diet and fatty acids was created to identify potential independent correlates of plasma Hcy. The only significant correlates of Hcy were age ($P=0{\cdot}001$), vitamin B_{12} ($P=0{\cdot}009$), folate ($P=0{\cdot}015$), height ($P=0{\cdot}010$), glucose ($P=0{\cdot}007$), SFA ($P=0{\cdot}040$) and 22 : 4*n*-6 ($P=0{\cdot}024$). These variables explained 49% of the variance in plasma Hcy (Table 4).

We further examined the prevalence of HHcy in vegetarians and omnivores. We observed that the prevalence of HHcy in vegetarians (26·47%) was significantly higher than that in omnivores (13·28%) ($P<0{\cdot}01$; Fig. 1).

We further examined the associations of PL fatty acids with the prevalence of HHcy. The results showed that PL SFA were significantly positively associated with the prevalence of HHcy in vegetarians ($P=0{\cdot}024$) and omnivores ($P=0{\cdot}018$) (Fig. 2). In addition, PL 22 : 4*n*-6 and 18 : 2*n*-6 were also significantly positively associated with the prevalence of HHcy in vegetarians (Figs. 3 and 4, respectively). We also found that PL 22 : 6*n*-3 was significantly negatively associated with the prevalence of HHcy in vegetarians ($P=0{\cdot}017$; Fig. 5).

Discussion

In the present study, we found that the prevalence of HHcy in vegetarians (26·47%) was significantly higher than that in omnivores (13·28%). Plasma PL *n*-3 PUFA, 22 : 6*n*-3 and SFA were significantly lower in vegetarians, while plasma PL *n*-6 PUFA and total PUFA were significantly higher in vegetarians than in omnivores. Plasma PL 22 : 6*n*-3 was significantly negatively associated with plasma Hcy. In addition, plasma PL SFA were significantly positively associated with the prevalence of HHcy in vegetarians and omnivores. To our knowledge, this is the first report; however, the mechanism at this stage is not known.

The risk for many chronic diseases, including CVD, is influenced by dietary fatty acid intake[18]. A higher degree of

Table 2. Plasma phospholipid (PL) fatty acid composition in vegetarians and omnivores

(Mean values and standard deviations)

	Vegetarians (*n* 103)		Omnivores (*n* 128)		
PL fatty acids (% of total fatty acids)	Mean	SD	Mean	SD	*P**†
14 : 0	0·37	0·47	0·25	0·15	<0·001
14 : 1*n*-5	0·44	0·69	0·22	0·15	0·002
15 : 0	0·45	0·43	0·28	0·21	<0·001
16 : 0	21·15	4·62	23·48	4·19	<0·001
16 : 1*n*-7	0·91	2·73	0·51	2·30	0·017
18 : 0	11·11	2·89	12·97	2·40	<0·001
18 : 1*n*-9	1·42	1·08	1·41	1·37	0·715
18 : 1*n*-7	7·33	2·36	8·16	2·00	0·015
18 : 2*n*-6	24·79	5·26	23·07	3·94	0·132
18 : 3*n*-6	0·76	2·84	0·37	0·38	0·012
18 : 3*n*-3	0·42	0·28	0·65	3·21	0·017
20 : 0	0·53	0·32	0·71	2·91	<0·001
20 : 1*n*-9	0·54	0·38	0·25	0·14	<0·001
20 : 2*n*-6	1·41	0·93	0·66	0·51	<0·001
20 : 3*n*-6	5·39	2·55	2·45	0·96	<0·001
20 : 4*n*-6	16·03	9·88	11·63	2·78	<0·001
20 : 5*n*-3	1·11	1·77	2·98	2·50	<0·001
22 : 1*n*-9	0·72	0·41	1·01	0·62	<0·001
22 : 2*n*-6	0·56	0·43	0·78	0·39	<0·001
22 : 4*n*-6	0·64	0·45	0·57	0·35	0·221
22 : 5*n*-6	1·08	0·43	1·26	0·35	0·021
22 : 5*n*-3	0·72	0·45	0·56	0·42	0·004
22 : 6*n*-3	2·10	1·04	5·61	1·36	<0·001
SFA	33·61	7·06	37·69	4·95	0·017
MUFA	11·35	4·93	11·57	4·60	0·094
PUFA	54·99	7·03	50·58	3·95	<0·001
n-3 PUFA	5·02	5·03	10·22	2·73	<0·001
n-6 PUFA	49·97	9·05	40·36	3·67	0·007
n-3:*n*-6 PUFA	0·16	0·13	0·26	0·09	0·064

* $P<0{\cdot}05$ is considered to indicate a significant difference.

† The difference between vegetarians and omnivores is determined by using the general linear model controlled for potential confounding factors (age and BMI).

Table 3. Partial correlations between plasma phospholipid (PL) fatty acid compositions and plasma homocysteine*

	Vegetarians		Omnivores	
PL fatty acids	Coefficient	*P*	Coefficient	*P*
14 : 0	0·055	0·666	0·023	0·835
14 : 1*n*-5	0·026	0·835	−0·008	0·942
15 : 0	−0·073	0·563	−0·117	0·288
16 : 0	0·220	0·146	0·012	0·915
16 : 1*n*-7	−0·028	0·822	0·030	0·784
18 : 0	0·108	0·392	0·078	0·479
18 : 1*n*-9	0·016	0·899	0·031	0·778
18 : 1*n*-7	−0·006	0·965	0·237	0·030
18 : 2*n*-6	0·084	0·504	−0·055	0·622
18 : 3*n*-6	−0·072	0·569	−0·023	0·833
18 : 3*n*-3	−0·067	0·598	0·008	0·940
20 : 0	−0·075	0·551	0·017	0·875
20 : 1*n*-9	−0·133	0·291	0·081	0·465
20 : 2*n*-6	−0·001	0·993	−0·103	0·350
20 : 3*n*-6	0·091	0·473	−0·088	0·424
20 : 4*n*-6	−0·160	0·204	−0·023	0·834
20 : 5*n*-3	−0·085	0·501	0·004	0·972
22 : 1*n*-9	−0·123	0·328	−0·228	0·037
22 : 2*n*-6	0·113	0·370	−0·011	0·920
22 : 4*n*-6	0·117	0·355	0·048	0·662
22 : 5*n*-6	−0·151	0·229	−0·024	0·830
22 : 5*n*-3	0·126	0·057	−0·043	0·698
22 : 6*n*-3	−0·257	0·046	−0·217	0·048
SFA	0·188	0·133	0·055	0·621
MUFA	−0·039	0·757	0·099	0·369
PUFA	−0·143	0·254	−0·171	0·121
n-3 PUFA	−0·216	0·093	−0·105	0·342
n-6 PUFA	0·249	0·045	−0·104	0·344
n-3:*n*-6	−0·213	0·098	−0·024	0·832

* The associations of plasma PL fatty acid compositions with plasma homocysteine concentration were tested by using Pearson's partial correlation after controlling for confounding factors (age, BMI, vitamin B_{12} and folate).

incorporation of *n*-3 PUFA into myocardial membranes reduces deaths following myocardial ischaemia[19]. The *n*-3 PUFA content in erythrocyte membranes reflects that in cardiac membranes[20]. A low erythrocyte 20 : 5*n*-3 and 22 : 6*n*-3 percentage content has been identified as a risk indicator for death from CVD[21]. Because fatty acid profiles of platelet and plasma/serum PL reflect an individual's type of dietary fat intake[7], in addition, the database on the individual fatty acid content of all foods is not available for Chinese populations, no method has been developed to accurately estimate the dietary intake of individual fatty acids since it is rare for people to prepare all meals at home. The amount of fat intake is highly variable depending on the ingredients used to prepare dishes in restaurants or canteens. Thus, compositions of platelet PL FA were used as a surrogate marker of dietary intake of FA[22]. In the present study, we observed a higher level of plasma PL 20 : 4*n*-6 but lower levels of *n*-3 PUFA, 20 : 5*n*-3 and 22 : 6*n*-3, and the *n*-3:*n*-6 ratio in vegetarians than those in omnivores, which may tend to promote thrombotic risk. However, lower concentrations of plasma PL SFA in vegetarians may provide beneficial effects on CVD risk[13].

Table 4. Independent predictors of plasma homocysteine after adjustment for other clinical characteristics and risk factors

Predictors (R^2 0·49)	β-Coefficient	*P*
Age	0·193	0·001
Vitamin B_{12}	−0·887	0·009
Folate	−0·058	0·015
Height	0·449	0·010
Glucose	0·513	0·007
SFA	0·374	0·040
22 : 4*n*-6	0·390	0·024

Previous studies and the present study demonstrated that *n*-3 PUFA and 22 : 6*n*-3 in plasma or erythrocytes were significantly lower in vegetarians. Sanders[12] reported that the proportions of 22 : 6*n*-3 in plasma, erythrocytes, breast milk and tissues are substantially lower in vegans and vegetarians compared with omnivores. Kornsteiner *et al.*[23] also demonstrated that vegetarians and vegans, who do not eat meat or fish, tend to have very low or negligible intakes of 20 : 5*n*-3 as well as 22 : 6*n*-3. Fokkema *et al.*[24] investigated the PUFA status of Dutch vegans and omnivores in erythrocyte membranes. They did not find significant differences in total *n*-3 PUFA in the erythrocytes; however, 20 : 5*n*-3 and 22 : 6*n*-3 were significantly reduced in vegans compared with omnivores. On the other hand, the results of Dutch vegans showed a higher 22 : 5*n*-3 content compared with Dutch omnivores[24], which is consistent with the present results. Rosell *et al.*[25] investigated *n*-3 PUFA in the plasma of British meat-eating, vegetarian and vegan men, and found that 20 : 5*n*-3, docosapentaenoic acid (22 : 5*n*-3) and 22 : 6*n*-3 were markedly decreased. Li *et al.*[13] demonstrated a decreased content of 20 : 5*n*-3, 22 : 6*n*-3 and total *n*-3 PUFA in Australian vegetarian females. Sanders *et al.*[26] showed that erythrocytes from vegans contained lower proportions of 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3 and higher proportions of 18 : 2*n*-6, 20 : 2*n*-6 and 22 : 4*n*-6. Kornsteiner *et al.*[23] documented that the imbalance in the *n*-6:*n*-3 ratio and the limited dietary sources of 20 : 5*n*-3 and 22 : 6*n*-3 in vegans and vegetarians led to reductions in 20 : 5*n*-3, 22 : 5*n*-3, 22 : 6*n*-3 and *n*-3 PUFA in phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine compared with omnivores and semi-omnivores. Animal studies and human intervention studies have demonstrated that a high *n*-3 PUFA intake increases the level of *n*-3 PUFA in tissues[23,27]. Therefore, the low content of plasma PL *n*-3 PUFA reflects the limited *n*-3 PUFA dietary intake in vegetarians[4].

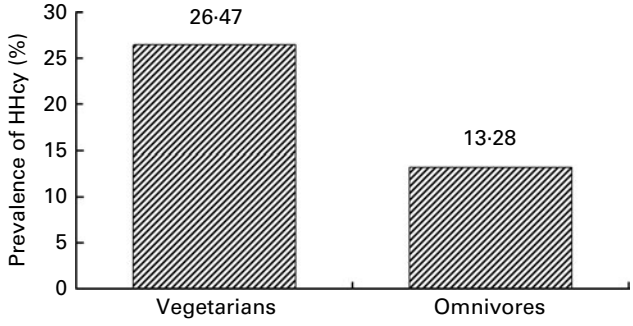

Fig. 1. Prevalence of hyperhomocysteinaemia (HHcy) in vegetarians (*n* 103) and omnivores (*n* 128). The prevalence of HHcy in vegetarians was significantly higher than that in omnivores ($P<0·01$; χ^2 test).

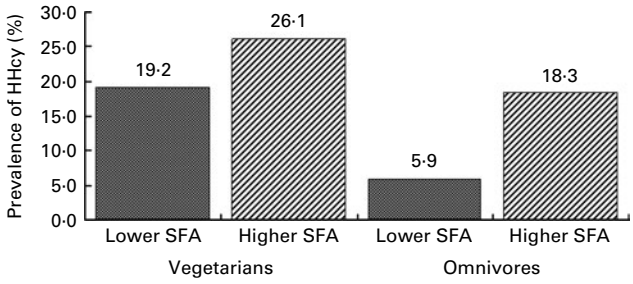

Fig. 2. Association of plasma phospholipid SFA with the prevalence of hyperhomocysteinaemia (HHcy) in vegetarians (lower SFA, *n* 34; higher SFA, *n* 36) and omnivores (lower SFA, *n* 48; higher SFA, *n* 46). Phospholipid SFA were significantly positively associated with the prevalence of HHcy in vegetarians ($P=0{\cdot}024$; χ^2 test) and omnivores ($P=0{\cdot}018$; χ^2 test).

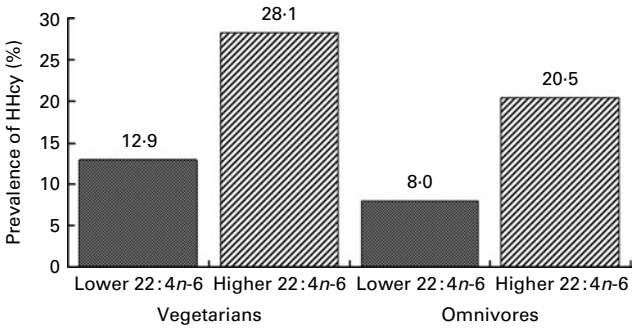

Fig. 3. Association of plasma phospholipid 22 : 4*n*-6 with the prevalence of hyperhomocysteinaemia (HHcy) in vegetarians (lower 22 : 4*n*-6, *n* 38; higher 22 : 4*n*-6, *n* 39) and omnivores (lower 22 : 4*n*-6, *n* 50; higher 22 : 4*n*-6, *n* 54). Phospholipid 22 : 4*n*-6 was significantly positively associated with the prevalence of HHcy in vegetarians ($P=0{\cdot}004$) and omnivores ($P=0{\cdot}017$).

Ovo-lacto-vegetarians consume minimal amounts of 20 : 5*n*-3 and varying amounts of 22 : 6*n*-3 from eggs, milk and dairy products. Vegans consume negligible amounts of long-chain *n*-3 PUFA (20 : 5*n*-3 and 22 : 6*n*-3) and rely entirely on the *in vivo* biosynthesis of *n*-3 PUFA from the precursor 18 : 3*n*-3, but the conversion via desaturation and elongation, especially to 22 : 6*n*-3, is not efficient[28]. Previous studies with stable isotopically labelled 18 : 3*n*-3 have shown the conversion of 18 : 3*n*-3 to 20 : 5*n*-3 varying from 6–21 % to much lower values (0·1–0·2 %)[29]. In one study, the conversion of 18 : 3*n*-3 to 22 : 6*n*-3 has been reported to range from 4–9 % to 0·04 %[30], or with undetectable 22 : 6*n*-3 synthesis. The lack of 20 : 5*n*-3 and 22 : 6*n*-3 in vegetarian diets is reflected in reduced amounts of these fatty acids in platelets, erythrocytes and plasma[23]. Thus, the uptake of preformed 22 : 6*n*-3 from the diet may be critical for maintaining adequate membrane 22 : 6*n*-3 concentrations in vegetarians[23].

In recent years, many epidemiological studies have investigated the associations of *n*-3 PUFA with CVD risk factors such as plasma Hcy. In the present study, we demonstrated that plasma Hcy concentration in vegetarians was significantly higher than that in omnivores. The prevalence of HHcy in vegetarians (26·47 %) was significantly higher than that in omnivores (13·28 %). This observation can be explained by the lower level of plasma vitamin B_{12} and *n*-3 PUFA in vegetarians. Previous studies suggested that *n*-3 PUFA play an important role in Hcy metabolism[9–11,31]. In the present study, plasma PL 22 : 6*n*-3 was significantly negatively associated with plasma Hcy. In our previous study, plasma Hcy concentration was negatively correlated with *n*-3 PUFA and the ratio of *n*-3:*n*-6 PUFA intake in Puerto Rican adults[11]. Li *et al.*[7] also found that increased total *n*-3 PUFA and *n*-3 : *n*-6 PUFA in platelet PL are associated with decreased plasma Hcy in middle-aged and geriatric hyperlipaemia patients in Hangzhou, China. In addition, plasma PL 22 : 6*n*-3, *n*-3 PUFA and the *n*-3:*n*-6 PUFA ratio were also negatively correlated with plasma Hcy in healthy Australian males[8]. Over the past two decades, several intervention studies of small sample size and short duration have documented the effects of *n*-3 PUFA on plasma Hcy concentration[32–34]. However, these results were not consistent: some studies have not shown a significant decrease in plasma Hcy[35,36], while other studies have documented a plasma Hcy-lowering effect after *n*-3 PUFA supplementation[32,33,37]. Therefore, we conducted a meta-analysis to increase the sample size and demonstrated that a high consumption of *n*-3 PUFA decreases plasma Hcy[38].

The potential mechanisms by which *n*-3 PUFA decrease plasma Hcy have been investigated in our animal and population studies[9–11]. In our animal study[9], we found that plasma Hcy was significantly decreased by tuna oil rich in 22 : 6*n*-3. Methionine adenosyl transferase (MAT) activity was significantly increased and MAT mRNA expression was significantly up-regulated by 22 : 6*n*-3; cystathionine-γ-lyase mRNA expression was significantly up-regulated by 22 : 6*n*-3. We suggested that 22 : 6*n*-3 decreases the concentration of Hcy despite increasing MAT activity and up-regulating MAT mRNA expression through compensatory cystathionine-γ-lyase mRNA expression, both of which are involved in Hcy metabolism[9]. However, HHcy 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. Our previous population studies[10,11] 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

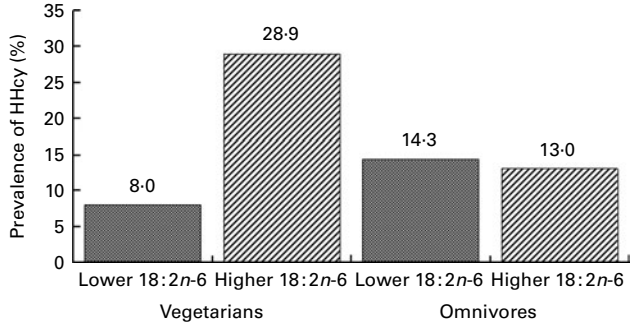

Fig. 4. Association of plasma phospholipid 18 : 2*n*-6 with the prevalence of hyperhomocysteinaemia (HHcy) in vegetarians (lower 18 : 2*n*-6, *n* 40; higher 18 : 2*n*-6, *n* 45) and omnivores (lower 18 : 2*n*-6, *n* 57; higher 18 : 2*n*-6, *n* 47). Phospholipid 18 : 2*n*-6 was significantly positively associated with the prevalence of HHcy in vegetarians ($P=0{\cdot}008$) and omnivores ($P=0{\cdot}164$).

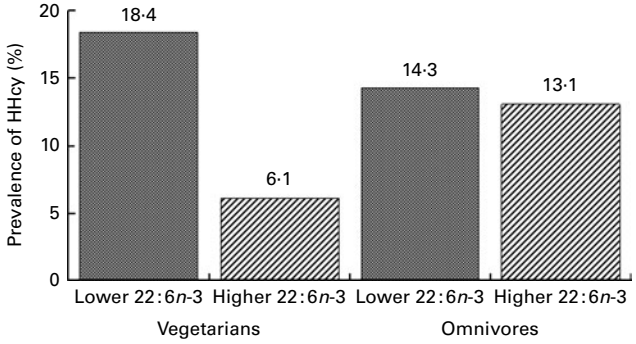

Fig. 5. Association of plasma phospholipid 22:6*n*-3 with the prevalence of hyperhomocysteinaemia (HHcy) in vegetarians (lower 22:6*n*-3, *n* 40; higher 22:6*n*-3, *n* 38) and omnivores (lower 22:6*n*-3, *n* 51; higher 22:6*n*-3, *n* 53). Phospholipid 22:6*n*-3 was significantly negatively associated with the prevalence of HHcy in vegetarians ($P=0{\cdot}017$) and omnivores ($P=0{\cdot}264$).

n-3:*n*-6 PUFA ratio of the diet in determining plasma Hcy concentration. Participants with combined genotypes of both 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). Therefore, it has been suggested that dietary PUFA intake modulates the effect of MTHFR variants on plasma Hcy[11]. Moreover, the genetic variant MAT1A 3U1510 displays 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. Also, two other MAT1A variants (d18777 and i15752) show significant interactions with different constituents of dietary fat in influencing Hcy concentration. Furthermore, haplotypes consisting of three variants display a strong interaction with the *n*-3:*n*-6 ratio influencing Hcy concentrations[10].

Based on the present data, vegetarians should probably increase their relatively low dietary vitamin B_{12} and *n*-3 PUFA and decrease dietary SFA, and thereby reduce the risk of HHcy.

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