ORIGINAL ARTICLE

Correlations between concentrations of plasma homocysteine and phospholipid fatty acids in healthy male Australian

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Abstract

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An increased plasma homocysteine level has been claimed as an independent risk factor, and increased levels of tissue membrane omega-3 polyunsaturated fatty acid (PUFA) has been suggested to have a protective effect on cardiovascular diseases. However, there is no data on the relationship between the concentrations of plasma homocysteine and phospholipid fatty acid. The aim of this study was to investigate the relationship of plasma homocysteine with phospholipid fatty acids in healthy male Australian. One hundred and thirty six healthy male subjects aged 20-55 years were recruited from Melbourne metropolitan area. Each volunteer completed a semi-quantitative food frequency questionnaire and gave a blood sample. Plasma homocysteine concentrations were determined using HPLC, and plasma phospholipid fatty acids were determined with standard methods. Plasma homocysteine concentration was significantly negatively correlated with plasma phospholipid concentration of PUFA 20:5n-3 (r = -0.226, P = 0.009), 22:5n-3 (r = -0.182, P = 0.036), 22:6n-3 (r = -0.286, P = 0.001), total n-3 (r = -0.270, P = 0.002) and ratio n-3/n-6 PUFA (r = -0.265, P = 0.002), and significantly positively correlated with 20:4n-6 (r = 0.180, P = 0.037). Present results showed that increased concentration of n-3 PUFA in tissues was negatively correlated with plasma homocysteine levels.

Key words : homosysteine, correlation, n-3 fatty acid, cardiovascular disease

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Increased plasma homocysteine levels have been suggested to be an independent risk factor for cardiovascular diseases (CVD) (Stampfer *et al*., 1992; Arnesen *et al*., 1995; Moleerergpoom *et al*., 2004). Homocysteine (Hcy) is an intermediate metabolite of methionine metabolism to cysteine. Normal metabolism of Hcy involves two pathways: remethylation and transsulfuration. Remethylation of Hcy to methionine requires vitamin B_{12} (methylcobalamin form) as a coenzyme for Hcy methyltransferase (methionine synthetase) and N^5-methyltetrahydrofolate as a methyl donor. The transsulfuration pathway of Hcy to cysteine requires vitamin B_6 as a coenzyme for both cystathionine β-synthase (converts Hcy to cystathionine) and cystathionine lyase (converts cystathionine to cysteine). A lack of vitamin B_{12} and/or folic acid or vitamin B_6 results in elevation of plasma Hcy (Finkelstein, 1990). We have previously reported that plasma Hcy concentration was significantly negatively correlated with serum vitamin B_{12} concentration, but no significant correlation with folate in healthy male Australian (Mann *et al*., 1999).

Increased dietary intake of n-3 polyunsaturated fatty acids (PUFA) will lead to an increased tissue levels of these fatty acids, and later has a beneficial effect on cardiovascular morbidity and mortality (Hu *et al*., 1999; Iso *et al*., 2001) via reduction in blood pressure (Morris *et al*., 1993) and serum/plasma triacylglycerol levels (Svaneborg *et al*., 1994; Zampelas *et al*., 1994). Furthermore, PUFA may have an anti-thrombotic effect (Schacky *et al*., 1985; Ferretti *et al*., 1998), anti-inflammatory effect (Ziboh *et al*., 2000), increase heart rate variability (Christensen *et al*., 2001), and secondary prevention of CVD (de Lorgeril *et al*., 1999, GISSI-Prevenzione Investigators 1999). As reviewed by Li (2003) and Li *et al*. (2002), n-3 PUFA can alter gene expression, such as down-regulating proteoglycan degrading enzyme (aggrecanases), inflammation-inducible cytokines (interleukin (IL)-1 α and tumor necrosis factor (TNF)-α), cyclooxygenase 2 (COX-2), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), S14 protein and stearoyl-CoA desaturase (SCD), up-regulating lipoprotein lipase (LPL) fatty acid-binding protein, acyl-CoA synthetase (ACS), carnitine palmitoyltransferase 1, acyl-CoA dehydrogenese, acyl-CoA oxidase, cytochrome P450 4A2 and peroxisome proliferator-activated receptor α.

Maternal plasma total Hcy concentrations were positively correlated with erythrocyte phospholipid docosahexaenoic acid of their offspring (Bohles *et al*., 1999). A recent 12-weeks double-blind intervention study found that plasma Hcy levels has not significantly changed in hyperlipidaemia subjects receiving 4g/day of either a n-3 PUFA which was composed of 85% eicosapentaenoic acid (EPA, 20:5n-3)/docosahexaenoic acid (DHA, 20:6n-3) (n = 28), or corn oil (n = 29). However, there is no data on the relationship between habitual n-3 PUFA intake and plasma Hcy levels. The aim of this study was to investigate the relationship of plasma Hcy with phospholipid fatty acid concentrations (as biomarker) in healthy male Australian.

Materials and Methods

Subjects

The study protocol was approved by the Human Ethics Committee, RMIT University, and all subjects were volunteers who gave their written consent prior to participation in the study. A total of 136 subjects were recruited from the Melbourne metropolitan area, they were all non-smoking healthy males in the age range of 22-55 years, without family history of cardiovascular diseases, and not taking any vitamin fortified supplements six months prior to the study.

Blood collection and dietary analysis

All subjects attended the RMIT University Medical Clinic in the morning following a 12-h overnight fast. Blood collection and dietary analysis were described previously (Li *et al*., 1999).

Laboratory measurements

Total plasma Hcy was determined as the ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate derivative (Araki and Sako, 1993), using a HPLC separation, and fluorometric detection

method as described by Dudman *et al.* (1996). Total plasma lipid was extracted with solvents. The plasma phospholipid (PL) fraction was then separated with thin-layer chromatography and the methyl esters of the fatty acids of the plasma PL fraction were prepared and separated with gas-liquid chromatography as described previously (Li *et al*., 1999).

Statistics

Data analyses were performed using a STATVIEW software program (Abacus Concepts Inc, Berkeley, CA, USA). Correlation between Hcy and plasma PL fatty acid concentration was performed by bivariate analysis. The level of significance is $P<0.05$.

Results

Figure 1 shows the distribution of plasma Hcy concentrations. There were 5.9% of the subjects that had a plasma Hcy concentration greater than 20 mmol/L, 16.2% between 15 to 20 mmol/L, 52.9% between 10 to 14.9 and 25% less than 10 mmol/L.

Plasma PL fatty acid concentrations of the subjects are shown in Table 1. The concentrations of total PUFA was 48.8±8.2 mg/100 mL plasma, total n-3 PUFA was 5.9±2.0 mg/100 mL plasma, total n-6 PUFA was 42.8±6.6 mg/100 mL, total MUFA was 14.3±2.9 mg/100 mL and total SFA was 46.1±8.3 mg/100 mL.

Table 2 shows the correlations between the concentrations of plasma Hcy concentration and PL fatty acids. Plasma Hcy concentration was negatively correlated with plasma phospholipid concentration of 20:5n-3 ($r = -0.226$, $P = 0.009$), 22:5n-3 ($r = -0.182$, $P = 0.036$), 22:6n-3 ($r = -0.286$, $P = 0.001$), total n-3 ($r = -0.270$, $P = 0.002$) and ratio n-3/n-6 PUFA ($r = -0.265$, $P = 0.002$), and positively correlated with 20:4n-6 ($r = 0.180$, $P = 0.037$).

Discussion

Results from epidemiological, case-control and cross-sectional studies have indicated that elevated concentrations of plasma Hcy is an independent risk factor for CVD. In a large prospective study from USA (Physician's Health Study) (Stampfer *et al*., 1992), 14,916 male physicians, aged 40 to 84 y, with no prior myocardial infarction (MI) or stroke, provided plasma samples at baseline and were followed up for 5 years. The results indicated that the 271 subjects who later had MI had significantly higher mean baseline levels of Hcy than matching paired controls who remained free of MI. Subjects whose Hcy levels were in the highest 5% had about three times the risk of MI compared with those having lower Hcy levels ($p=0.005$), even after adjustment for a variety of coronary risk factors. In a large-scale prospective nested case control study (Tromsø Study) (Arnesen *et al*., 1995), 21,826 subjects (10,963 males and

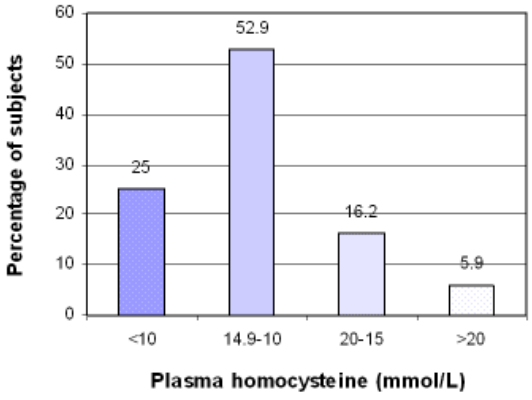

Figure 1. Distribution of plasma homocysteine in the study subjects.

Table 1. Plasma phospholipid fatty acid concentrations (mg/100 mL) in the study subjects.

Fatty Acid	Concentration (Mean ± SD)
Total SFA	46.1±8.3
Total MUFA	14.3±2.9
18:2n-6	26.5±4.7
20:3n-6	3.9±1.2
20:4n-6	11.6±3.0
22:4n-6	0.5±0.1
22:5n-6	0.3±0.1
Total n-6	42.8±6.6
18:3n-3	0.3±0.1
20:5n-3	1.0±0.6
22:5n-3	1.3±0.4
22:6n-3	3.3±1.3
Total n-3	5.9±2.0
Total PUFA	48.8±8.2
n3 : n-6	0.14±0.04

10,863 females), aged 12 to 61 y, who were free from MI at the screening were studied. Three years later, 123 subjects developed coronary heart disease (CHD). Four controls were selected for each case. They found levels of serum Hcy were higher in the cases than in the controls (p=0.002), but with no threshold level. In a cross-sectional study (Framingham Heart Study) of 1,041 elderly subjects (418 men and 623 women; aged 67-96 y) conducted by Selhub *et al*. (1995), the results showed a clearly graded increase in the prevalence of carotid-artery stenosis with increasing plasma levels of Hcy. The individuals in the group having the highest levels of Hcy had twice the risk of severe stenosis when compared with the group with lowest Hcy levels. Similar findings were reported by Malinow *et al*. (1993) for the risk of carotid-artery thickening, by Genest *et al*. (1990) for angiographically defined coronary-artery stenosis, by Bachmann *et al*. (1995) for occlusive arterial disease, by Arnesen *et al*. (1995) and Landgren *et al*. (1995) for MI, and Lindgren *et al*. (1995) for stroke. A study (Glueck *et al*., 1995) in 482 patients already at high risk of atherosclerosis from hyperlipidemia, assessed by logistic regression, indicated that high Hcy levels were an independent risk

Table 2. Correlates of plasma homocysteine in the study subjects.

Correlates	Standardised Coefficients (r)	P Value
20:4n-6	0.180	0.037
20:5n-3	-0.226	0.009
22:5n-3	-0.182	0.036
22:6n-3	-0.286	0.001
Total n-3	-0.270	0.002
n-3 : n-6	-0.265	0.002

factor for atherosclerotic vascular disease. The relative risk for atherosclerotic events was almost three times higher (p=0.0004) in patients with top (≥11.4 mmol/L) than with bottom (<6.9 mmol/L) quintile of Hcy. There were significant Hcy/HDL (p=0.012) and Hcy/triacyglycerol (p=0.02) interaction terms. The highest risk of an atherosclerotic event was seen when Hcy was high, while HDL and TAG were low. Reis *et al*. (1994) investigated the correlation between the Hcy and early cerebrovascular disease in 33 (19 male, 14 female) patients under 55 years old who suffered a stroke within 3 months to 1 year before the study. The patients were matched for age and sex. They found that hyperhomocysteinemia was a risk factor for thrombotic cerebrovascular disease before the age of 55 (Reis *et al*., 1994). In a recent nested case-control study (Perry *et al*., 1995), 5,661 British men aged 40-59 during 12 years follow-up; there were 141 incident cases of stroke among men with no history of stroke at screening. Serum Hcy concentrations were significantly higher in 107 cases than 118 controls (who did not develop a stroke or myocardial infarction during follow-up) (p=0.004). Hcy is an intermediate metabolite of methionine metabolism to cysteine. Normal metabolism from Hcy to cysteine involves two pathways: remethylation and transsulfuration, which require vitamin B_{12} and B_6 as coenzymes. A lack of vitamin B_{12} and/or folic acid or vitamin B_6 will results in elevation of plasma Hcy (Finkelstein, 1990). Previously, we have reported a significant negative correlation between plasma Hcy concentration and serum vitamin B_{12} concentration, however there was no significant correlation with

plasma folate in healthy male Australian (Mann *et al*., 1999). A similar finding has been reported by Mezzano *et al*. (1999).

In a double-blind intervention study, 57 healthy subjects were randomly divided to receive either a daily dose of 4g of 85% 20:5n-3/22:6n-3 (n = 28) or corn oil (n = 29) for 12 weeks. Plasma Hcy levels has not significantly changed from baseline in both groups after 12-week (Grundt *et al*., 1999). Bohles *et al*. (1999) determined the plasma Hcy and erythrocyte PL fatty acid in maternal (n=60, age ranged from 21 to 39 years) and their newborn children. Maternal plasma Hcy concentration was significantly positively correlated with those of their newborns ($r = 0.71$; $P<0.0001$). The maternal plasma Hcy levels was significantly negatively correlated with their offspring erythrocyte PL 22:6n-3 concentrations ($r = -0.51$; $P<0.0003$). However, there is no data on the influence of habitual n-3 PUFA intake on plasma Hcy levels in the literature.

Results from the present study showed that plasma Hcy concentration was significantly negatively correlated with the concentrations of plasma PL 20:5n-3, 22:5n-3, 22:6n-3, total n-3 PUFA and the ratio of n-3 to n-6 PUFA, and significantly positively correlated with 20:4n-6. We do not know if the metabolism of plasma Hcy was influenced by dietary n-3 PUFA intake at this stage.

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