Original Article

Green vegetable juice increases polyunsaturated fatty acid of erythrocyte membrane phospholipid in hypercholesterolaemic patients

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Treatment with green vegetable juice rich in dark-green leafy and cruciferous vegetables, such as cabbage, was tested in 31 hypercholesterolaemic patients for three weeks. Green vegetable juice supplementation significantly increased the content of polyunsaturated fatty acids (PUFA; P); 18:2n-6, 20:3n-6, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-3 and 22:6n-3 of erythrocyte membrane phospholipid and decreased saturated fatty acids (SFA; S): 14:0 and 18:0, and therefore increased the polyunsaturated fatty acids/saturated fatty acids (P/S) ratio markedly from 0.26 ± 0.01 to 0.49 ± 0.01 ($P < 0.001$). Green vegetable juice supplementation increased both n-6 and n-3 PUFA, but the increase of n-3 PUFA was greater than that of n-6 PUFA. Therefore, the n-6/n-3 ratio decreased significantly from 4.29 ± 0.42 to 3.00 ± 0.16 ($P < 0.05$). Plasma thiobarbituric acid reactive substance production decreased, following the green vegetable juice supplementation. These results suggest that green vegetable juice may protect the erythrocyte membrane from oxidative stress, and the decrease in n-6/n-3 and increase in P/S ratio in the erythrocyte membrane phospholipid may be accompanied by rheological and functional changes in erythrocytes in hypercholesterolaemic patients.

Key words: hypercholesterolaemia, erythrocyte membrane, green vegetable juice, phospholipid, polyunsaturated fatty acid.

Introduction

Pro-oxidant states may play an important role in atherogenicity and may also promote certain types of cancer.[1] Much research has focused on anti-oxidant nutrients, which act as scavengers of oxygen-derived free radicals.[2] The beneficial effects of vegetables have been attributed, in part, to the anti-oxidants they provide. Among the anti-oxidants contained in vegetables and fruits, vitamins C and E, carotenes and other phytochemicals have been discussed for their possible role in the prevention of cardiovascular disease and cancer.[3–6] In univariate analysis of various cardiovascular risk parameters and age-specific ischemic heart disease mortality in 12 populations with normal cholesterol, highly significant correlation coefficients were found for plasma vitamin E and vitamin C levels.[7] Increased carotenoid-rich fruit and vegetable intake seemed to have an inhibitory effect on susceptibility of low-density lipoprotein (LDL) to oxidation.[8]

To investigate the health benefits of green vegetable intake, the present study examined the effects of green vegetable juice on fatty acid composition in the erythrocyte membrane in hypercholesterolaemic patients.

Methods

Subjects

Twenty males (39.7 ± 2.0 years) and 11 females (50.0 ± 3.4 years) with hypercholesterolaemia, who had no episodes of ischemic heart disease or any administered drug treatment, and had serum total cholesterol levels higher than 240 mg/dL (mean: 259.6 ± 5.3 mg/dL), were included in the present study. Mean body mass indices (BMI) in the male and female subjects were 22.8 ± 0.8 and 21.1 ± 0.7, respectively. Control blood samples were obtained from healthy adult volunteers having serum cholesterol levels lower than 240 mg/dL (8 males and 8 females, 38.9 ± 2.5 years, BMI; 21.6 ± 0.6). This study complied with the code of ethics of the World Medical Association (Declaration of Helsinki), and informed consent was obtained from all of the subjects.

Protocol

Green vegetable juice (Oishii Aojiru, Sunstar, Takatsuki, Japan) used in the present study was made from broccoli, cabbage, Japanese radish leaves, celery, spinach, lettuce and parsley. Apple and lemon juices were added to improve the taste. The green vegetable juice was packaged in 160 g cans. The subjects were given two cans per day to be drunk at breakfast and dinner for three weeks. The green vegetable

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juice was well accepted and all subjects consumed two cans per day. Nutrient contents of the green vegetable juice analysed are shown in Table 1. During the study, subjects did not receive any dietary counselling and were not forced to undergo exercise.

Blood samples were obtained in disodium EDTA-containing tubes prior to green vegetable juice administration (week 0), immediately following the administration (week 3) and 12 weeks following the start of the examination.

Dietary energy, protein, fat, vitamins, minerals and fatty acid consumption were calculated according to the quantitative food frequency questionnaire method[9] prior to and immediately following the supplementation of green vegetable juice.

Fatty acid analysis

After erythrocytes were separated by centrifugation at 1000× g for 15 min at 4°C, the erythrocyte ghosts were obtained according to the method of Hanahan and Ekholm.[10] Total lipid was extracted from the erythrocyte ghosts by the method of Bligh and Dyer[11] with some modifications as described in our previous report.[12] Phospholipid was separated by one-dimensional thin-layer chromatography (TLC) using a solvent system of petroleum ether/ethyl ether/acetic acid (75 : 25 : 1).[13] The spots corresponding to phospholipids were scraped from the TLC plates and transmethylated using acetyl chloride/methanol (5 : 50). A known amount of heptadecanoic acid was used as an internal standard. Following transmethylation, fatty acid methyl esters were extracted with petroleum ether and quantified, using a model GC14 A gas chromatograph (Shimadzu, Kyoto, Japan). The fatty acid methyl esters were identified according to their retention times by comparison with known standards.

α-Tocopherol analysis

α-Tocopherol concentrations were determined by high performance liquid chromatography (HPLC) according to a modification of the method of Milne and Botnen.[14]. α-Tocopherol was extracted from plasma with ethanol and n-hexane and the extracts were evaporated under nitrogen. The residue was redissolved in ethanol, and the samples were injected into a 250 × 4 mm column of TSK-gel ODS-80Ts (Tosoh, Tokyo, Japan) at room temperature (20°C–24°C). A mixture of methanol and 1-butanol (80 : 20) including 0.1% 10 mM sodium acetate buffer (pH 3.6) was used as the mobile phase. α-Tocopherol was monitored at an excitation wavelength of 292 nm and an emission wavelength of 320 nm. The α-tocopherol peak was identified and quantified against authentic tocopherols used as external standards.

Measurement of thiobarbituric acid reactive substances (TBARS)

Plasma thiobarbituric acid reactive substances (TBARS), primarily malondialdehyde (MDA), were measured as an indicator of lipid peroxidation according to the method described by Yagi.[15]

Statistical analysis

Statistical analysis was carried out with an Apple Macintosh computer using the Prism Ver. 2 package (GraphPad Software, CA, USA). The data are given as the mean ± SEM. Where appropriate, the data were analysed by one-way, repeated-measures analysis of variance followed by the Tukey test. Differences between controls and hypercholesterolaemic patients were examined by *t*-test (two-tailed). Significance was set at $P < 0.05$.

Results

Dietary consumption of foods, nutrients and fatty acids

Dietary food intake is shown in Table 2. Vegetable intakes, both green and others, were very low at week 0. No significant differences were recognised in food intake between weeks 0 and 3.

Mean dietary consumption of energy, nutrients and fatty acids from diets and green vegetable juice is summarised in Table 3. There were no significant differences in energy and major nutrient intake between weeks 0 and 3. However, vitamin C and vitamin E/polyunsaturated fatty acid (PUFA) (mg/g) intake increased significantly at week 3 compared with that at week 0. These results may be attributed mostly to the green vegetable juice intake, because two cans of juice provided 146 mg vitamin C and 0.96 mg vitamin E. A small, but not significant, increase in carotene intake at week 3 may have resulted from the green vegetable juice intake and a slight increase in dietary vegetables.

Fatty acid composition in erythrocyte membrane

Fatty acid composition in erythrocyte membrane phospholipids is summarised in Table 4. Significantly lower levels in

Table 1. Energy and major nutrients provided by the green vegetable juice used in the study

	(/two cans/day)
Energy (kcal)	106
Protein (g)	2.0
Fat (g)	0.64
Carbohydrate (g)	26.0
Vitamin A (IU)	160
Carotene (μg)	266
Vitamin C (mg)	146
Vitamin E (mg)	0.96
Dietary fibre (g)	2.6

One can contains 160 g green vegetable juice.

Table 2. Dietary intake of foods prior to (week 0) and 3 weeks following the green vegetable juice supplementation

	week 0 (*n* = 31)	week 3 (*n* = 31)
	g/day	
Cereals and potatoes	454 ± 30	413 ± 32
Soy bean and products	61 ± 6	64 ± 8
Oil and fat	14 ± 1	13 ± 1
Vegetables (Green)	53 ± 7	56 ± 5
Vegetables (Others)	97 ± 8	101 ± 9
Fruit	62 ± 11	49 ± 8
Fish	39 ± 4	39 ± 4
Meat	56 ± 6	55 ± 5
Eggs	22 ± 2	22 ± 2
Milk and other dairy products	179 ± 40	186 ± 41

Mean ± SEM.

20:4n-6, 22:4n-6, 22:5n-3 and 22:6n-3 and higher levels in 14:0 and 18:0 were recognised at week 0 in hypercholesterolaemic patients compared with those in controls. Green vegetable juice supplementation for 3 weeks in the hypercholesterolaemic patients significantly increased the content of polyunsaturated fatty acids: 18:2n-6, 20:3n-6, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-3 and 22:6n-3 of erythrocyte membrane phospholipid, and decreased saturated fatty acids: 14:0 and 18:0. After cessation of the green vegetable juice supplementation (week 12), fatty acid content mostly returned to pretreatment levels. Therefore, the molar ratio of polyunsaturated fatty acids/saturated fatty acids (P/S) was elevated markedly from 0.26 ± 0.01 at week 0 to 0.49 ± 0.01 at week 3, which was as high as in the control group, and lowered to 0.27 ± 0.01 at week 12 (Fig. 1). Both n-6 and n-3 PUFA were increased by green vegetable juice supplementation, but the increase in n-3 PUFA was greater than that of n-6 PUFA. Therefore, the n-6/n-3 PUFA ratio (n-6/n-3) decreased significantly from 4.29 ± 0.42 (week 0) to 3.00 ± 0.16 ($P < 0.05$). This compared to the same level as the control group, at week 3, as shown in Fig. 2. By week 12, the n-6/n-3 returned to the ratio (5.25 ± 0.72) observed at week 0. There were no significant changes in 16:1 throughout the test period. The content of 22:0, 24:0 and 24:1 increased at week 3, and 22:0 and 24:1 decreased at week 12.

Table 3. Dietary consumption of energy, nutrients and fatty acids reported prior to (week 0) and 3 weeks following the green vegetable juice supplementation

	Week 0 (*n* = 31)	Week 3 (*n* = 31)
Energy (kcal/kg IBW)	34.4 ± 2.0	32.8 ± 1.5
Protein (g/kg IBW)	1.19 ± 0.07	1.14 ± 0.06
Fat (g/kg IBW)	0.83 ± 0.07	0.79 ± 0.04
Carbohydrate (g/kg IBW)	5.21 ± 0.43	4.91 ± 0.25
Vitamin A (IU/day)	1593 ± 144	1862 ± 122
Carotene (µg/day)	1899 ± 221	2364 ± 174
Vitamin C (mg/day)	53 ± 5	205 ± 4†
Vitamin E (mg/day)	6.4 ± 0.4	6.8 ± 0.30
Vitamin E/PUFA (mg/g)	0.51 ± 0.02	0.60 ± 0.01*
Fatty acids (g/day)		
Saturated fatty acids	14.1 ± 1.0	13.5 ± 0.9
Monounsaturated fatty acids	17.0 ± 1.1	15.9 ± 0.9
Polyunsaturated fatty acids	12.7 ± 0.8	11.9 ± 0.5
18:2n-6	10.0 ± 0.6	9.14 ± 0.42
18:3n-3	1.47 ± 0.08	1.45 ± 0.07
20:5n-3	0.27 ± 0.03	0.26 ± 0.03
22:6n-3	0.50 ± 0.05	0.50 ± 0.04
Total n-6 fatty acids	10.2 ± 0.6	9.29 ± 0.43
Total n-3 fatty acids	2.41 ± 0.14	2.38 ± 0.13
n-6/n-3 ratio	4.42 ± 0.19	4.11 ± 0.16
P/S ratio	0.97 ± 0.05	0.95 ± 0.04

Nutrients provided by green vegetable juice were included in the values at week 3; Mean ± SEM, †$P < 0.0001$, *$P < 0.001$ compared with week 0, IBW, ideal body weight; IU, international unit; PUFA, polyunsaturated fatty acid; P/S, polyunsaturated fatty acid/saturated fatty acid.

Plasma α-tocopherol concentration

Plasma α-tocopherol concentration was unchanged with green vegetable juice supplementation (9.23 ± 0.45 µg/mL at week 0 and 9.76 ± 0.40 µg/mL at week 3, respectively).

Plasma TBARS production

The TBARS production in the plasma at week 3 (1.04 ± 0.14 nmol/mL) was significantly lower than that at week 0 (0.74 ± 0.09 nmol/mL, $P < 0.05$).

Discussion

The data presented here demonstrate that green vegetable juice causes profound changes in the phospholipid fatty acid pattern of the erythrocyte membrane in hypercholesterolaemic patients. It is well known that the types of fatty acids consumed in the diet affect plasma fatty acids and

Table 4. Fatty acid composition of erythrocyte membrane phospholipid

	Controls (*n* = 16)	Hypercholesterolaemic patients (*n* = 31) Week 0	Week 3	Week 12	ANOVA
		mol%			*P*
14:0	2.86 ± 0.29	4.21 ± 0.42[a*]	0.92 ± 0.05[c§]	2.22 ± 0.18[b]	0.0001
16:0	28.52 ± 0.90	29.65 ± 0.76[b]	27.03 ± 0.54[b]	33.65 ± 0.66[a‡]	0.0001
16:1	0.87 ± 0.08	1.33 ± 0.51[a]	0.41 ± 0.03[a§]	0.58 ± 0.07[a*]	NS
18:0	17.86 ± 0.54	20.48 ± 0.51[a*]	16.42 ± 0.18[b*]	19.38 ± 0.29[a*]	0.0016
18:1	13.63 ± 0.56	14.84 ± 0.63[b]	15.24 ± 0.17[ab*]	15.94 ± 0.30[a†]	0.0361
18:2n-6	6.69 ± 0.49	6.01 ± 0.27[b]	7.88 ± 0.13[a*]	6.71 ± 0.20[b]	0.0001
20:0	0.79 ± 0.16	0.96 ± 0.10[ab]	0.56 ± 0.02[b]	1.42 ± 0.13[a*]	0.0001
20:1	0.24 ± 0.07	0.50 ± 0.11[a]	0.24 ± 0.03[ab]	0.12 ± 0.03[b]	0.0363
20:2n-6	0.20 ± 0.05	0.30 ± 0.08[ab]	0.37 ± 0.02[a†]	0.06 ± 0.02[b]	0.0133
20:3n-6	0.73 ± 0.10	0.54 ± 0.10[b]	0.99 ± 0.03[a]	0.52 ± 0.03[b]	0.0002
20:4n-6	8.79 ± 0.40	5.12 ± 0.26[b§]	8.53 ± 0.16[a]	4.50 ± 0.19[b§]	0.0001
22:0	1.43 ± 0.16	1.31 ± 0.11[b]	1.95 ± 0.04[a†]	1.33 ± 0.07[b]	0.0003
20:5n-3	1.21 ± 0.24	0.81 ± 0.09[b]	1.26 ± 0.07[a]	0.88 ± 0.12[b]	0.0393
22:4n-6	1.20 ± 0.39	0.21 ± 0.05[b*]	1.03 ± 0.04[a]	0.23 ± 0.04[b*]	0.0001
24:0	2.92 ± 0.17	2.91 ± 0.19[b]	4.57 ± 0.17[a§]	4.07 ± 0.15[a‡]	0.0001
24:1	3.54 ± 0.28	3.18 ± 0.16[b]	4.98 ± 0.16[a‡]	3.80 ± 0.16[b]	0.0001
22:5n-3	1.20 ± 0.15	0.62 ± 0.09[b†]	1.12 ± 0.06[a]	0.51 ± 0.06[b‡]	0.0001
22:6n-3	4.59 ± 0.34	2.06 ± 0.17[b§]	4.43 ± 0.20[a]	2.15 ± 0.16[b§]	0.0001

Values are mean ± SEM. Within each row in hypercholesterolaemic patients, means not sharing a common superscript letter are significantly different (paired one-way ANOVA following Tukey test: $P < 0.05$). § $P < 0.0001$, ‡$P < 0.001$, †$P < 0.01$, *$P < 0.05$; compared with controls (unpaired *t*-test).

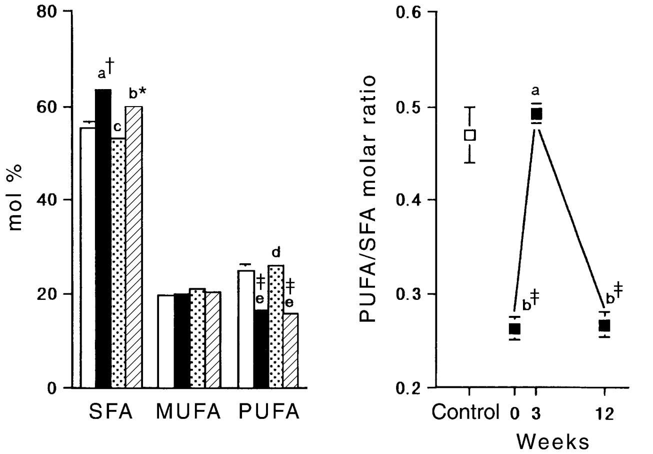

Figure 1. Changes in polyunsaturated (PUFA), monounsaturated (MUFA) and saturated (SFA) fatty acid composition, and polyunsaturated and saturated fatty acid molar ratio (P/S) in erythrocyte membrane phospholipids following green vegetable juice supplementation. Data are mean ± SEM. Means not sharing a common letter are significantly different (P < 0.05). PUFA/SFA molar ratio; paired one-way ANOVA: P < 0.0001. ‡P < 0.0001, †P < 0.01, *P < 0.05; compared with controls. HCh, hypercholesterolaemic patients; □, control; ■, HCh week 0; ⊡, HCh week 3; ▨, HCh week 12.

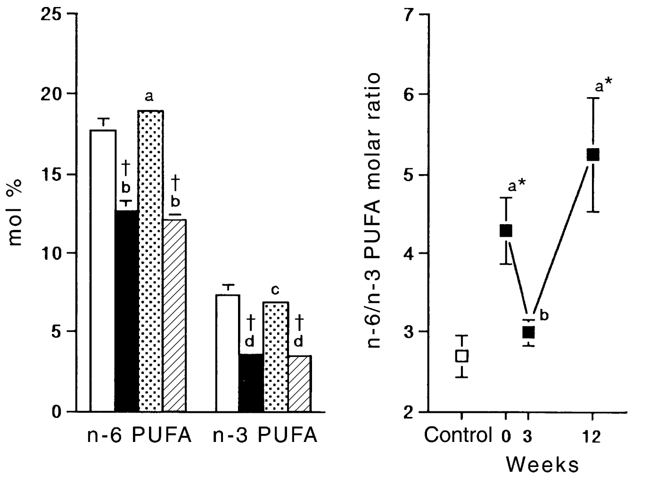

Figure 2. Changes in n-6 and n-3 polyunsaturated fatty acid composition and n-6/n-3 molar ratio in erythrocyte membrane phospholipids following green vegetable juice supplementation. Data are mean ± SEM. Means not sharing a common letter are significantly different (P < 0.05). n-6/n-3 PUFA molar ratio; paired one-way ANOVA: P < 0.05. † P < 0.0001, *P < 0.05; compared with controls. HCh; hypercholesterolaemic patients; □, control; ■, HCh week 0; ⊡, HCh week 3; ▨, HCh week 12.

erythrocyte membrane phospholipid composition and function.[16,17] Tynan *et al.* also reported that restriction of energy intake and increase in dietary P/S ratio induced an increase in P/S ratio of the erythrocyte membrane.[18] In the present study, although no restriction of energy intake, loss of body weight or change in dietary fatty acid consumption were observed during the green vegetable juice supplementation, the P/S ratio in the erythrocyte membranes increased, and n-6/n-3 ratio decreased. Therefore, our present data suggest that increased intake of vitamin C, vitamin E/PUFA or some other components, caused by green vegetable juice supplementation influenced not only the P/S ratio, but also the n-6/n-3 ratio of the erythrocyte membrane phospholipid in hypercholesterolaemic patients.

Vitamin C protects lipids in human plasma and low-density lipoprotein against oxidative damage more than other water-soluble plasma anti-oxidants. Vitamin C is also reactive enough to effectively intercept oxidants in the aqueous phase before they can cause detectable oxidative damage to lipids.[19] Harats *et al.* supplemented citrus fruit to young men and observed an increased plasma ascorbate level and reduced lipoprotein oxidation, and speculated an interaction between vitamins C and E *in vivo*.[20] In the present study, we did not measure plasma ascorbate, but supplementation of 146 mg vitamin C/day for 3 weeks with green vegetable juice presumably increased plasma ascorbate.

Vitamin E is the principal fat-soluble anti-oxidant in membranes, and it has been reported that vitamin E protects endothelial cells from oxidative injury.[21] It seems that increased vitamin E/PUFA (mg/g) intake following the green vegetable juice supplementation protected polyunsaturated fatty acid oxidation, accounting for the increase in P/S ratio in the erythrocyte membrane.

The lower production of TBARS following the green vegetable juice supplementation indicates the anti-oxidant activities of the juice. Other phytochemicals, β-carotene and oxygenated carotenoids, primarily lutein,[22] and plant phenol,[22] provided with the green vegetable juice can be factors in the changes in the erythrocyte membrane fatty acid composition.

In erythrocyte membrane phospholipid, 24:0 and 24:1 increased, following green vegetable juice supplementation, suggesting an increase of sphingomyelin content, as sphingomyelin contains high levels of 24:0 and 24:1 (about 50% of total fatty acids). No other erythrocyte membrane phospholipids contain significant levels of 24:0 and 24:1. Sphingomyelin inhibited human type II secretory phospholipase A_2 ($sPLA_2$), and the sphingomyelin-to-cholesterol ratio was suggested to account for the variable susceptibility of cell membranes to $sPLA_2$ *in vivo*.[23,24] The increase in erythrocyte membrane polyunsaturated fatty acids, such as 20:4n-6, 20:5n-3 and 22:6n-3, may have been induced by an inhibitory effect of sphingomyelin on $sPLA_2$, but this possibility should be assessed by further investigation.

We found significant decrease in 20:4n-6 and increase in 18:0 of erythrocyte membrane phospholipid in hypercholesterolaemic patients prior to supplementation, and recognised significant improvement in these fatty acid levels following green vegetable juice supplementation. Clifton and Nestel reported an inverse relationship between fasting plasma insulin and the percentage of 20:4n-6 and of total n-6 fatty acids in erythrocytes and suggested that n-6 fatty acids, in particular 20:4n-6, modify the membrane environment of the insulin receptor or the glucose transporters.[25] In the erythrocyte, 18:0 may be unfavourable with respect to the rheological properties of erythrocyte, because increased poportion of 18:0 in phosphatidylcholine was correlated with a high red cell transit time.[26] An increase in membrane n-3/n-6 ratio with highly unsaturated n-3 fatty acid supplementation demonstrated a decrease in blood viscosity, and an increase in membrane deformability and filterability.[17, 27]

In conclusion, the decrease in saturated fatty acids, 14:0 and 18:0, and n-6/n-3 and increase in n-3 and n-6 PUFAs and

P/S ratio found in erythrocyte membrane phospholipids following green vegetable juice supplementation, may be accompanied by rheological and functional changes in the erythrocytes in hypercholesterolaemic patients. Further examination is needed to clarify the role of green vegetable juice on phospholipid composition and the function of the erythrocyte membrane.

References

1. Diplock AT. Antioxidant nutrients and disease prevention: an overview. Am J Clin Nutr 1991; 53: 189S–193S.
2. Decker EA. The role of phenolics, conjugated linoleic acid, carnosine and pyrroloquinoline quinone as nonessential dietary antioxidants. Nutr Rev 1995; 53: 49–58.
3. Gey KF, Brubacher GB, Stähelin HB. Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. Am J Clin Nutr 1987; 45: 1368–1377.
4. Wald NJ. Retinol, beta-carotene and cancer. Cancer Surv 1987; 6: 635–651.
5. Ishikawa T, Suzukawa M, Ito T, Yoshida H, Ayaori M, Nishiwaki M, Yonemura A, Hara Y, Nakamura H. Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. Am J Clin Nutr 1997; 66: 261–266.
6. Nanjee MN, Verhagen H, Poppel G, Rompelberg CJM, Bladeren PJ, Miller N. Do dietary phytochemicals with cytochrome P-450 enzyme-inducing activity increase high-density-lipoprotein concentrations in humans? Am J Clin Nutr 1996; 64: 706–711.
7. Gey KF, Puska P, Jordan P, Moser UK. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. Am J Clin Nutr 1991; 53: 326S–334S.
8. Hininger I, Chopra M, Thurnham DI, Laporte F, Richard M-J, Favier A, Roussel A-M. Effect of increased fruit and vegetable intake on the susceptibility of lipoprotein to oxidation in smokers. Eur J Clin Nutr 1997; 51: 601-606.
9. Suzuki K, Sasagawa T, Okita M, Fujiwara Y, Mori K, Saito M, Abe Y. Development of new simplified-food frequency method for use in the surveillance on lifestyle in order to promote healthy condition in Okayama Prefecture, computed by Microsoft Excel. Bull Facul Health Welfare Sci, Okayama Prefec Univ, 1996; 3: 143-153.
10. Hanahan DJ, Ekholm JE. The preparation of red cell ghosts. In: Fleischer S, Packer L, eds. Methods in enzymology, vol. 31 (Part A). New York and London: Academic Press, 1974; 168–172.
11. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37: 911–917.
12. Okita M, Gaudette DC, Mills GB, Holub BJ. Elevated levels and altered fatty acid composition of plasma lysophosphatidylcholine (lysoPC) in ovarian cancer patients. Int J Cancer 1997; 71: 31–34.
13. Okita M, Yoshida S, Yamamoto J, Suzuki K, Kaneyuki T, Kubota M, Sasagawa T. N-3 and n-6 fatty acid intake and serum phospholipid fatty acid composition in middle-aged women living in rural and urban areas in Okayama Prefecture. J Nutr Sci Vitaminol (Tokyo) 1995; 41: 313–323.
14. Milne DB, Botnen J. Retinol, alpha-tocopherol, lycopene and alpha- and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. Clin Chem 1986; 32: 874–876.
15. Yagi K. Assay for serum lipid peroxide and its clinical significance. In: Yagi K, ed. Lipid peroxides in biology and medicine. New York: Academic Press, 1982; 223–242.
16. Dougherty RM, Galli C, Ferro-Luzzi A, Iacono JM. Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. Am J Clin Nutr 1987; 45: 443–455.
17. Mills DE, Murthy M, Galey WR. Dietary fatty acids, membrane transport and oxidative sensitivity in human erythrocytes. Lipids 1995; 30: 657–663.
18. Tynan MB, Nicholls DP, Maguire SM, Steele IC, McMaster C, Moore R, Trimble ER, Pearce J. Erythrocyte membrane fatty acid composition as a marker of dietary compliance in hyperlipidaemic subjects. Atherosclerosis 1995; 117: 245–252.
19. Frei B. Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. Am J Clin Nutr 1991; 54: 1113S–1118S.
20. Harats D, Chevion S, Nahir M, Norman Y, Sagee O, Berry EM. Citrus fruit supplementation reduces lipoprotein oxidation in young men ingesting a diet high in saturated fat: presumptive evidence for an interaction between vitamins C and E *in vivo*. Am J Clin Nutr 1998; 67: 240–245.
21. Martin A, Wu D, Meydani SN, Blumberg JB, Meydani M. Vitamin E protects human aortic endothelial cells from cytotoxic injury induced by oxidized LDL *in vitro*. J Nutr Biochem 1998; 9: 201–208.
22. Yang Y, Huang C-Y, Peng S-S, Li J. Carotenoid analysis of several dark-green leafy vegetables associated with a lower risk of cancers. Biomed Environ Sci 1996; 9: 386–392.
23. Koumanov K, Wolf C, Béréziat G. Modulation of human type II secretory phospholipase A_2 by sphingomyelin and annexin VI. Biochem J 1997; 326: 227–233.
24. Koumanov KS, Quinn PJ, Béréziat G, Wolf C. Cholesterol relieves the inhibitory effect of sphingomyelin on type II secretory phospholipase A_2. Biochem J 1998; 336: 625–630.
25. Clifton PM, Nestel PJ. Relationship between plasma insulin and erythrocyte fatty acid composition. Prostag Leuk Essent Fatty Acids 1998; 59: 191-194.
26. Persson SU, Wohlfart G, Larsson H, Gustafson A. Correlations between fatty acid composition of the erythrocyte membrane and blood rheology data. Scand J Clin Lab Invest 1996; 56: 183–190.
27. Cartwright IJ, Pockley AG, Galloway JH, Greaves M, Preston FE. The effects of dietary omega-3 polyunsaturated fatty acids on erythrocyte membrane phospholipids, erythrocyte deformability and blood viscosity in healthy volunteers. Atherosclerosis 1985; 55: 267–281.