Effects of a Long-Term Vegetarian Diet on Biomarkers of Antioxidant Status and Cardiovascular Disease Risk

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OBJECTIVE: We compared plasma biomarkers of antioxidant status, oxidative stress, inflammation, and risk for coronary heart disease in long-term vegetarians and age- and sex-matched omnivores.

METHODS: Thirty vegetarians (mean age ± standard deviation: 44.2 ± 9.0 y) were recruited. The subjects had been vegetarian for 5 to 55 y (21.8 ± 12.2 y). The control group comprised 30 adults selected by age-stratified sampling from a community health project (mean age: 44.0 ± 9.2 y). Fasting plasma total antioxidant status (ferric-reducing antioxidant power), ascorbic acid (AA), α-tocopherol (total and lipid standardized), malondialdehyde, total cholesterol, triacylglycerol, uric acid (UA), and high-sensitivity C-reactive protein (hsCRP) were measured.

RESULTS: Plasma AA was significantly higher in the vegetarians than in the omnivores (90.5 ± 21.0 and 61.8 ± 17.0 μM; $P < 0.001$). The vegetarians had lower concentrations of triacylglycerol, UA, and hsCRP. Plasma total and lipid-standardized α-tocopherol concentrations were also lower in the vegetarians: 22.0 ± 5.9 and 27.0 ± 7.9 μM versus 3.76 ± 0.57 and 4.23 ± 0.58 μM per millimoles per liter of total cholesterol plus triacylglycerol, respectively. There was a significant inverse correlation between AA and UA ($r = -0.343$, $P < 0.01$; $n = 60$) and between AA and hsCRP ($r = -0.306$, $P < 0.05$; $n = 55$). Plasma ferric-reducing antioxidant power and malondialdehyde did not differ significantly between groups; however, the contribution of AA to the total antioxidant capacity of plasma was approximately 50% greater in the vegetarians.

CONCLUSIONS: A long-term vegetarian diet is associated with markedly higher fasting plasma AA concentrations and lower concentrations of TAG, UA, and hsCRP. Long-term vegetarians have a better antioxidant status and coronary heart disease risk profile than do apparently healthy omnivores. Plasma AA may act a useful marker of overall health status. *Nutrition* 2004;20:863–866. ©Elsevier Inc. 2004

KEY WORDS: vegetarian, ascorbic acid, antioxidant, vitamin E, cardiovascular, biomarker

INTRODUCTION

Diets rich in fruit and vegetables protect against chronic, degenerative disease. These beneficial effects are believed to be due to plant-based antioxidant compounds, such as ascorbic acid, carotenoids, and flavonoids, that may protect key biological sites, such as lipoproteins, membranes, and DNA, from oxidative damage.[1,2] Evidence of benefit is so strong that the World Cancer Research Fund recommends five or more servings of fruit and vegetables each day,[3] and the recommended daily intake of vitamin C in the United States was recently revised upward to 75 mg/d for women and 90 mg/d for men.[4] Although the identity of the protective component or components in fruit and vegetables is not yet clear, evidence exists that intakes and plasma concentrations of vitamin C (ascorbic acid) correlate inversely with all-cause mortality rate and may act as biomarkers of health status.[5] In addition, the lipophilic antioxidant vitamin E (mainly α-tocopherol) is an anti-atherogenic and anti-inflammatory agent.[6,7] Increased antioxidant status, therefore, may account for at least part of the benefit of plant-based diets. One cross-sectional study has suggested that vegetarians are healthier than non-vegetarians,[8] and we were interested in determining whether vegetarians exhibit a more favorable profile in terms of antioxidant status and risk of chronic disease than do omnivores of similar age. Therefore, we compared biomarkers of antioxidant status, oxidative stress, inflammation, and coronary heart disease (CHD) risk in a group of long-term vegetarians with those of age- and sex-matched omnivores.

MATERIALS AND METHODS

Thirty vegetarians (27 women and 3 men; mean age ± standard deviation: 44.2 ± 9.0 y) were recruited with their informed consent. These subjects had been vegetarian for 5 to 55 y (21.8 ± 12.2 y). They ate no meat or fish owing to their religious (Taoist) beliefs, but some occasionally consumed eggs and milk in small amounts. The control group comprised 30 non-Taoist adults (27 women and 3 men, 44.0 ± 9.2 y) matched by age- and sex-stratified sampling from a pool of participants in a community-based health project. Venous blood was collected from fasting subjects into heparin-containing tubes, and plasma was separated within 2 h of collection. Plasma total antioxidant status (as the ferric-reducing antioxidant power [FRAP] value) and ascorbic acid concentrations were measured within 30 min of separation by using a modification of the FRAP assay, known as FRASC, as

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previously described in detail.[9] Plasma was portioned and stored at −70°C until assayed for α-tocopherol (total and lipid standardized), total cholesterol, triacylglycerol, uric acid, malondialdehyde, and high-sensitivity C-reactive protein (hsCRP).

α-Tocopherol was measured by using a slight modification of the high-performance liquid chromatographic (HPLC) procedure of Brandt et al.[10] Briefly, 100 μL of plasma was mixed with 100 μL of ethanol (Riedel de Haen, Seelze, Germany) and 100 μL of 150 μM α-tocopherol acetate (internal standard; Merck, Darmstadt, Germany). The mixture was agitated in a vortex mixer, and 200 μL of hexane (Fisher Scientific, Loughborough, UK) containing 0.5 g/L of butylated hydroxytoluene (Sigma, St. Louis, MO, USA) was added to each calibrator or plasma sample, and the samples were again agitated in a vortex mixer for 30 s. The mixtures were then centrifuged at 2500*g* for 10 min at 4°C. One hundred twenty microliters of the hexane layer was transferred to a glass test tube, and the hexane was evaporated under nitrogen at about 50°C. Sixty microliters of mobile phase was added to dissolve the extracts. The mobile phase consisted of 80:20 methanol:toluene, by volume (Riedel de Haen). Each calibrator or sample extract was transferred to a small glass vial fitted with a micro glass insert, and the vial was placed inside the HPLC autosampler at 4°C. Twenty microliters of each calibrator was injected into the HPLC system. The HPLC system consisted of an Alliance 2690 Separations Module with a temperature-controlled column chamber (Waters, Milford, MA, USA), a 996 Photodiode Array Detector and Millennium-32 PDA 3.05.01 (Waters), a reversed-phase C_{18} analytical column (5 μm, 250 × 5 mm internal diameter; ISCO Inc, Lincoln, NE, USA), and a Sentry guard column (Symmetry C_{18} 3.5 m, 3.9 × 20 mm internal diameter; Waters). Detection was at 292 nm, and the flow rate was 1.0 mL/min. The between-run coefficient of variation (CV) was less than 8% at 25 μM (n = 5).

Malondialdehyde was measured by HPLC according to the HPLC protocol of Chirico et al.[11] and the extraction procedure of Jentzsch et al.[12]. One hundred microliters of pooled heparin-treated plasma was mixed with 100 μL of 1,1,3,3-tetraethoxypropane standard (Sigma; which degrades to malondialdehyde upon heating) in ethanol (0 to 1.5 μM), or a 100-μL sample was mixed with 100 μL of ethanol, followed by 25 μL of 90 mM butylated hydroxytoluene in ethanol and 200 μL of 0.2 M orthophosphoric acid (BDH Laboratory Supplies, Poole, UK). The mixture was then agitated in a vortex mixer for 10 s, and 25 μL of 0.11 M freshly prepared 2-thiobarbituric acid (Sigma) was added. The mixtures were then heated at 90°C for 45 min. After a brief cooling on ice, 200 μL of butanol was added to each tube, followed by vortex mixing for 20 s. The mixtures were then centrifuged at 2500*g* for 15 min at 10°C. One hundred microliters of the butanol layer was mixed with 100 μL of methanol, and 20 μL of each calibrator was injected into the Waters HPLC system. Detection was at 532 nm, and the flow rate was 0.5 mL/min. The between-run CV was 9.2% at 0.5 μM (n = 5).

Concentrations of uric acid (between-run CV = 2.6% at 304 mM/L), total cholesterol (between-run CV = 2.6% at 6.7 mM/L), and triacylglycerol (between-run CV = 5.4% at 2.6 mM/L) were measured by using commercially available enzymatic methods (Unimate, Roche Diagnostics Ltd., Basel, Switzerland) and a Cobas Fara centrifugal analyzer (Roche Diagnostics). High-sensitivity CRP was assayed in one batch by use of an immunoassay method (BN ProSpec System, Dade Behring, Marburg, Germany); the within-run CV was 3.8% at 13.2 mg/L.

Associations between variables were investigated with Pearson's correlation analysis.

Differences between the vegetarian and non-vegetarian groups were investigated by using Student's *t* test for unpaired data, and significance was sought at the 5% level. Non-normally distributed data (hsCRP and triacylglycerol) were log-transformed before analysis. Statistical analyses were conducted with PRISM 3.0 (GraphPad, San Diego, CA, USA).

TABLE I.

VARIABLES OF INTEREST IN FASTING PLASMA OF VEGETARIANS AND OMNIVORES*

	Vegetarians (n = 30)	Non-vegetarians (n = 30)
Ascorbic acid (μM)	90.5 (21.0)‡	61.8 (17.0)
α-Tocopherol (μM)†	22.0 (5.9)§	27.0 (7.9)
Lipid-standardized α-tocopherol (μMol/mM TC+TAG)	3.76 (0.57)§	4.23 (0.58)
Cholesterol (mM)	4.8 (1.1)	4.9 (1.3)
Triacylglycerol (mM)†	1.06 (0.45)‡	1.35 (0.57)
Uric acid (μM)	239 (87.7)§	306 (68.3)
FRAP value (μM)	1028 (180)	1040 (178)
C-reactive protein (mg/L)†	0.77 (1.29)§	1.30 (1.38)
MDA (μM)	0.56 (0.15)	0.61 (0.17)

* Data are mean (standard deviation).
† Analysis performed on log-transformed data.
‡ $P < 0.0001$, versus non-vegetarian group (Student's t test).
§ $P < 0.01$, versus non-vegetarian group (Student's t test).
FRAP, ferric-reducing antioxidant power; MDA, malondialdehyde; TC+TAG, total cholesterol + triacylglycerol

The university human subjects ethics committee approved this study, and all procedures involving human subjects complied with the Declaration of Helsinki as revised in 2000.

RESULTS

As shown in Table I, plasma ascorbic acid concentrations were significantly higher in the vegetarians than in the omnivores ($P < 0.0001$). Malondialdehyde concentrations were slightly but non-significantly lower in the vegetarians. No significant difference in the total antioxidant capacity of plasma (as the FRAP value) was seen; however, the contribution of ascorbic acid to the FRAP value of the vegetarian subjects was 50% higher than that in the omnivores (averaging 16.6% and 11.8% for vegetarians and omnivores, respectively). Plasma hsCRP, triacylglycerol, uric acid, and α-tocopherol concentrations were significantly lower in the vegetarians, but total cholesterol did not differ significantly between groups. Overall, significant inverse correlations were seen between ascorbic acid and uric acid (Pearson's $r = -0.343$, $P < 0.01$; n = 60) and between ascorbic acid and hsCRP (Pearson's $r = -0.306$, $P < 0.05$; n = 55). No significant correlations were found between any of the other biomarkers measured.

DISCUSSION

Individuals who eat a diet rich in fruit and vegetables or who have high plasma concentrations of antioxidant micronutrients have a low risk of cardiovascular disease and stroke.[13–16] This observational evidence has led to the recommendation that high-risk individuals or populations with a high incidence of CHD and stroke should substantially increase their intakes of dietary antioxidants. It has been shown that plasma concentrations of antioxidants, such as α- and β-carotenoids and ascorbic acid, increase appreciably in those individuals with low intakes of fruit and vegetables when they follow such recommendations.[17] Key et al.[18] examined dietary factors associated with mortality rate among 11 000 health-conscious individuals followed for an average of 17 y. After adjusting for smoking, those who ate fresh fruit daily compared with those who ate fresh fruit less frequently had a 24%

lower mortality rate from CHD and a 32% lower mortality rate from stroke. Although other factors, including smoking, socioeconomic status, physical activity, and body mass index, affect the risk of disease,[19] the positive findings of an association between a diet rich in fruit and vegetables and a lower risk of chronic degenerative disease are strong, consistent, and convincing.[3,5,19,20] Therefore, it is not surprising that vegetarians have been found to have lower rates of morbidity and mortality from degenerative disease.[18,21,22]

The results of the small, cross-sectional comparison study presented here lend further support to the literature. Various biomarkers of antioxidant status and CHD risk were compared in long-term vegetarians and in a sample of the general public matched for age and sex. Plasma ascorbic acid concentrations were nearly 50% higher in the vegetarians than in the non-vegetarians and made a significantly larger contribution to the total antioxidant capacity of plasma in the vegetarian group.

Plasma ascorbic acid concentrations have been shown to be inversely correlated with mortality rate from cardiovascular disease and cancer, and in one study, a 20-μM increase in fasting plasma ascorbic acid was linked to a 20% decrease in mortality rate.[5] Increased intakes of fruit and vegetables and supplementation with vitamin C have also been reported to decrease blood pressure.[20,23] In addition, high ascorbic acid intake and high plasma ascorbic acid concentrations are inversely related to stroke incidence and mortality rate,[5,24] whereas low plasma ascorbic acid reportedly predicts unstable coronary artery disease activity in patients with established cardiovascular disease.[25] These findings support a role for ascorbic acid, or something strongly associated with it, in the promotion of cardiovascular health. Further, we suggest that the fasting plasma ascorbic acid concentration may be a useful prospective biomarker in terms of health maintenance, with decreasing concentrations indicating the onset or an increased risk of chronic degenerative disease. This concept of using plasma ascorbic acid as an indicator of overall health status requires further study in prospective trials.

Gey[26] summarized the published results relating to the plasma status of ascorbic acid and CHD and suggested that ascorbic acid concentrations of at least 50 μM are cardioprotective. Interestingly, all but six of our healthy subjects had fasting plasma ascorbic acid concentrations above this suggested threshold; the six subjects with values below the threshold were all in the omnivorous group. It is also of interest that the plasma ascorbic acid concentration in our local population, in which mortality rate from heart disease is relatively low (all-age mortality rate $<$ 75 per 100 000 persons),[27] appears to be markedly higher than that in Western groups. A British study by John et al.[23] reported a mean $\pm$ standard deviation fasting plasma ascorbic acid concentration of 34 $\pm$ 15 μM. The mean concentrations in our omnivorous and vegetarian subjects were 62 $\pm$ 17 and 91 $\pm$ 21 μM, respectively. However, even though the association between ascorbic acid and improved health is apparently strong, it is not yet clear whether it is ascorbic acid that is responsible, alone or in part. The apparent relation may be mediated by synergistic effects between ascorbic acid and other agents in plant-based food or may be due to other as yet unknown components of healthy diets. It is also likely that the putative beneficial effect of increased ascorbic acid or other antioxidants is mediated by protection over many years. This may account for the apparent lack of benefit seen in a recently published supplementation study.[28]

In addition to having higher concentrations of ascorbic acid, the vegetarian group had lower concentrations of plasma uric acid, hsCRP, and triacylglycerol. Uric acid and triacylglycerol are independent risk factors for CHD, whereas hsCRP is a marker of inflammation and has been reported to be predictive of CHD.[29–31] Together, our results indicate a more favorable CHD risk profile in the vegetarian group, even though total cholesterol concentrations did not differ significantly between groups.

The role of uric acid in CHD is double edged. Uric acid is an important endogenous antioxidant that is present in relatively large amounts throughout the body.[32] However, high uric acid concentrations are associated with an increased risk of CHD,[29] and uric acid has been reported to correlate directly with plasma thiobarbituric acid–reactive substances, which are an index of oxidative stress.[33] The high content of nucleic acids in meat results in the formation of more purine-derived uric acid in meat eaters; thus, the lower uric acid concentration in our vegetarian group is not unexpected. However, the apparent lack of one antioxidant (uric acid) was compensated for by another (ascorbic acid), so that, although the relative contributions of the individual antioxidants differed, the plasma FRAP values of both groups were similar. Interestingly, we found a significant inverse correlation between uric acid and ascorbic acid in the population overall. It is possible that increased ascorbic acid decreases cell turnover and purine degradation.[34] However, ascorbic acid has been reported to have a uricosuric effect,[35] which could also account for an inverse relation. Regardless of the route, we suggest that, in view of the direct association of uric acid with insulin resistance, hypertension, and CHD risk, a decrease in the contribution of uric acid to a total antioxidant capacity maintained by ascorbic acid indicates improved antioxidant status and lower CHD risk.

The Taoist vegetarian group, somewhat surprisingly, had lower plasma concentrations of α-tocopherol, although not low enough to indicate deficiency. The lower concentrations could not be explained completely by lower lipid concentrations, because lipid-standardized α-tocopherol concentrations were also lower. The Taoist group did not generally include nuts and seeds in their diet. These are rich sources of vitamin E, and the lower plasma concentrations may simply be due to a low intake of α-tocopherol. With this in mind, it may be advisable for these and other vegetarians to increase their dietary intake of vitamin E, e.g., by eating nuts and seeds. It is worth noting that zinc deficiency has been reported to affect the absorption of α-tocopherol.[36] Because the richest food source of zinc is meat,[37] zinc deficiency is not uncommon in vegetarians.[38,39] In addition, it is possible that the higher phytate content of vegetarian diets inhibits the absorption of zinc, thus further lowering zinc status.[40] This may help to account for the lower plasma α-tocopherol concentrations in our vegetarian group. However, the abundance of ascorbic acid may help to conserve and recycle the limited α-tocopherol supplies, because evidence exists that these antioxidant vitamins act in cooperation.[41]

Malondialdehyde is a product of peroxidized polyunsaturated fatty acids and is used as a biomarker of oxidative stress.[33] However, we found no significant difference in malondialdehyde concentrations between the two groups of healthy subjects tested.

C-reactive protein is an acute-phase protein. Increased concentrations reflect inflammation and increased CHD risk, and plasma CRP has been validated as a sensitive, independent biomarker and predictor of CHD.[31] In the present study, we used a highly sensitive method (hsCRP) that enables the detection of subclinical inflammation and allowed us to revisit the comparison of CRP-related assessment of cardiovascular disease risk in vegetarians and non-vegetarians. A previous study reported no significant difference in plasma CRP between healthy vegetarians and non-vegetarians.[42] In the present study, however, we found lower hsCRP concentrations in the vegetarians. This finding indicates that a long-term vegetarian diet may be cardioprotective and anti-inflammatory. Interestingly, our data also showed lower hsCRP in association with higher ascorbic acid, implying a possible anti-inflammatory role for ascorbic acid.[43] There are previous reports of an inverse correlation between ascorbic acid and CRP in inflammatory disorders such as rheumatoid arthritis and atherosclerosis;[44,45] however, to our knowledge, this is the first report of a significant inverse correlation in apparently healthy subjects found with the use of a highly sensitivity method. It is of interest that supplementation with α-tocopherol for 3 mo (1200 IU/d) was reportedly associated with a decrease in CRP, from around 6 to 4 mg/L, in healthy subjects.[7] However, in light of the relatively low α-tocopherol concentrations of our vegetarian subjects, it is unclear whether this effect on CRP is due to α-tocopherol specifically or to a generic increase in antioxidant status.

In conclusion, the results of this small cross-sectional study support and extend the findings of other studies on the health benefits of vegetarian diets. Our results should not be overstated, however, owing to our lack of data on the body mass index, smoking habits, and supplement use of the subjects and to the undoubted influence of other lifestyle factors on the risk of cardiovascular disease. However, these new data do show that the long-term vegetarian subjects had markedly higher plasma ascorbic acid concentrations than did the omnivores and had lower concentrations of three independent risk factors for CHD: triacylglycerol, uric acid, and hsCRP. Further, our data show previously unreported inverse relations between ascorbic acid and uric acid and between ascorbic acid and hsCRP in healthy subjects, which supports the hypothesis that the plasma ascorbic acid concentration may be a useful marker of overall health status. Therefore, our results indicate that long-term adherence to a vegetarian diet is associated with an improved CHD risk profile. We suggest that the fasting plasma ascorbic acid concentration may be a sensitive and dynamic marker of health status, particularly in relation to the cardiovascular system, and this requires further study.

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REFERENCES

1. Strain JJ, Benzie IFF. Diet and antioxidant defence. In: Sadler M, Caballero B, Strain JJ, eds. *The encyclopedia of human nutrition.* London: Academic Press, 1998:p. 95
2. Collins AR. Oxidative damage, antioxidants and cancer. Bioessays 1999;21:238
3. World Cancer Research Fund and the American Institute for Cancer Research. *Food, nutrition and the prevention of cancer: a global perspective*. Washington, DC: American Institute for Cancer Research, 1997
4. Food and Nutrition Board, Panel on Dietary Antioxidants and Related Compounds. Vitamin C. In: *Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids*. Washington, DC: National Academy Press; 2000, p. 95.
5. Khaw KT, Bingham S, Welch A, et al. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. European Prospective Investigation into Cancer and Nutrition. Lancet 2001;357:657
6. Yoshida N, Manabe H, Terasawa Y, et al. Inhibitory effects of vitamin E on endothelial-dependent adhesive interactions with leukocytes induced by oxidized low density lipoprotein. Biofactors 2000;13:279
7. Devaraj S, Jialal I. Alpha tocopherol supplementation decreases serum C-reactive protein and monocyte interleukin-6 levels in normal volunteers and type 2 diabetic patients. Free Radic Biol Med 2000;29:790
8. Fraser GE. Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. Am J Clin Nutr 1999;70(suppl):532S
9. Benzie IFF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In: Packer L, ed. *Methods in enzymology.* Orlando, FL: Academic Press, 1999:p. 15
10. Brandt RB, Kaugars GE, Riley WT, Bei RA, et al. Evaluation of serum and tissue levels of α-tocopherol. Biochem Mol Med 1996;57:64
11. Chirico S, Smith C, Marchant C, Mitchinson MJ, Halliwell B. Lipid peroxidation in hyperlipidaemic patients. A study of plasma using an HPLC-based thiobarbituric acid test. Free Radic Res Commun 1993;19:51
12. Jentzsch AM, Bachmann H, Furst P, Biesalski HK. Improved analysis of malondialdehyde in human body fluids. Free Radic Biol Med 1996;20:251
13. Pandey DK, Shekelle R, Selwyn BJ, Tangney C, Stamler J. Dietary vitamin C and β-carotene and risk of death in middle-aged men: the Western Electric Study. Am J Epidemiol 1995;142:1269
14. Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. Epidemiology 1992;3:194
15. Gaziano JM, Manson JE, Branch LG, et al. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. Ann Epidemiol 1995;5:255
16. Gillman MW, Cupples LA, Gagnon D, et al. Protective effect of fruits and vegetables on development of stroke in men. JAMA 1995;273:1113
17. Zino S, Skeaff M, Williams S, Mann J. Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. BMJ 1997;314:1787
18. Key TJA, Thorogood M, Appleby PN, Burr ML. Dietary habits and mortality in 11000 vegetarians and health conscious people: results of a 17 year follow up. BMJ 1996;313:775
19. Kilander L, Berglund L, Boberg M, Vessby B, Lithell H. Education, lifestyle factors and mortality from cardiovascular disease and cancer. A 25-year follow-up of Swedish 50-year-old men. Int J Epidemiol 2001;30:1119
20. Duffy SJ, Gokce N, Holbrook M, et al. Treatment of hypertension with ascorbic acid. Lancet 1999;354:2048
21. Messina VK, Burke KI. Position of the American Dietetic Association: vegetarian diets. J Am Diet Assoc 1997;97:1317
22. Knutsen SF. Lifestyle and the use of health services. Am J Clin Nutr 1994; 59(suppl):1171S
23. John JH, Ziebland S, Yudkin P, Roe LS, Neil HAW. Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomized controlled trial. Lancet 2002;35:1969
24. Gale CR, Martyn CN, Winter PD, Cooper C. Vitamin C and risk of death from stroke and coronary heart disease in cohort of elderly people. BMJ 1995;310: 1563
25. Vita JA, Keaney JF, Jr, Raby KE, et al. Low plasma ascorbic acid independently predicts the presence of an unstable coronary syndrome. J Am Coll Cardiol 1998;31:980
26. Gey KF. Vitamin E plus C, and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. Biofactors 1998;7:113
27. Department of Health annual report. Hong Kong: Department of Health; 1999.
28. Collins R, Peto R, Armitage J. The MRC/BHF Heart Protection Study: preliminary results. Int J Clin Pract 2002;56:53
29. Bickel C, Rupprecht HJ, Blankenberg S, et al. Serum uric acid as an independent predictor of mortality in patients with angiographically proven coronary artery disease. Am J Cardiol 2002;89:12
30. Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. Am J Cardiol 2000;86:943
31. Yu H, Rifai N. High-sensitivity C-reactive protein and atherosclerosis: from theory to therapy. Clin Biochem 2000;33:601
32. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci USA 1981;78:6858
33. Benzie IFF, Strain JJ. Uric acid: friend or foe? Redox Rep 1996;2:231
34. Lugli SM, Lutz WK. Stimulation of cell division in the rat by NaCl, KCl, $MgCl_2$, and $CaCl_2$, and inhibition of the sodium chloride effect on the glandular stomach by ascorbic acid and beta-carotene. J Cancer Res Clin Oncol 1999;125:209
35. Sutton JL, Basu TK, Dickerson JW. Effect of large doses of ascorbic acid in man on some nitrogenous components of urine. Hum Nutr Appl Nutr 1983;37:136
36. Kim ES, Noh SK, Koo SI. Marginal zinc deficiency lowers the lymphatic absorption of α-tocopherol in rats. J Nutr 1998;128:265
37. Brown JE. *The science of human nutrition*. Orlando, FL: Harcourt Brace Jovanovich, 1990
38. Rauma AL, Mykkanen H. Antioxidant status in vegetarians versus omnivores. Nutrition 2000;16:111
39. Ball MJ, Ackland ML. Zinc intake and status in Australian vegetarians. Br J Nutr 2000;83:27
40. Hunt JR. Moving toward a plant-based diet: are iron and zinc at risk? Nutr Rev 2002;60:127
41. Hamilton IM, Gilmore WS, Benzie IFF, Mulholland CW, Strain JJ. Interactions between vitamins C and E in human subjects. Br J Nutr 2000;84:261
42. Mezzano D, Munoz X, Martinez C, et al. Vegetarians and cardiovascular risk factors: hemostasis, inflammatory markers and plasma homocysteine. Thromb Haemost 1999;81:13
43. Grimble RF. Effect of antioxidative vitamins on immune function with clinical applications. Int J Vitam Nutr Res 1997;67:312
44. Jacobsson L, Lindgarde F, Manthorpe R, Akesson B. Correlation of fatty acid composition of adipose tissue lipids and serum phosphatidylcholine and serum concentrations of micronutrients with disease duration in rheumatoid arthritis. Ann Rheum Dis 1990;49:901
45. Langlois M, Duprez D, Delanghe J, De Buyzere M, Clement DL. Serum vitamin C concentration is low in peripheral arterial disease and is associated with inflammation and severity of atherosclerosis. Circulation 2001;103:1863