Long-term vitamin C supplementation has no markedly favourable effect on serum lipids in middle-aged Japanese subjects

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Antioxidant vitamins have been reported to be associated with an improvement in blood lipid profiles, but results are not consistent. The present study was designed to determine whether long-term vitamin C supplementation could alter serum lipid concentrations in subjects who completed a 5-year population-based double-blind intervention trial. A total of 439 Japanese subjects with atrophic gastritis initially participated in the trial using vitamin C and β-carotene to prevent gastric cancer. Before and upon early termination of β-carotene supplementation, 134 subjects dropped out of the trial; finally, 161 subjects assigned to the high-dose group (500 mg vitamin C/d) and 144 subjects assigned to the low-dose group (50 mg vitamin C/d) were studied. No favourable effect of vitamin C supplementation on serum concentrations of total cholesterol, HDL- and LDL-cholesterol, and triacylglycerol was observed, although high-dose vitamin C supplementation increased serum vitamin C concentrations substantially. Among women, the mean change in serum triacylglycerol decreased (−0.12 mmol/l, 95 % CI −0.32, 0.09) in the high-dose group, but increased (+0.12 mmol/l, 95 % CI 0.03, 0.22) in the low-dose group. In addition, the mean change in serum triacylglycerol among women with hypertriacylglycerolaemia was statistically significant (−1.21, 95 % CI −2.38, −0.05) after high-dose vitamin C supplementation. The 5-year vitamin C supplementation had no markedly favourable effects on the serum lipid and lipoprotein profile. However, our present results do not preclude the possibility that vitamin C supplementation may decrease triacylglycerol concentrations among women with hypertriacylglycerolaemia.

Vitamin C supplementation: Cholesterol: Triacylglycerol: Hypertriacylglycerolaemia: Intervention trial

The hypothesis that antioxidant vitamins may reduce the risk of CHD has been the focus of considerable research (Buring & Gaziano, 1997). Fundamental research (Lynch *et al.* 1996; Bok *et al.* 1999) and epidemiological studies (Trout, 1991; Carr & Frei, 1999) have provided evidence for mechanisms that may explain the effect of antioxidants on CHD. Two possible mechanisms of vitamin C in prevention of CHD have been postulated: (1) vitamin C may inhibit the oxidation of LDL-cholesterol both *in vivo* and *in vitro* (Steinberg & Chait, 1998); (2) improve the serum lipid levels by modulating the activity of several enzymes involved in lipid metabolism (e.g. cholesterol 7α-hydroxylase, 3-hydroxy-3-methyl-glutaryl-CoA reductase and lipoprotein lipase) (Bok *et al.* 1999).

Many epidemiological studies and a limited number of intervention trials (Jacques *et al.* 1995; Kurowska *et al.* 2000; Singhal *et al.* 2001) have provided evidence

indicating that antioxidant vitamins are associated with an improvement in blood lipid and lipoprotein profile; however, the findings are not entirely consistent. Most authors using the non-pharmacological lipid-lowering approach (Jacques *et al.* 1995; Walden *et al.* 1997; Kurowska *et al.* 2000; Singhal *et al.* 2001) have studied small numbers of normolipidaemic subjects with short-term follow-up, mainly focused on dietary modification of fats and fatty acids, and their results have been inconsistent.

The Hiraka Chemoprevention Study (Tsubono *et al.* 1997; Kim *et al.* 2002) in Japan was originally designed as a population-based double-blind randomized controlled trial to examine the effect of vitamin C and/or β-carotene supplementation on the primary prevention of gastric cancer in a population at high-risk for gastric cancer and stroke. The objective of the present report was to examine the effect of 5-year vitamin C supplementation on serum lipid levels.

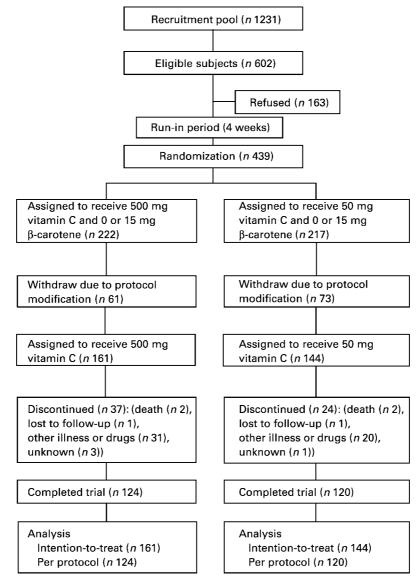
Abbreviation: TG, triacylglycerol.
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Methods

Subjects

The rationale, design and methodology of the study and the characteristics of the participants were published in detail by Tsubono *et al.* (1997) and Kim *et al.* (2002). Briefly, target subjects were men and women aged 40–69 years living in a village within the Yokote Public Health Centre district in Akita Prefecture, one of the regions with the highest mortality from stroke and gastric cancer in Japan (Tsugane *et al.* 1992). They regularly participated in annual screening programmes for circulatory diseases, conducted by each municipality under the National Health and Welfare Services Law for the Aged. Subject eligibility required: a diagnosis of chronic atrophic gastritis (defined as pepsinogen I <70 ng/ml and pepsinogen I:pepsinogen II ratio <3.0); no past history of gastric cancer or related surgery; no previous history of liver cancer, cirrhosis, or of other cancers within the last 5 years; no abnormal liver function; no use of diet supplements containing β-carotene or vitamin C; no expectation of moving outside the study area within 1 year. Of 1231 individuals screened, 1214 provided serum for pepsinogen measurements, and 635 (52 %) were diagnosed with chronic atrophic gastritis. Thirty-three people were ineligible, since they failed to meet the inclusion criteria. Of the remaining 602 eligible individuals, 439 (73 %) consented to take part in the trial. Of the 439 persons initially accepted into the study, 134 subjects (seventy-three in the high-dose and sixty-one in the low-dose group) dropped out early, either before or upon the study protocol modification: the modification followed a report by Omenn *et al.* (1996) suggesting that β-carotene supplementation had potential harmful effects among individuals at high risk for lung cancer. Of the 305 remaining participants, 244 (124 in the high-dose and 120 in the low-dose group) completed the present study (Fig. 1).

Fig. 1. Design of the randomized controlled diet.

Methods

In September 1995, 439 subjects were randomly assigned to one of four treatment groups using a 2×2 factorial design, whereby subjects were given 0 or 15 mg β-carotene/d and 50 or 500 mg vitamin C/d and were supplemented in a double-blind manner. The study capsule contained half of the dose of each of the two nutrients, and we instructed the subjects to take two capsules per d after their evening meal. However, because of the possible harmful effect of β-carotene supplementation (Omenn *et al.* 1996), we were forced to modify the initial study protocol. The β-carotene component of the trial was terminated early, on January 18 1996, after the mean treatment duration of 4 months. However, the vitamin C trial continued for 5 years. In addition, the primary endpoint of the trial was changed from the 10-year cumulative incidence of gastric cancer to the 5-year change in serum levels of pepsinogens and several biomarkers. Informed consent was obtained again from individuals willing to take part in the modified trial, and they were provided with new capsules containing vitamin C only (50 or 500 mg/d).

The vitamin C doses were set based on the pilot study (Sasaki *et al.* 2000) in which we examined the serum response to 3-month oral supplementation of vitamin C (0, 50 or 1000 mg/d). In that study, the high-dose group (1000 mg/d) showed a significant increase in serum concentrations at each point during supplementation (1, 2 and 3 months), with no adverse effect. However, the serum vitamin C concentrations in the placebo (0 mg/d) and even in the low-dose (50 mg/d) group did not significantly increase. Furthermore, we found that no significant differences in serum vitamin C concentrations were observed between the placebo and the low-dose group at any point of supplementation. Thus, we set 50 mg and 500 mg as the doses for the low- and high-dose groups respectively.

Compliance with pill-taking was determined on the basis of average pill counts (observed/expected number of pills consumed $\times$ 100; %) at every follow-up visit. We also monitored adverse effects using a questionnaire at every visit. The study protocol was approved by institutional review boards at the National Cancer Centre and the Hiraka General Hospital before the start of the study.

Measurements

Twelve-hour fasting blood samples collected upon entering the study and at annual health check-ups for circulatory diseases for 5 years; the samples were analysed for serum lipids. Serum concentrations of total cholesterol, triacylglycerol (TG) and HDL-cholesterol were analysed immediately after blood sampling. Enzymatic colorimetric methods were used to determine total cholesterol (Determiner-L TC II; Kyowa Medix, Tokyo, Japan), HDL-cholesterol (Cholestest N HDL; Daiichi Kagaku Yakuhin, Tokyo, Japan) and TG (Determiner-L TG II; Kyowa Medix) in serum using a commercial kit. The interassay CV of the control serum samples were (%): total cholesterol 0·69, HDL-cholesterol 1·66, TG 0·93. The LDL-cholesterol concentration was calculated according to the Friedewald equation (Friedewald *et al.* 1972). Because this equation is not valid when the TG concentration >4·5 (400 mg) mmol, no LDL-cholesterol concentrations were calculated under these circumstances (four subjects at baseline and two at the 5th year).

For vitamin C measurements, fasting blood samples collected on entering the study in 1995 and after 5 years in 2000 were analysed. Serum for ascorbic acid measurement was stabilized by addition of metaphosphoric acid (Wako Pure Chemical, Osaka, Japan). Serum (1 ml) was mixed with 0·25 ml metaphosphoric acid–dithiothreitol solution (17 g/l and 0·17 g/l respective final concentrations after dilution with serum) and stored at −80°C until assayed (measurements (Zannoni *et al.* 1974; Kim *et al.* 2003) were performed simultaneously (February 1997 for serum sampled at baseline and November 2000 for serum sampled at 5 years)). The interassay CV for vitamin C was 2·2 %. All assays were conducted by laboratory personnel blinded to the intervention assignment and the questionnaire data.

At recruitment and after the completion of the supplementation study, participants provided information on weight, height and demographic details such as marital and occupational status, education attainment, smoking status, alcohol consumption, disease history, family history of disease, and general health status. They also completed a 138-item semi-quantitative food-frequency questionnaire to provide information on their food habits and average daily consumption of food and beverages during 1 year at enrolment in the trial and after the completion of the supplementation program (5th year) (Tsubono *et al.* 1995).

Statistical analyses

For the primary analysis we followed the intention-to-treat analysis, which included all subjects remaining in the study after the protocol modification and completed lipid data, irrespective of compliance (161 in high-dose and 144 in low-dose group). In addition, a per-protocol analysis was also performed, which included the subjects who completed the study for 5 years (124 in high-dose and 120 in low-dose group), because the study aim was to examine the 5-year effect of vitamin C supplementation on serum lipids among those who complied with the supplementation of vitamin C throughout the study period. Therefore, those sixty-one subjects (thirty-seven in high-dose and twenty-four in low-dose group) who dropped out for personal reasons after the protocol modification were excluded from the per-protocol analysis.

Baseline comparisons between the high- and low-dose groups and drop-out group were examined by one-way ANOVA for continuous variables and by χ^2 test for categorical variables. Adjusted analyses of the mean changes in serum lipids for covariates were performed by one-way analysis of covariance. Results were adjusted for age, gender and baseline serum lipid level. Mean values for changes in serum lipids with 95 % CI are given. Hypertriacylglycerolaemia in subgroup analysis was defined as subjects with serum TG ≥ 1·70 mmol/l (150 mg/dl) at baseline. All statistical analyses were done using the

Statistical Analysis Systems statistical software package, version 8.01 (SAS Institute Inc., Cary, NC, USA).

Results

The baseline characteristics of the subjects are shown in Table 1. There were no significant differences in baseline characteristics and serum lipid concentrations between the high- and low-dose groups. Randomization appeared to be effective for baseline lipid measures and other characteristics, even though we excluded 134 subjects who dropped out early in the study before the protocol modification. In addition, baseline characteristics of the subjects who completed the 5-year trial did not differ between the high- (*n* 124) and low-dose groups (*n* 120) (results not shown). The compliance rate for vitamin C pill-taking, measured by means of pill counts, was 92·2 % in the high-dose group and 92·6 % in the low-dose group.

Table 2 shows the dietary intake and serum concentration of vitamin C at baseline and after 5 year of supplementation for female subjects, male subjects who smoked and male subjects who did not smoke. Dietary intakes of vitamin C did not differ between the high- and the low-dose groups at baseline and after supplementation. At baseline, the mean serum vitamin C concentration was similar between two supplemental groups for each subgroup, but after 5 years of supplementation, serum vitamin C levels were significantly greater in the high-dose group compared with those at baseline ($P<0.01$). The increases in serum vitamin C between the high- and the low-dose groups at 5 years for female and male non-smokers were significantly different ($P<0.05$), but the increase among male smokers was not significant.

Table 1. Baseline characteristics of study subjects

	High dose (*n* 161)*		Low dose (*n* 144)*	
	Mean	SD	Mean	SD
Age (years)	56·6	8·74	58·6	6·64
BMI (kg/m²)	23·2	2·69	23·4	2·92
Total cholesterol (mmol/l)	5·32	0·83	5·43	0·82
HDL-cholesterol (mmol/l)	1·55	0·37	1·55	0·38
LDL-cholesterol (mmol/l)†	3·18	0·77	3·33	0·85
Triacylglycerol (mmol/l)	1·29	1·00	1·25	0·80
Nutrient intake				
Energy (MJ/d)	8·80	3·21	8·67	2·58
Carbohydrate (% energy)	54·3	8·64	55·1	8·84
Fat (% energy)	25·6	6·88	25·0	6·88
Ethanol (g/d)	11·5	19·8	11·8	21·2
Current smoker‡	26§	44·8‖	18§	33·3‖
Drinker (≥ 5 times/week)	46§	28·6‖	34§	23·6‖
Cholesterol-lowering medication	12§	7·5‖	19§	13·2‖
Compliance (%)¶	92·2		92·6	

* High dose: male *n* 58, female *n* 103; low dose: male *n* 54, female *n* 90.
† LDL-cholesterol was calculated according to the Friedewald equation (Friedewald *et al.* 1972). Data for four subjects was excluded because no LDL-cholesterol could be calculated when the triacylglycerol concentration was >4·5 mmol/l.
‡ Based on male subjects, because no female subjects smoked.
§ *n*.
‖ %.
¶ Compliance was determined by pill counts at each 3-month follow-up visit, and the percentage was averaged over the treatment period.

After vitamin C supplementation, mean changes in serum lipids were not significantly different between the high- and the low-dose groups in the intention-to-treat analysis (Table 3). Nevertheless, in the per-protocol analysis, the mean serum TG concentration decreased by 0·09 mmol/l (95 % CI −0·27, 0·09) in the high-dose group, but increased by 0·11 mmol/l (95 % CI −0·01, 0·23) in the low-dose group ($P=0.02$ between groups). HDL-cholesterol significantly increased in both groups ($P<0.05$) compared with values at baseline. However, the net increase in HDL-cholesterol did not significantly differ between the high- and the low-dose groups.

Mean changes in serum TG according to gender and baseline TG level are shown in Table 4. Among women after vitamin C supplementation, serum TG concentration decreased by 0·12 mmol/l (95 % CI −0·32, 0·09) in the high-dose group and increased by 0·12 mmol/l (95 % CI 0·03, 0·22) in the low-dose group, but the difference was not statistically significant between groups. However, among men there was no difference in the mean change of serum TG between groups. Within the high-dose group, the mean serum TG decreased (−0·12 mmol/l, 95 % CI −0·32, 0·09) in women, but increased (+0·13 mmol/l, 95 % CI −0·08, 0·35) in men. Adjusted for age and baseline TG level, mean changes in serum TG were different between normolipidaemic and hypertriacylglycerolaemic subjects for each gender. The high dose of vitamin C resulted in decrease in the mean change of serum TG in subjects with hypertriacylglycerolaemia compared with the normolipidaemic subjects. The TG reduction in hypertriacylglycerolaemic female subjects was statistically significant (−1·21 mmol/l (95 % CI −2·38, −0·05) in the high-dose group, $P<0.05$). The difference in TG reduction between the high-dose and low-dose groups among hypertriacylglycerolaemic female subjects was significant in the per-protocol analysis ($P=0.04$), but not in intention-to-treat analysis. Furthermore, serum TG in female hypertriacylglycerolaemic subjects decreased throughout the 5-year period in the high-dose group (Fig. 2). The baseline level of TG in female hypertriacylglycerolaemic subjects averaged 3·21 mmol/l (284·5 mg/dl) and decreased to 1·55 mmol (137·3 mg/dl) after 5 years with 500 mg vitamin C/d.

The present study examined the effect of vitamin C supplementation on the serum lipids in smokers and non-smokers. There were no significant differences in change of lipids during the 5-year supplementation period between the two treatment groups according to smoking status (results not shown).

Discussion

Although the high-vitamin C supplementation increased serum vitamin C concentrations substantially, there was no marked favourable effect of vitamin C supplementation on the serum concentrations of total cholesterol, HDL- and LDL-cholesterol, and TG after a treatment duration of 5 years in the Hiraka Chemoprevention Study, a population-based double-blind randomized controlled trial conducted among subjects with atrophic gastritis using two different doses of vitamin C (50 mg or 500 mg/d) in

Table 2. Dietary and serum vitamin C at baseline and after 5 years of vitamin C supplementation‡

(Mean values and standard deviations)

	Intention-to-treat analysis§								Per-protocol analysis‖							
	High dose				Low dose				High dose				Low dose			
	Baseline		5-year		Baseline		5-year		Baseline		5-year		Baseline		5-year	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Male (smoker)¶																
Dietary vitamin C (mg/day)**	95·2	59·7	87·8	60·4	85·4	43·8	79·5	35·0	107·2	63·6	98·8	66·1	87·2	28·1	82·3	31·6
Serum vitamin C (mmol/l)	64·4	15·9	77·5†	21·1	62·8	17·7	67·5	12·4	62·6	15·9	81·8†	21·6	65·0	14·8	68·2	11·4
Male (non-smoker)††																
Dietary vitamin C (mg/day)**	116·9	81·7	115·7	75·3	92·2	43·3	94·2	32·0	114·4	60·1	108·3	49·8	91·5	46·7	94·9	33·4
Serum vitamin C (mmol/l)	74·3	22·7	85·3*†	23·4	70·9	21·0	73·9	14·7	76·4	23·6	90·2*†	23·2	69·9	15·3	75·1	15·3
Female‡‡																
Dietary vitamin C (mg/d)**	157·9	81·3	156·4	86·4	165·5	82·0	166·4	76·6	150·2	68·3	152·7	81·2	165·7	83·2	164·5	77·7
Serum vitamin C (mmol/l)	80·4	20·8	97·0*†	27·4	83·7	17·2	89·1†	14·2	80·4	19·8	102·7*†	26·6	83·9	17·6	90·0†	14·6

Mean values were significantly different from those of the low-dose group: *$P < 0.05$.
Mean values were significantly different from those at baseline: †$P < 0.05$.
‡ For details of subjects and procedures, see Table 1, Fig. 1 and pp. 82–83.
§ Intention-to-treat comparison with missing data added from initial pretreatment values.
‖ Based on the subjects who completed 5-year vitamin C supplementation.
¶ Intention to treat analysis: high dose n 26, low dose n 18; per-protocol analysis: high dose n 19, low dose n 12.
** From foods, without supplement (Energy-adjusted nutrient intake assessed with semi-quantitative food frequency).
†† Intention to treat analysis: high dose n 32, low dose n 36; per-protocol analysis: high dose n 26, low dose n 29.
‡‡ Intention to treat analysis: high dose n 103, low dose n 90; per protocol analysis: high dose n 79, low dose n 79.

Japan. Thus, these results do not support the hypothesis of improvement in serum lipid levels by long-term vitamin C supplementation.

The antioxidant vitamin-cardiovascular disease hypothesis has been explored in several observational studies (Sahyoun *et al.* 1996; Simon & Hudes, 1999; Joshipura *et al.* 2001) and intervention trials (Jacques *et al.* 1995; Kurowska *et al.* 2000; Singhal *et al.* 2001; Brown *et al.* 2001). However, although many prospective cohort studies have found inverse associations between dietary intake (Pandey *et al.* 1995; Sahyoun *et al.* 1996) or plasma levels (Sahyoun *et al.* 1996; Nyyssonen *et al.* 1997; Simon *et al.* 1998) of vitamin C and risk of cardiovascular disease, the overall results of the intervention trials have been disappointing and differ from the results of observational studies (Jacques *et al.* 1995; Sahyoun *et al.* 1996; Kurowska *et al.* 2000; Brown *et al.* 2001; Singhal *et al.* 2001; Heart Protection Study, 2002). A recent 5-year randomized placebo-controlled trial (Heart Protection Study Collaborative Group, 2002), in which 20 536 randomized patients at high risk of CHD (coronary disease, either occlusive vascular disease of non-coronary arteries or diabetes mellitus) failed to show any significant effect of anti-oxidant vitamins (600 mg vitamin E, 250 mg vitamin C and 20 mg β-carotene) on the 5-year mortality from, or incidence of, any type of vascular disease and cancer, as well as plasma lipids. A 3-year, double-blind trial of 160 patients with established coronary disease (Brown *et al.* 2001) reported that a combination of antioxidants (537 mg α-tocopherol, 1000 mg vitamin C, 25 mg β-carotene, 100 μg Se) resulted in no beneficial changes in coronary stenosis, the occurrence of a first cardiovascular event or serum lipids. In contrast to these null findings (Brown *et al.* 2001; Heart Protection Study Collaborative Group, 2002), in the Antioxidant Supplementation in Atherosclerosis Prevention study 520 non-smoking and smoking men and postmenopausal women with serum cholesterol ≥5·0 mmol (193 mg/dl) were randomized to receive 91 mg α-tocopherol and/or 250 mg slow-release vitamin C, or a placebo for 3 years (Salonen *et al.* 2000) and 6 years (Salonen *et al.* 2003); the results showed that a combined supplementation with reasonable doses of both α-tocopherol and vitamin C can retard the progression of common carotid atherosclerosis in men, but not in women. Similarly, several small-scale randomized trials showed that vitamin C supplementation (1 or 2 g/d) improved the concentrations of total cholesterol (Tofler *et al.* 2000) or HDL-cholesterol and apo A–I (Jacques *et al.* 1995) in clinically healthy subjects with low plasma vitamin C levels.

There is disagreement among studies that have examined the effect of vitamin C supplementation on the improvement of serum lipid levels or cardiovascular events (Jacques *et al.* 1995; Kurowska *et al.* 2000; Brown *et al.* 2001; Singhal *et al.* 2001; Salonen *et al.* 2003). The differences in the baseline risk profiles of subjects, the type and dosage of antioxidant tested, and the duration of treatment and follow-up may account for the discrepancies among the antioxidant trials.

One possible reason for these inconclusive findings may be the fact that the subjects studied in many trials varied in terms of baseline risk profiles; the subjects in some trials

Table 3. Effect of dose of vitamin C supplementation on change in serum lipids (mmol/l) from baseline to end of intervention*†
(Mean values and 95 % confidence intervals)

| | Intention-to-treat analysis‡ | | | | | Per-protocol analysis | | | | |
| | High dose (n 161) | | Low dose (n 144) | | Statistical significance of effect: P§ | High dose (n 124) | | Low dose (n 120) | | Statistical significance of effect: P§ |
	Mean	95 % CI	Mean	95 % CI		Mean	95 % CI	Mean	95 % CI	
Total cholesterol	−0·03	−0·14, 0·07	−0·03	−0·13, 0·08	0·50	−0·02	−0·14, 0·10	−0·04	−0·15, 0·07	0·85
HDL-cholesterol	0·08	0·04, 0·12	0·07	0·02, 0·12	0·83	0·09	0·05, 0·14	0·06	0·01, 0·10	0·36
LDL-cholesterol||	−0·11	−0·21, −0·02	−0·16	−0·28, −0·04	0·96	−0·08	−0·18, 0·02	−0·16	−0·27, −0·04	0·62
Triacylglycerol	−0·03	−0·18, 0·12	0·13	0·02, 0·23	0·11	−0·09	−0·27, 0·09	0·11	−0·01, 0·23	0·02

* For details of subjects and procedures, see Table 1, Fig. 1 and pp. 82–83.
† To convert mmol/l to mg/dl, multiply cholesterol by 38·67 and triacylglycerol by 88·57. Within-group changes are significant ($P < 0.05$) when 95 % CI do not overlap zero.
‡ Intention-to-treat comparison with missing data added from initial pretreatment values.
§ P values for comparison between the high- and low-dose groups by analysis of covariance, (adjusted for age, gender and baseline serum lipid level).
|| LDL-cholesterol was calculated according to the Friedewald equation, (Friedewald et al. 1972).

Table 4. Effect of dose of vitamin C supplementation on change in serum triacylglycerol (mmol/l) from baseline to end of intervention*†
(Mean values and 95 % confidence intervals)

| | Intention-to-treat analysis | | | | | | | Per-protocol analysis | | | | | | |
| | High dose | | | Low dose | | | Statistical significance of effect: P‡ | High dose | | | Low dose | | | Statistical significance of effect: P‡ |
	n	Mean	95 % CI	n	Mean	95 % CI		n	Mean	95 % CI	n	Mean	95 % CI	
Male														
Total	58	0·13	−0·08, 0·35	54	0·13	−0·12, 0·37	0·79	45	0·00	−0·22, 0·23	41	0·15	−0·15, 0·46	0·24
Normal (<1·70 mmol/l)	42	0·22	0·06, 0·38	36	0·36	0·15, 0·57	0·22	34	0·18	0·00, 0·37	28	0·43	0·17, 0·69	0·08
High TG (≧1·70 mmol/l)§	16	−0·11	−0·81, 0·60	18	−0·37	−0·97, 0·23	0·42	11	−0·55	−1·24, 0·14	13	−0·40	−1·13, 0·32	0·97
Female														
Total	103	−0·12	−0·32, 0·09	90	0·12	0·03, 0·22	0·15	79	−0·14	−0·39, 0·11	79	0·09	−0·01, 0·20	0·09
Normal (<1·70 mmol/l)	88	0·08	−0·01, 0·18	82	0·10	0·01, 0·19	0·52	69	0·09	−0·02, 0·20	72	0·07	−0·03, 0·16	0·90
High TG (≧1·70 mmol/l)§	15	−1·21	−2·38, −0·05	8	0·42	−0·51, 1·36	0·14	10	−1·66	−3·31, −0·02	7	0·42	−0·51, 1·36	0·04

TG, triacylglycerol.
* For details of subjects and procedures, see Table 1, Fig. 1, and pp. 82–83.
† To convert mmol/l to mg/dl, multiply cholesterol by 38·67 and triacylglycerol by 88·57. Within-group changes are significant ($P < 0.05$) when 95 % CI do not overlap zero.
‡ P values for comparison between the high- and low-dose groups by analysis of covariance (adjusted for age and baseline serum TG level).
§ Hypertriacylglycerolaemia was defined as TG ≥1·70 mmol/l at baseline.
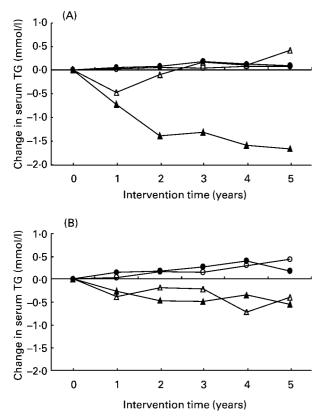
Fig. 2. The effect of vitamin C supplementation on the change in serum triacylglycerol (TG) for 5 years according to gender ((A), female subjects; (B), male subjects), based on per-protocol analysis. ○, normal baseline TG and 50 mg vitamin C/d; ●, normal baseline TG and 500 mg vitamin C/d; △, high baseline TG and 50 mg vitamin C/d; ▲, high baseline TG and 500 mg vitamin C/d (high baseline TG was defined as ≥1·70 mmol TG/l). For details of subjects and procedures, see Table 1, Fig. 1 and pp. 82–83.

(Brown et al. 2001; Singhal et al. 2001; Heart Protection Study Collaborative Group, 2002) had advanced degrees of CHD, whereas in other trials (Salonen et al. 2000; Tofler et al. 2000) the study subjects were clinically healthy but had hyperlipidaemia. Antioxidant vitamin trials to prevent CHD may be more important in earlier stages of lesion development, but less effective for established atherosclerosis (Gotto, 2003). The non-randomized observational studies that found a lower incidence of cardiovascular events to be associated with higher intakes of different antioxidant vitamins were chiefly on subjects without known coronary or other vascular disease (Pandey et al. 1995; Sahyoun et al. 1996; Nyyssonen et al. 1997; Simon et al. 1998). The lack of benefit in the high-risk population study (Heart Protection Study Collaborative Group, 2002) suggests that antioxidant vitamins might be protective only before occlusive disease has developed. As seen in the previous trials (Salonen et al. 2000; Brown et al. 2001; Heart Protection Study Collaborative Group, 2002), the subject's profile may be an important consideration in the antioxidant vitamin trial for prevention of CHD. Active antioxidant supplementation may prove to be a specialized approach for cardiovascular prevention in certain kinds of patients.

Another possible explanation may be the different doses of vitamin C: 500 mg/d in the high-dose group and 50 mg/d

in the low-dose group of the present trial. The vitamin C doses used in the other trials were 200–1500 mg/d. The treatment duration was 5 years in the present study, which is relatively long-term compared with other trial durations of several weeks at most. Long-term effects of vitamin C may be different from those of short-term trials. In addition, most studies conducted in western countries have focused mainly on the cholesterol level rather than the TG level, which is so important for many Asian people whose staple food is rice.

The effect of vitamin C supplementation on blood lipids may vary according to subgroups of gender and baseline serum lipid concentrations. The possibility of a differential lipid-lowering effect in female subjects compared with male subjects should be considered. In the present study, TG concentrations in the high-dose group (500 mg vitamin C/d) showed a decrease in mean TG (−0·12 mmol/l) in female subjects as compared with an increase in male subjects (+0·13 mmol/l), indicating a significant gender difference ($P=0·002$). There is evidence (Geil et al. 1995; Walden et al. 1997; Salonen et al. 2000) that women and men may respond differently to dietary changes or supplementations. The effect of vitamin supplementation on the progression of carotid atherosclerosis was significant in men, but not in women (Salonen et al. 2000). Two dietary studies reported a differential lipid response to the diet (American Heart Association step 1 diet and the National Cholesterol Education Program step 2 diet) by gender for TG (Geil et al. 1995) and for HDL-cholesterol (Walden et al. 1997).

In addition, the possibility of a differential lipid-lowering effect in hyperlipidaemic subjects compared with normolipidaemic subjects should also be considered. In the present study, the effect of vitamin C supplementation was much more apparent in female subjects with hypertriacylglycerolaemia. Supplementation for 5 years with a high dose (500 mg/d) of vitamin C significantly decreased the serum TG concentrations (−1·21 mmol/l) in female subjects with hypertriacylglycerolaemia, as compared with normolipidaemic females (+0·08 mmol/l). The observed 5-year changes in serum TG among hypertriacylglycerolaemic male subjects may possibly be explained to some extent by what is called 'regression to the mean' including physiological fluctuation. On the other hand, the observed 5-year changes in serum TG among hypertriacylglycerolaemic female subjects with high-dose vitamin C may reflect the regression to the mean effect, in addition to the real reduction due to vitamin C supplementation and the real biological change, even though we cannot quantitatively measure the absolute amount of each portion (Fig. 2).

The reduction in TG (−1·21 mmol/l) in hypertriacylglycerolaemic female subjects after 5 years of high-dose vitamin C may has a significant clinical benefit in the reduction of CHD risk. As has been observed in previous studies (Assmann et al. 1998; Pedersen et al. 1998), reducing TG levels in hypertriacylglycerolaemic subjects may have a marked effect on CHD risk reduction and the incidence of coronary events (Assmann et al. 1998). The reduced risk of CHD post-therapy observed in patients with normal LDL-cholesterol levels and high baseline

TG may be due to a reduction in TG levels (Pedersen *et al.* 1998).

A possible limitation of the present study may be that our study subjects had been diagnosed with chronic atrophic gastritis on the basis of serum pepsinogen levels. The prevalence of atrophic gastritis was 55·4 % (866 of 1564 subjects) among screening programme participants aged 40–59 years in another village within the same Yokote Public Health Centre district (S Tsugane, unpublished results). Although the prevalence of atrophic gastritis was relatively higher than in other areas, the present study subjects were not a specially selected group in Japan. Yokote is one of the regions with the highest mortality from stroke and gastric cancer (Tsugane *et al.* 1992). One study (Waring *et al.* 1996), however, found no differences in plasma vitamin C concentration between subjects with and without chronic gastritis in either vitamin C-supplemented or unsupplemented groups ($P > 0.05$ in all cases), and suggested that the plasma and mucosal concentrations were unaffected by the presence of chronic gastritis. Nevertheless, we cannot exclude the possibility that the effect of vitamin C supplementation on serum lipids may be influenced by the presence of atrophic gastritis.

The percentage of subjects who did not complete the 5-year vitamin C supplementation was 20 % (23 % in high-dose group and 17 % in low-dose group). Although the number of subjects who dropped out might alter the effect of vitamin C supplementation, there were no differences in baseline characteristics between subjects who completed and did not complete this trial. However, even though we observed a significant difference in the change in serum TG between the two groups in the per-protocol analysis, the result from those of the intention-to-treat analysis was not significant. This may be because the effect of vitamin C supplementation was diluted in the intention-to-treat analysis, because of the subjects who dropped out.

The rationale for the vitamin C dose was based on previous pharmacokinetic studies (Levine *et al.* 1996; Blanchard *et al.* 1997), in which plateau plasma vitamin C was close to maximum at 500 mg vitamin C/d and plasma vitamin C was completely saturated at the 1000 mg/d. The RDA of vitamin C was 50 mg at the time this study was designed, according to the Ministry of Health, Labour and Welfare of Japan (1994). As a prophylactic antioxidant, we chose to administer ten times the daily dose recommended in Japan and gave 500 mg ascorbic acid/d in capsule form. According to a pharmacokinetic study (Levine *et al.* 1996), safe doses of vitamin C are < 1000 mg/d, while bioavailability declines and the absorbed amount is largely excreted at a single dose of ≥ 500 mg. Thus, we set 500 mg as the dosage for the high-dose group. This amount of vitamin C is half of that which we tested in a pilot study (Sasaki *et al.* 2000), in which no adverse effect was observed from taking 1000 mg vitamin C. In most randomized clinical trials of vitamin C, there was no apparent adverse effect of a higher dose of vitamin C (< 1000 mg/d). Results from other large intervention trials with higher doses of vitamin C suggest no evidence of any potential hazard with < 1000 mg/d (Brown *et al.* 2001).

The lack of a placebo group may be a limitation in evaluating the supplemental effect of vitamin C. However, the mean dietary intakes of vitamin C were 139·7 (SD 81·8) mg/d for the high-dose group and 137·2 (SD 78·9) mg/d for the low-dose group, respectively. The supplementation of 50 mg vitamin C/d for the low-dose group was similar to or within 1SD of the estimated vitamin C intake level from foods. In addition, in the pilot study for this trial (Sasaki *et al.* 2000), we found no significant differences in serum vitamin C concentrations between the placebo (0 mg/d) and the low-dose (50 mg/d) groups at any point of supplementation (1, 2 and 3 months). Moreover, the purpose of the present study was to evaluate the effect of vitamin C supplementation (much higher than usual intake level: 500 mg/d in the present study) compared with the normal level (average consumption level of Japanese). Likewise, the mean dietary intake of vitamin C in the placebo group was 150 mg/d in a placebo-controlled trial of vitamin C (500 mg ascorbate/d; Huang *et al.* 2002). Thus, the dose of vitamin C for the low-dose group (50 mg/d) may be interpreted as allowing this group to play a similar role as a placebo group.

There are some limitations in the subgroup findings, even though we have found a significant net reduction in serum TG in hypertriacylglycerolaemic female subjects with a high dose of vitamin C. First, the number of subjects for subgroup analysis was small. Second, subgroup analyses are vulnerable to maldistribution of confounding variables. However, we confirmed that the baseline characteristics of the hypertriacylglycerolaemic subjects were similar between the high- and low-dose groups.

CHD remains the leading cause of death in some Asian countries including Japan, as well as in most developed countries, accounting for approximately one in four deaths. For this reason, even the modest reductions in CHD risk suggested by studies to date, if real, could yield substantial public health benefits. At present, however, results remain inadequate to draw firm conclusions regarding the possible role of antioxidant vitamins in the prevention of CHD (Carr & Frei, 1999).

In conclusion, the 5-year vitamin C supplementation had no markedly favourable effects on the serum lipid and lipoprotein profile. However, the results do not preclude the possibility that vitamin C supplementation may decrease triacylglycerol concentrations among women with hypertriacylglycerolaemia in a high-risk population for stroke, an issue that needs further study.

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