*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 × 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 $\chi^2$ 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/m$^2$) | 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 antioxidant 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.

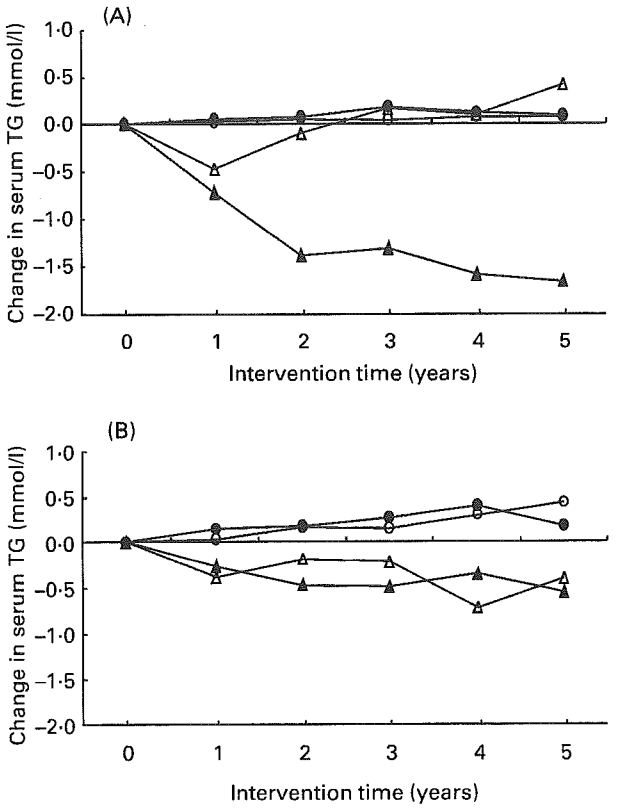


**Fig. 2.** The effect of vitamin C supplementation on the change in serum triacylglycerol (TG) for 5 years according to gender ((A), female subjects; (B), male subjects), based on per-protocol analysis. ○, normal baseline TG and 50 mg vitamin C/d; ●, normal baseline TG and 500 mg vitamin C/d; △, high 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 ($P>0{\cdot}05$ 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 $\geq 500$ 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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# Alcohol Consumption, Smoking, and Subsequent Risk of Colorectal Cancer in Middle-Aged and Elderly Japanese Men and Women: Japan Public Health Center-based Prospective Study

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## Abstract

**Few studies have examined the association of alcohol consumption and cigarette smoking with colorectal cancer in Asian populations whose genetic susceptibility to these factors are different from Western populations. We investigated this association and the joint effect of these factors, and estimated the population-attributable fraction to clarify the public health impact on a Japanese population, based on a prospective study. We analyzed the 10-year (cohort I) and 7-year (cohort II) follow-up data of the Japan Public Health Center-based prospective study on cancer and cardiovascular disease, derived from 90,004 (42,540 male and 47,464 female) middle-aged and elderly Japanese. We identified 716 (457 in men and 259 in women) newly diagnosed cases of colorectal cancer. Both alcohol consumption and smoking were clearly associated with colorectal cancer in men, after adjusting for age, family history of colorectal cancer, body mass index, and physical exercise. Regular heavy drinking of 150 g/week or more of ethanol showed a statistically significant increased risk compared with nondrinkers: relative risks (RRs) were 1.4 [95% confidence interval (CI), 1.1–1.9] for 150–299 g/week and 2.1 (95% CI, 1.6–2.7) for 300 g/week or more. On the contrary, regular ethanol consumption was not associated with colorectal cancer (RR, 0.7; 95% CI, 0.4–1.1) in women. In terms of smoking, the RRs were 1.4 (95% CI, 1.1–1.8) for current smokers and 1.3 (95% CI, 0.98–1.7) for ex-smokers compared with never-smokers in men. The risk of smoking in women was similar to that in men, although not statistically significant. The colorectal cancer risk with 300 g/week or more of ethanol in current smokers was estimated at 3.0 (95% CI, 1.8–5.1) compared with nondrinkers among nonsmokers in men. Colorectal cancer attributable to alcohol consumption or smoking was estimated to be 46%. In conclusion, approximately half of the colorectal cancer cases may be preventable by tobacco and alcohol controls in middle-aged and elderly Japanese men.**

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## Introduction

Colorectal cancer is one of the most common cancers in Western countries, and its incidence rate has increased recently in Asian countries, especially in Japan (1), as Japan has been westernized over the past few decades. In fact, the high incidence in Japanese migrants to Hawaii may suggest that a change of environmental factors, including the westernization of dietary habits and lifestyle, may contribute to this increase (1, 2).

Many epidemiological studies have reported the association of alcohol consumption with colorectal cancer (3) and adenoma. Recent prospective studies using incidence data have consistently supported this association (4–11). However, most of such studies of the incidence data have targeted Western populations; an Asian population has been investigated in only

one study (11). Alcohol consumption has increased in Asian populations, especially Japanese, so as to now reach the levels of Western populations (12). At the same time, half of all Japanese people have an atypical allele of the aldehyde dehydrogenase 2 gene (*ALDH2*; Ref. 13), which catalyzes the acetaldehyde metabolism less (14), resulting in a high blood level of acetaldehyde after drinking (15). Because of this genetic polymorphism, Japanese may have a susceptibility to alcohol consumption different from that in Western populations. Therefore, a study using a Japanese population would be expected to detect a stronger effect of alcohol consumption in relation to colorectal cancer than in Western populations.

Studies over the past decade have consistently reported a positive association between smoking and colorectal cancer (16–20). In addition, it has been revealed that smoking requires a long induction period to lead to colorectal carcinogenesis (19, 20). However, evidences of the association and the public health impact of smoking are only available for Western populations (19, 21). It is important to clarify the public health impact of smoking in populations with a high prevalence of smoking like Japanese men (53.5% of males ≥20 years of age in 2000; Ref. 22).

Therefore, we investigated the association of alcohol consumption, smoking, and their joint effect with colorectal cancer and estimated the population-attributable fraction (PAF) to clarify their public health impact, based on a population-based prospective cohort study.

## Materials and Methods

**Study Population.** The Japan Public Health Center-based prospective study on cancer and cardiovascular disease (JPHC study) started in 1990 for the first group (cohort I) and in 1993 for the second group (cohort II). Cohort I covered 5 areas administered by the Public Health Centers (PHC) in 5 prefectures (Iwate, Akita, Nagano, Okinawa, and Tokyo). Cohort II included 6 PHC areas in 6 prefectures (Ibaraki, Niigata, Kochi, Nagasaki, Okinawa, and Osaka). Cohort I comprised all residents aged 40–59 as of January 1, 1990 except for Tokyo, and cohort II comprised all residents aged 40–69 as of January 1, 1993 except for Osaka. The study subjects were identified by population registries maintained by local municipalities. When analyzing the present data, we excluded the subjects in Tokyo whose incidence data were not available, and those in Osaka who were not all within the specific age range. Thus, we defined a population-based cohort of 57,714 men (27,063 in cohort I and 30,651 in cohort II) and 59,182 women (27,435 in cohort I and 31,747 in cohort II). Those deemed ineligible during this study period were excluded, such as non-Japanese (29 men and 20 women), those who had already moved away at baseline (94 men and 57 women), and those outside of the 40–59 age parameters in cohort I (2 women). This left 57,591 men and 59,103 women eligible subjects. This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan. The study design is described in detail elsewhere (23).

**Baseline Survey.** A self-administered questionnaire was distributed mostly by hand and partly by mail to the JPHC study subjects in 1990 for cohort I and in 1993–1994 for cohort II. They were asked about their personal and familial medical histories, smoking, alcohol consumption, dietary habits, and other lifestyle factors (24–26). Among the eligible subjects, 45,452 men (79%) and 49,924 women (84%) returned the questionnaire. From them, we excluded subjects with a self-reported medical history of cancer and with a diagnosis of colorectal cancer before the survey began (687 men and 1,363 women). This additionally reduced the number of eligible subjects to 44,765 men and 48,561 women. Finally, we excluded subjects with incomplete alcohol and/or smoking items (2,225 men and 1,097 women), leaving 42,540 men and 47,464 women as study subjects.

**Assessment of Exposure.** The average frequency of alcohol consumption was reported in six categories by cohort I: "less than 1 day/month," "1–3 days/month," "1–2 days/week," "3–4 days/week," "5–6 days/week," and "everyday." Subjects consuming alcoholic beverages at least once a week were also asked about types of drinks and average consumption. Subjects in cohort II were asked about drinking status, *i.e.,* never-, ex-, or current drinkers. Ex- and current drinkers provided information on average frequency, types of drinks, and average consumption per day. The average frequency was divided into four categories: "1–3 days/month," "1–2 days/week," "3–4 days/week," and "almost everyday." We assigned a score to each category of frequency as follows: 1.5 for "1–2/week," 3.5 for "3–4/week," 6 for "5–6/week," and "everyday" in the cohort I questionnaire, and 1.5 for "1–2/week," 3.5 for "3–4/week," and 6 for "almost everyday" in the cohort II questionnaire. The amount of ethanol in each type of alcoholic beverages was calculated as follows: 180 ml sake (rice wine) as 23 g ethanol, 180 ml shochu or awamori (white spirits) as 36 g, 633 ml beer as 23 g, 30 ml whiskey or brandy as 10 g, and 60 ml wine as 6 g. Finally, weekly ethanol intake was estimated by multiplying the amount by the score.

Alcohol consumption was classified into five groups in cohort I: nondrinkers (<1 day/month), occasional drinkers (1–3 days/month), and three groups of regular drinkers (1–149 g/week ethanol, 150–299 g/week, and 300 g/week or more; Table 1). Cohort II was categorized into six groups, because nondrinkers were divided into two groups, ex- and never-drinkers. When analyzing the two cohorts together, we combined ex- and never-drinkers into nondrinkers. Three groups of regular drinkers were combined in the analyses of women (Table 2).

To evaluate the validity of alcohol consumption, we compared the estimates from the questionnaires with the 28-day dietary records (7 days in 4 seasons) provided by volunteers in each cohort. Spearman's rank correlations were 0.79 in 94 men and 0.44 in 107 women of cohort I (27), and 0.59 in 176 men and 0.40 in 178 women of cohort II. The reproducibility of the responses on alcohol intake was 0.78 in men and 0.66 in women of cohort I between 1990 and 1995 (5-year interval; Ref. 27), and 0.72 in men and 0.63 in women of cohort II between 1993 and 1997 (4-year interval). Because we also confirmed that assigning a score of 6 to "5–6/week" and "everyday" was as valid as 5.5 to "5–6/week" and 7 to "everyday" in the comparison with the dietary records in cohort I, we used the score 6 in cohort I as well as in cohort II.[3]

The questions on smoking habits included current and former smoking status, age at initiation of smoking, and average number of cigarettes smoked per day. Smoking intensity for current smokers was evaluated by pack-year defined by multiplying the years of smoking times the average number of cigarettes divided by 20 (28). We classified current smokers by the following categories of smoking intensity: <20 pack-years, 20–29 pack-years, 30–39 pack-years, and ≥40 pack-years.

A high prevalence of current smokers was found in both

[3] Unpublished observations.

*Table 1* Baseline characteristics by categories on alcohol consumption and smoking status in men

| | Alcohol consumption | | | | | | Smoking status | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ex | Never | Occasional | Regular (g/week ethanol) | | | Never | Ex | Current |
| | (Non) | | | 1–149 | 150–299 | 300+ | | | |
| Cohort I *n* (%) | 4,191 (21.1) | | 2,162 (10.9) | 4,578 (23.1) | 4,501 (22.7) | 4,426 (22.3) | 4,788 (24.1) | 4,543 (22.9) | 10,527 (53.0) |
| Age (years) Mean (SD) | 50.0 (6.0) | | 48.8 (5.9) | 49.1 (5.9) | 49.4 (5.9) | 49.1 (5.8) | 49.5 (5.6) | 50.2 (6.0) | 48.9 (6.0) |
| Family history of colorectal cancer (%) | 1.0 | | 0.9 | 0.8 | 1.3 | 0.9 | 0.9 | 1.1 | 1.0 |
| Body mass index (kg/m$^2$) Mean (SD) | 23.6 (3.0) | | 24.0 (3.2) | 23.6 (2.8) | 23.5 (2.7) | 23.6 (2.9) | 24.0 (2.9) | 24.0 (2.9) | 23.2 (2.8) |
| Current smokers (%) | 47.6 | | 46.2 | 45.9 | 57.9 | 63.9 | – | – | – |
| Regular drinkers (%) | – | | – | – | – | – | 59.0 | 69.4 | 71.6 |
| Physical exercise (% of 1/week or more) | 15.5 | | 19.3 | 21.0 | 16.8 | 16.6 | 19.6 | 21.5 | 15.3 |
| Green vegetables (%)[a] | 69.0 | | 69.9 | 71.8 | 70.9 | 67.2 | 72.7 | 71.8 | 67.5 |
| Yellow vegetables (%)[a] | 49.2 | | 48.7 | 49.4 | 44.2 | 42.3 | 51.5 | 50.0 | 42.8 |
| Fruits (%)[a] | 57.2 | | 55.7 | 58.7 | 52.8 | 47.0 | 62.4 | 57.6 | 48.8 |
| Beef (%)[a] | 12.5 | | 11.7 | 11.3 | 10.9 | 12.3 | 11.1 | 11.9 | 12.0 |
| Pork (%)[a] | 30.4 | | 25.2 | 29.9 | 33.1 | 32.3 | 30.3 | 29.4 | 31.6 |
| Chicken (%)[a] | 24.1 | | 20.2 | 21.5 | 23.0 | 24.1 | 24.3 | 22.3 | 22.4 |
| Fish (%)[a] | 44.0 | | 37.4 | 49.2 | 54.2 | 56.3 | 49.1 | 49.7 | 49.7 |
| Cohort II *n* (%) | 952 (4.2) | 4,886 (21.5) | 1,936 (8.5) | 5,263 (23.2) | 4,785 (21.1) | 4,860 (21.4) | 5,461 (24.1) | 5,532 (24.4) | 11,689 (51.5) |
| Age (years) Mean (SD) | 58.2 (8.3) | 55.5 (8.9) | 51.3 (8.3) | 52.2 (8.7) | 53.1 (8.6) | 52.3 (8.2) | 53.4 (8.3) | 55.7 (8.9) | 52.1 (8.6) |
| Family history of colorectal cancer (%) | 1.8 | 1.5 | 2.2 | 1.6 | 1.4 | 1.4 | 1.1 | 1.7 | 1.7 |
| Body mass index (kg/m$^2$) Mean (SD) | 23.1 (3.0) | 23.3 (3.6) | 24.1 (3.1) | 23.3 (2.8) | 23.5 (2.8) | 23.8 (3.1) | 24.0 (2.9) | 23.8 (2.9) | 23.2 (3.2) |
| Current smokers (%) | 44.4 | 47.6 | 47.6 | 45.8 | 56.7 | 60.5 | – | – | – |
| Regular drinkers (%) | – | – | – | – | – | – | 59.9 | 66.0 | 69.1 |
| Physical exercise (% of 1/week or more) | 20.0 | 17.4 | 18.8 | 21.7 | 19.8 | 19.0 | 22.3 | 24.0 | 16.1 |
| Green vegetables (%)[a] | 56.2 | 48.4 | 45.3 | 50.1 | 50.8 | 47.2 | 54.0 | 53.5 | 44.8 |
| Carrot (%)[a] | 41.1 | 33.3 | 31.7 | 32.5 | 30.5 | 29.0 | 37.7 | 35.1 | 27.4 |
| Apple (%)[a] | 25.0 | 22.0 | 17.8 | 19.8 | 16.2 | 11.9 | 21.3 | 23.5 | 13.6 |
| Citrus fruits (%)[a] | 42.4 | 44.5 | 38.0 | 40.1 | 34.1 | 27.2 | 42.8 | 43.3 | 31.2 |
| Beef (%)[a] | 3.9 | 5.2 | 3.5 | 4.1 | 4.1 | 4.8 | 4.5 | 3.5 | 4.8 |
| Pork (%)[a] | 16.1 | 16.0 | 14.5 | 14.0 | 16.3 | 18.2 | 16.4 | 14.6 | 16.4 |
| Chicken (%)[a] | 11.9 | 9.0 | 7.2 | 7.9 | 8.1 | 9.0 | 8.5 | 9.3 | 8.1 |
| Fish (%)[a] | 52.7 | 42.7 | 41.5 | 47.4 | 52.8 | 57.9 | 48.7 | 53.0 | 48.2 |

[a] Percentage of 3 days/week or more intake.

male and female regular drinkers (Tables 1 and 2). As the level of weekly regular consumption was higher, the percentage of current smokers was higher in males. As for potential confounding factors, we examined age at baseline, body mass index (kg/m$^2$; Ref. 29), subjects with a family history of colorectal cancer, those exercising once a week or more, and intake frequency of foods such as vegetables, fruits, meats, and fish. However, the impact of these factors showed no positive or negative trend by categories on alcohol consumption and smoking status (Tables 1 and 2). Baseline characteristics by categories on alcohol consumption have also been shown elsewhere (30).

**Follow-Up.** We followed study subjects until December 31, 1999. When subjects died, we used mortality data from the Ministry of Health, Labor, and Welfare. Subjects moving to other municipalities were also annually identified through residential registers in PHC areas. Among study subjects, 5.0% moved away and 0.04% were lost to follow-up during the study period.

**Identification of Colorectal Cancer Incidence.** After January 1, 1990 in cohort I and January 1, 1993–1994 in cohort II, incidence data on colorectal cancer were collected for the JPHC cancer registry through two data sources, local major hospitals and population-based cancer registries. Death certificates were used to supplement the information on cancer incidence.

Cases of colorectal cancer were extracted from the JPHC cancer registry based on site codes [International Classification of Diseases for Oncology, second edition (ICD-O-2) code: C180–189 (colon) and C199, 209 (rectum); Ref. 31]. Up to December 31, 1999, 772 incident cases of colorectal cancer were identified. For multiple primary cancers in colon or rectum at different times, the earliest diagnosis was applied. For those occurring simultaneously, the most advanced and most invasive diagnosis was applied. Among these incident cases, 716 were pathologically confirmed as adenocarcinoma (M: 8140, 8210, 8211, 8240, 8243, 8260, 8261, 8262, and 8263 for ICD-O-2). Such cases were additionally classified into two groups according to the depth of tumor invasion, *i.e.*, invasive cancer over a mucosal layer corresponding to code 3 (malignant, primary site) in "behavior code for neoplasms" (298 colon cases and 206 rectal cases), and noninvasive cancer within a mucosal layer corresponding to code 2 (carcinoma *in situ*; 165 colon and 38 rectum) in ICD-O-2 (the depth in 5 colon and 4 rectal tumors were unknown).

In our cancer registry system, the proportion of cases for which information was available only from death certificates was 1.0% for colorectal cancer and 3.1% for all of the cancers during the study period. These figures were considered of satisfactory quality for the present study based on the international standard (1).

*Table 2* Baseline characteristics by categories on alcohol consumption and smoking status in women

| | Alcohol consumption | | | | Smoking status | | |
|---|---|---|---|---|---|---|---|
| | Ex | Never (Non) | Occasional | Regular | Never | Ex | Current |
| Cohort I *n* (%) | 16,668 (77.5) | | 2,567 (11.9) | 2,281 (10.6) | 19,934 (92.6) | 369 (1.7) | 1,213 (5.6) |
| Age (years) Mean (SD) | 49.9 (5.8) | | 48.3 (5.8) | 48.2 (5.8) | 49.5 (5.8) | 49.2 (6.4) | 48.5 (5.9) |
| Family history of colorectal cancer (%) | 0.8 | | 1.5 | 1.1 | 0.9 | 0.6 | 0.8 |
| Body mass index (kg/m$^2$) Mean (SD) | 23.7 (3.3) | | 23.5 (2.9) | 23.2 (2.9) | 23.6 (3.2) | 24.2 (3.4) | 23.4 (3.8) |
| Current smokers (%) | 4.1 | | 6.6 | 16.2 | – | – | – |
| Regular drinkers (%) | – | | – | – | 9.2 | 24.4 | 30.3 |
| Physical exercise (% 1/week or more) | 13.4 | | 17.6 | 17.4 | 14.3 | 17.2 | 13.7 |
| Green vegetables (%)[a] | 78.4 | | 75.3 | 78.3 | 78.5 | 71.3 | 72.4 |
| Yellow vegetables (%)[a] | 65.5 | | 63.6 | 61.5 | 65.5 | 61.0 | 55.0 |
| Fruits (%)[a] | 73.7 | | 79.1 | 73.0 | 75.4 | 67.0 | 59.2 |
| Beef (%)[a] | 10.0 | | 9.1 | 11.2 | 9.7 | 13.2 | 14.0 |
| Pork (%)[a] | 32.4 | | 35.6 | 34.6 | 33.1 | 32.7 | 31.4 |
| Chicken (%)[a] | 29.9 | | 29.3 | 28.1 | 30.0 | 27.5 | 25.2 |
| Fish (%)[a] | 53.8 | | 55.2 | 59.9 | 55.2 | 47.9 | 48.4 |
| Cohort II *n* (%) | 223 (0.9) | 21,112 (81.4) | 2,112 (8.1) | 2,501 (9.6) | 24,133 (93.0) | 278 (1.1) | 1,537 (5.9) |
| Age (years) Mean (SD) | 53.7 (8.4) | 54.9 (8.7) | 49.1 (7.5) | 50.1 (8.0) | 54.0 (8.8) | 53.9 (9.6) | 51.4 (8.6) |
| Family history of colorectal cancer (%) | – | 1.3 | 1.4 | 1.6 | 1.4 | 1.3 | 0.8 |
| Body mass index (kg/m$^2$) Mean (SD) | 23.6 (3.8) | 23.6 (3.3) | 23.5 (3.1) | 23.1 (3.2) | 23.5 (3.2) | 23.8 (3.4) | 23.2 (3.8) |
| Current smokers (%) | 28.1 | 3.9 | 9.0 | 18.6 | – | – | – |
| Regular drinkers (%) | – | – | – | – | 8.5 | 26.7 | 31.2 |
| Physical exercise (% 1/week or more) | 20.2 | 18.5 | 19.6 | 21.5 | 19.1 | 17.9 | 16.0 |
| Green vegetables (%)[a] | 61.6 | 61.2 | 56.4 | 60.8 | 61.5 | 59.6 | 49.9 |
| Carrot (%)[a] | 52.2 | 53.0 | 49.2 | 45.9 | 52.9 | 45.8 | 38.4 |
| Apple (%)[a] | 29.6 | 33.6 | 31.2 | 30.7 | 33.9 | 26.3 | 21.7 |
| Citrus fruits (%)[a] | 55.2 | 60.9 | 60.9 | 57.6 | 61.6 | 51.3 | 44.8 |
| Beef (%)[a] | 6.9 | 4.2 | 5.1 | 5.3 | 4.4 | 5.0 | 5.4 |
| Pork (%)[a] | 15.8 | 18.8 | 18.8 | 17.3 | 18.8 | 13.8 | 16.2 |
| Chicken (%)[a] | 8.4 | 10.2 | 9.6 | 9.4 | 10.2 | 11.3 | 8.5 |
| Fish (%)[a] | 52.7 | 49.1 | 45.9 | 52.9 | 49.7 | 51.7 | 42.5 |

[a] Percentage of 3 days/week or more intake.

**Statistical Analysis.** Person-years of follow-up were determined from January 1, 1990 (cohort I) or 1993–1994 (cohort II) until the date of diagnosis of colorectal cancer, the date of a subject's death, the date of moving from a PHC area, or December 31, 1999, whichever occurred first. Incidence rates of colorectal cancer were calculated using person-years as the denominators and standardized with a 5-year age distribution at baseline in each cohort (40–44, 45–49, 50–54, and 55–59 in cohort I, and 40–44, 45–49, 50–54, 55–59, 60–64, and 65–69 in cohort II; Ref. 32).

Relative risks (RRs) and 95% confidence intervals (CIs) for alcohol consumption and smoking were estimated by the Cox proportional hazards model, according to the SAS PHREG procedure (33). The estimates were adjusted for the following potential confounding factors incorporated into the model: age (5-year groups), family history of colorectal cancer (anyone or none), body mass index (quartiles in each cohort), physical exercise (less than once a week and once a week or more), smoking status (when calculating RR for alcohol consumption; never-, ex-, and current smokers), alcohol consumption (when calculating RR for smoking status and intensity; nondrinkers, occasional drinkers, 1–149 g/week, 150–299 g/week, and ≥300 g/week), and PHC area. The factors relating to dietary habits, which were slightly different between both cohorts, were not considered as confounding factors, because they hardly affected the RR of alcohol consumption and smoking status. The linear trend of alcohol consumption or smoking intensity was assessed by assignment of ordinal values to categories among drinkers or current smokers, respectively. *P*s for those trends were evaluated using the two-sided test with 0.05 as the significance level.

First, we estimated the RR of all cases of colorectal cancer in each cohort, because slightly different questionnaires were used. Second, in addition to all of the cases, we combined two cohorts and calculated the RRs and the linear trends of invasive colorectal, colon, and rectal cancer to obtain more power to detect the association after confirming the same risk trend in the two cohorts. When we estimated the RR of the invasive, we defined the noninvasive as a censored case. Similarly, we considered rectal cancer as a censored case in colon cancer end point and colon cancer as a censored case in rectal cancer end point.

We also calculated the RRs of colorectal cancer for combined categories of alcohol consumption and smoking status, and tested statistical interactions, using the differences between two likelihood ratios of the models with and without the interaction terms between alcohol consumption and smoking status (34).

*Table 3* Age-standardized incidence rate, multivariate-adjusted relative risk (RR), and 95% confidence interval (CI) of colorectal cancer by categories on alcohol consumption in Japan Public Health Center-based Prospective Study Cohort I men (1990–1999) and Cohort II men (1993–1999)

| | Ex-drinkers | Never-drinkers (Nondrinkers) | Occasional drinkers | Regular drinkers (g/week ethanol) | | | *P* for trend among drinkers |
|---|---|---|---|---|---|---|---|
| | | | | 1–149 | 150–299 | 300+ | |
| Cohort I (aged 40–59) | | | | | | | |
| No. of cases ($n$ = 244) | 42 | | 15 | 39 | 58 | 90 | |
| Person-years | 39,165 | | 20,305 | 42,812 | 42,470 | 41,134 | |
| Incidence rates[a] | 104.7 | | 78.7 | 91.8 | 135.2 | 226.4 | |
| RR[b] (95% CI) ($n$ = 240) | 1.0 (reference) | | 0.8 (0.4–1.4) | 0.9 (0.6–1.4) | 1.2 (0.8–1.8) | 2.0 (1.4–3.0) | <0.001 |
| Cohort II (aged 40–69) | | | | | | | |
| No. of cases ($n$ = 213) | 8 | 40 | 10 | 46 | 50 | 59 | |
| Person-years | 5,817 | 30,939 | 12,483 | 33,277 | 30,500 | 31,196 | |
| Incidence rates[a] | 99.0 | 109.8 | 92.2 | 149.6 | 166.0 | 207.7 | |
| RR[b] (95% CI) ($n$ = 207) | 0.9 (0.4–2.0) | 1.0 (reference) | 0.9 (0.4–1.9) | 1.5 (0.9–2.3) | 1.6 (1.1–2.5) | 2.0 (1.3–3.0) | 0.024 |

[a] Incidence rate (per 100,000 person-years) standardized by distribution of 5-year age groups at baseline in each cohort.
[b] Adjusted for age (5-year groups), family history of colorectal cancer, body mass index (quartiles in each cohort), smoking status (never-, ex-, and current smokers), physical exercise (less than once a week and once a week or more), and 4 Public Health Center (PHC) areas in Cohort I or 5 PHC areas in Cohort II.

The PAF was estimated by $P_e(RR_a - 1)/RR_a$, where $P_e$ was the prevalence of exposure among incident cases and $RR_a$ was the adjusted RR. The 95% CI of the PAFs were estimated by the formula of Greenland (35). We estimated the PAFs of drinkers to nondrinkers, current and ex-smokers to never-smokers, and drinkers currently and formerly smoked to nondrinkers never smoked.

In women, RRs were estimated only in both cohorts combined, because of the few cases and noncases in drinkers and/or smokers, and the insufficient statistical power by each cohort.

## Results

Age-standardized incidence rates increased among drinkers in both cohorts (Table 3). Drinkers consuming ≥300 g/week had a higher risk of colorectal cancer compared with nondrinkers in both cohort I (RR, 2.0; 95% CI, 1.4–3.0) and cohort II (RR, 2.0; 95% CI, 1.3–3.0). We observed linear positive trends of RR according to the level of alcohol consumption ($P < 0.001$ in cohort I and $P = 0.024$ in cohort II). The risk of ex-drinkers did not substantially differ from those of nondrinkers in cohort II.

The RR of invasive cancer for alcohol consumption showed the same trend as all of the cases of colorectal cancer (RR, 2.1 in all cases and 1.7 in invasive cancer for those consuming ≥300 g/week to nondrinkers; Table 4). The association with alcohol consumption was also shown in both colon and rectal cancer, as well as in all of the cases. Statistical significance in linear trends was consistent among all endpoints. The PAF% for alcohol consumption at least occasional drinking to nondrinking was 24% (95% CI, 8–38%) in all colorectal cancer (7% to 150–299 g/week and 17% to ≥300 g/week).

Meanwhile, the RRs of current smokers for colorectal cancer were 1.5 (95% CI, 0.9–2.1) in cohort I, 1.2 (95% CI, 0.8–1.8) in cohort II, and 1.4 (95% CI, 1.1–1.8) in two cohorts combined, compared with never-smokers (Table 5 shows only the combined results). The association did not change after exclusion of noninvasive cases and did not depend on the subsite. The nonsignificant linear trend was obtained according to smoking intensity except for rectal cancer. Furthermore, long-term smoking significantly elevated the risk compared with never-smoking: RR, 1.3 (95% CI, 0.7–2.2) for ≤25 years, 1.4 (0.9–2.2) for 25–29 years, 1.4 (0.99–2.1) for 30–34 years, and 1.5 (1.1–2.0) for ≥35 years. Smoking intensity in the remote past (before age 30 years) showed no dose-response relationship (data not shown). The PAF% for currently and formerly smoking to never-smoking was 22% (95% CI, 9–36%).

Next, we assessed the joint effect of alcohol consumption and smoking status in men (Table 6). Colorectal cancer risk for drinkers of ≥300 g/week of ethanol who smoked was estimated at 3.0 (95% CI, 1.8–5.1), compared with nondrinkers who never smoked. The association did not differ between colon and

*Table 4* Relative risk (RR) and 95% confidence interval (CI) of colorectal cancer by the depth of tumor invasion and the site in Japan Public Health Center-based Prospective Study Cohort I men (1990–1999) and Cohort II men (1993–1999) combined

| | Nondrinkers | Occasional drinkers | Regular drinkers (g/week ethanol) | | | *P* for trend among drinkers |
|---|---|---|---|---|---|---|
| | | | 1–149 | 150–299 | 300+ | |
| Person-years | 74,123 | 32,273 | 75,001 | 71,933 | 71,194 | |
| Colorectal cancer[a] ($n$ = 447) | 87 | 24 | 83 | 107 | 146 | |
| RR[b] (95% CI) | 1.0 (reference) | 0.8 (0.5–1.3) | 1.1 (0.8–1.5) | 1.4 (1.1–1.9) | 2.1 (1.6–2.7) | <0.001 |
| Invasive colorectal cancer ($n$ = 298) | 65 | 18 | 53 | 72 | 90 | |
| RR[b] (95% CI) | 1.0 (reference) | 0.9 (0.5–1.5) | 1.0 (0.7–1.5) | 1.3 (0.9–1.9) | 1.7 (1.2–2.4) | <0.001 |
| Colon cancer[a] ($n$ = 299) | 62 | 16 | 51 | 71 | 99 | |
| RR[b] (95% CI) | 1.0 (reference) | 0.8 (0.4–1.3) | 1.0 (0.7–1.4) | 1.3 (0.9–1.8) | 1.9 (1.4–2.7) | <0.001 |
| Rectal cancer[a] ($n$ = 148) | 25 | 8 | 32 | 36 | 47 | |
| RR[b] (95% CI) | 1.0 (reference) | 1.0 (0.5–2.3) | 1.6 (0.9–2.6) | 1.7 (1.01–2.8) | 2.4 (1.5–4.0) | 0.015 |

[a] Including noninvasive and invasive cancers.
[b] Adjusted for age (5-year groups), family history of colorectal cancer, body mass index (quartiles in each cohort), smoking status (never-, ex-, and current smokers), physical exercise (less than once a week and once a week or more), and 9 Public Health Center areas.

*Table 5* Relative risk (RR) and 95% confidence interval (CI) for smoking status and intensity in Japan Public Health Center-based Prospective Study Cohort I men (1990–1999) and Cohort II men (1993–1999) combined

| | Never-smokers | Ex-smokers | Current smokers | (Pack-years) | | | | *P* for trend among current smokers |
|---|---|---|---|---|---|---|---|---|
| | | | All | <20 | 20–29 | 30–39 | 40+ | |
| Person-years | 78,706 | 76,424 | 169,394 | 32,566 | 45,323 | 41,855 | 46,080 | |
| Colorectal cancer[a] ($n$ = 447) | 78 | 124 | 245 | 33 | 50 | 73 | 83 | |
| RR[b] (95% CI) | 1.0 (reference) | 1.3 (0.98–1.7) | 1.4 (1.1–1.8) | 1.1 (0.8–1.7) | 1.3 (0.9–1.9) | 1.4 (1.05–2.0) | 1.4 (0.99–1.8) | 0.47 |
| Invasive colorectal cancer ($n$ = 298) | 50 | 85 | 163 | 23 | 32 | 43 | 60 | |
| RR[b] (95% CI) | 1.0 (reference) | 1.5 (1.02–2.1) | 1.6 (1.1–2.1) | 1.3 (0.8–2.2) | 1.4 (0.9–2.2) | 1.4 (0.9–2.1) | 1.6 (1.1–2.3) | 0.64 |
| Colon cancer[a] ($n$ = 299) | 53 | 86 | 160 | 17 | 31 | 55 | 54 | |
| RR[b] (95% CI) | 1.0 (reference) | 1.4 (0.96–1.9) | 1.4 (0.99–1.9) | 0.9 (0.5–1.5) | 1.2 (0.8–2.0) | 1.7 (1.1–2.4) | 1.3 (0.9–2.0) | 0.16 |
| Rectal cancer[a] ($n$ = 148) | 25 | 38 | 85 | 16 | 19 | 18 | 29 | |
| RR[b] (95% CI) | 1.0 (reference) | 1.2 (0.7–2.0) | 1.4 (0.9–2.3) | 1.6 (0.9–3.0) | 1.5 (0.8–2.7) | 1.0 (0.6–1.9) | 1.4 (0.8–2.3) | 0.48 |

[a] Including noninvasive and invasive cancers.
[b] Adjusted for age (5-year groups), family history of colorectal cancer, body mass index (quartiles in each cohort), alcohol consumption (nondrinkers, occasional, 1–149 g, 150–299 g, and 300 g+), physical exercise (less than once a week and once a week or more), and 9 Public Health Center areas.

rectum. We detected no interaction between alcohol consumption and smoking status ($P$ for interaction = 0.88 in colorectum, 0.75 in colon, and 0.44 in rectum). The PAF% for alcohol consumption and/or currently or formerly smoking was estimated at 46% (95% CI, 14–66%), compared with nondrinking and never-smoking.

Female regular drinkers had no elevated risk of colorectal cancer compared with nondrinkers (RR, 0.7; 95% CI, 0.4–1.1; Table 7). Occasional drinkers to nondrinkers were inversely associated with colorectal cancer (RR, 0.5; 95% CI, 0.3–0.9). Female ex- and current smokers had a nonsignificant increased risk of colorectal cancer, as well as male ex- and current smokers (RR, 1.3; 95% CI, 0.5–3.6 for ex-smokers; RR, 1.4; 95% CI, 0.8–2.4 for current smokers).

## Discussion

Our results confirmed that alcohol consumption was positively associated with colorectal cancer in a Japanese population of middle-aged and elderly men. A clear linear trend of RR was observed among drinkers. However, the RR for 24 g/day increment of alcohol consumption did not substantially differ from the result of a previous meta-analysis (36), which estimated the pooled RR at 1.32 (against 1.11, 95% CI, 1.06–1.17 in the present study; data not shown in tables).

Female regular drinkers showed no increased risk of colorectal cancer. Eighty percent of them were categorized into the lowest group (1–149 g/week ethanol). In men, the lowest group of regular drinkers showed no significant risk of colorectal

*Table 6* Relative risk (RR) and 95% confidence interval (CI) for alcohol consumption and smoking status in Japan Public Health Center-based Prospective Study Cohort I men (1990–1999) and Cohort II men (1993–1999) combined

| | Nondrinkers | Occasional drinkers | Regular drinkers (g/week ethanol) | | | *P* for interaction |
|---|---|---|---|---|---|---|
| | | | 1–149 | 150–299 | 300+ | |
| Colorectal cancer[a] ($n$ = 447) | | | | | | |
| Never-smokers ($n$ = 78) | 17 | 8 | 20 | 15 | 18 | 0.88 |
| RR[b] (95% CI) | 1.0 (reference) | 1.2 (0.5–2.8) | 1.3 (0.7–2.5) | 1.4 (0.7–2.9) | 2.2 (1.1–4.3) | |
| Ex-smokers ($n$ = 124) | 30 | 6 | 20 | 28 | 40 | |
| RR[b] (95% CI) | 1.6 (0.9–3.0) | 1.2 (0.5–3.1) | 1.3 (0.7–2.5) | 1.9 (1.04–3.5) | 3.2 (1.8–5.7) | |
| Current smokers ($n$ = 245) | 40 | 10 | 43 | 64 | 88 | |
| RR[b] (95% CI) | 1.5 (0.8–2.6) | 1.1 (0.5–2.4) | 1.9 (1.1–3.4) | 2.2 (1.3–3.8) | 3.0 (1.8–5.1) | |
| Colon cancer[a] ($n$ = 299) | | | | | | |
| Never-smokers ($n$ = 53) | 11 | 5 | 12 | 13 | 12 | 0.75 |
| RR[b] (95% CI) | 1.0 (reference) | 1.1 (0.4–3.3) | 1.2 (0.5–2.7) | 1.9 (0.8–4.2) | 2.2 (0.97–5.0) | |
| Ex-smokers ($n$ = 86) | 21 | 3 | 11 | 21 | 30 | |
| RR[b] (95% CI) | 1.8 (0.9–3.7) | 0.9 (0.3–3.3) | 1.1 (0.5–2.6) | 2.2 (1.04–4.5) | 3.6 (1.8–7.3) | |
| Current smokers ($n$ = 160) | 30 | 8 | 28 | 37 | 57 | |
| RR[b] (95% CI) | 1.7 (0.9–3.4) | 1.3 (0.5–3.3) | 1.9 (0.96–3.9) | 2.0 (1.01–3.9) | 3.0 (1.6–5.7) | |
| Rectal cancer[a] ($n$ = 148) | | | | | | |
| Never-smokers ($n$ = 25) | 6 | 3 | 8 | 2 | 6 | 0.44 |
| RR[b] (95% CI) | 1.0 (reference) | 1.3 (0.3–5.4) | 1.6 (0.5–4.6) | 0.6 (0.1–2.9) | 2.3 (0.7–7.1) | |
| Ex-smokers ($n$ = 38) | 9 | 3 | 9 | 7 | 10 | |
| RR[b] (95% CI) | 1.4 (0.5–3.8) | 1.8 (0.4–7.2) | 1.7 (0.5–4.7) | 1.4 (0.5–4.2) | 2.4 (0.9–6.6) | |
| Current smokers ($n$ = 85) | 10 | 2 | 15 | 27 | 31 | |
| RR[b] (95% CI) | 1.0 (0.4–2.8) | 0.6 (0.1–3.1) | 1.9 (0.7–4.8) | 2.7 (1.1–6.5) | 3.1 (1.3–7.5) | |

[a] Including noninvasive and invasive cancers.
[b] Adjusted for age (5-year groups), family history of colorectal cancer, body mass index (quartiles in each cohort), physical exercise (less than once a week and once a week or more), and 9 Public Health Center areas.

*Table 7* Age-standardized incidence rate, multivariate-adjusted relative risk (RR) and 95% confidence interval (CI) of colorectal cancer by categories on alcohol consumption and smoking status in Cohort I women (1990–1999) and Cohort II (1993–1999) women combined

| | Alcohol consumption | | | Smoking status | | |
|---|---|---|---|---|---|---|
| | Nondrinkers | Occasional drinkers | Regular drinkers | Never-smokers | Ex-smokers | Current smokers |
| No. of cases ($n$ = 259) | 230 | 12 | 17 | 239 | 4 | 16 |
| Person-years | 300,634 | 38,181 | 37,706 | 350,470 | 5,209 | 20,841 |
| Incidence rate[a] | 76.3 | 40.2 | 52.9 | 69.9 | 78.4 | 91.0 |
| RR[b] (95% CI) ($n$ = 253) | 1.0 (reference) | 0.5 (0.3–0.9) | 0.7 (0.4–1.1) | 1.0 (reference) | 1.3 (0.5–3.6) | 1.4 (0.8–2.4) |

[a] Incidence rate (per 100,000 person-years) standardized by distribution of 5-year age group at baseline in both cohorts.
[b] Adjusted for age (5-year groups), family history of colorectal cancer, body mass index (quartiles in each cohort), smoking status (when calculating RR for alcohol consumption; never-, ex-, and current smokers), alcohol consumption (when calculating RR for smoking status; non-, occasional, regular drinkers), physical exercise (less than once a week and once a week or more), and 9 Public Health Center areas.

cancer. Thus, female regular drinkers may not be associated with colorectal cancer because of the small proportion of heavy drinkers.

On the basis of our estimate, 24% of colorectal cancer was attributable to alcohol consumption in men. Because relatively heavy drinkers (≥150 g/week = ≥1.5 drinks/day) contribute to a large part of the PAF, a reduction in the number of such drinkers may lead to a decrease in colorectal cancer. To our knowledge, no reported prospective studies estimated the PAF of alcohol consumption in colorectal cancer. One case-control study evaluated the PAF as 19% (37).

One reason for the high PAF may be the high prevalence of heavy drinkers. Men in the highest categories who weekly consumed ≥300 g/week of ethanol (≥3 drinks/day) accounted for 22% in our study. In the case-control study estimating the PAF (37), drinkers consuming only ≥0.7 drinks/day accounted for 33% of male controls. Moreover, based on calculation of the published numbers, in other cohort studies, the highest consumers either took at most "2 drinks/day or more" or accounted for a smaller percentage: 14% of subjects consumed ≥2.5 drinks/day in a Netherlands study (5); men accounted for 14% of person-years consuming ≥2 drinks/day in a Health Professionals Follow-up Study (6); 21% of subjects consumed ≥2 drinks/day in a Hawaiian-Japanese study (8); and only 8.7% of subjects consumed ≥3 drinks/day in a United States study (38).

Another reason may be the different distribution of the genetic polymorphisms on alcohol-related enzymes including *ALDH2* in Japanese, although we have not investigated the genetic polymorphisms in our subjects. The *ALDH2* genotypes with the atypical allele (Glu487Lys) [frequency: 0.28 in Japanese (13) *versus* <0.03 in Caucasian (39)] exert little ALDH2 activity (40) and cause a high acetaldehyde levels in blood (15). Although acetaldehyde has not been concluded to be a human colorectal carcinogen, some *in vitro* and animal studies suggest that acetaldehyde triggers carcinogenesis in the colorectum (41–43) via folate deficiency (44, 45). However, because the magnitude of RR in our study population was not higher than that of a pooled one as mentioned above (36), the effect of such genetic susceptibility may be limited.

Current and ex-smokers had an increased risk of colorectal cancer in men and women. The risk showed a nonsignificant linear trend according to smoking intensity in men. We also confirmed that the long-term smoking elevated the risk in men. Smoking intensity before age 30 years, however, failed to show the dose-response relationship seen in a previous study (19), possibly due to estimating the remote past pack-years using current numbers of cigarettes smoked per day.

Recent prospective studies consistently reported a positive association of smoking adjusted for potential confounding factors (17, 18), especially when accounting for the long induction period (19, 20). In addition, tobacco smoke includes various carcinogens such as polynuclear aromatic hydrocarbons and *N*-nitrosamines. These carcinogens in tobacco smoke are reasonable risks for colorectal carcinogenesis (46, 47). As mentioned in a recent review regarding the causality (16), evidence has been sufficiently accumulated to add colorectal cancer to the list of tobacco-associated malignancies. In the present study of a Japanese population, we could attribute 22% of colorectal cancer to currently and formerly smoking. In the Health Professionals Follow-up Study, smoking was responsible for 21% of the incidence of this cancer (19). The Cancer Prevention Study II reported that 12% of colorectal cancer deaths were attributable to smoking (21). Therefore, we can expect to reduce a large part of colorectal cancer by eliminating tobacco consumption, especially in the population with the high prevalence of smoking.

Many previous studies have defined invasive adenocarcinoma as "colorectal cancer." However, in our opinion, "colorectal cancer" should be defined as not only invasive adenocarcinoma but also noninvasive adenocarcinoma. Thus, we needed to confirm that our definition is comparable with the Western definition. As a result, the RR of all cases (including the noninvasive type) approximately corresponded to those of only the invasive type. However, 2 case-control studies showed that pack-years as smoking intensity was associated significantly with the noninvasive type rather than the invasive type (48, 49). Additional studies will be needed to determine whether or not the association of some risk factors differs in terms of these two definitions.

The major strengths of our study include its prospective design, a general population with a high response rate (80%), and the relatively low proportion of subjects lost to follow-up (0.04%). Information on alcohol consumption and smoking was collected before any subsequent diagnosis of colorectal cancer, thus avoiding the exposure recall bias inherent in case-control studies. The findings of this study can be generalized to middle-aged and elderly Japanese men, because the study subjects were selected from the general population, and there was a high response rate. Moreover, two cohorts starting at different times produced the same results.

The adjustment for the frequencies of food intake did not change the RR estimates of alcohol consumption and smoking status (data not shown). In addition, recent prospective studies have reported the weak association of fruits and vegetables (50), and meats (51). Thus, no food variables were used in the final multivariate model. However, we could not examine whether or not some nutrients, such as folate and methionine (6), affected the association of alcohol consumption and smoking status because of the inavailability of these nutrients.

In conclusion, alcohol consumption dose-dependently in-

creased the risk of colorectal cancer in men. Smoking was also associated significantly with colorectal cancer in men and not significantly in women. From the risk estimates, 46% of colorectal cancer is attributable to alcohol consumption and smoking in middle-aged and elderly Japanese men.

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# A population-based dietary intervention trial in a high-risk area for stomach cancer and stroke: changes in intakes and related biomarkers

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**Abstract**

*Background.* Dietary intervention is one of the important fields in cancer and cardiovascular disease prevention. The Hiraka Dietary Intervention Study is a community-based randomized cross-over trial designed to develop an effective dietary modification tool and system in an area with high mortality of stomach cancer and stroke.

*Methods.* The subjects were 550 healthy volunteers and were randomized into two groups with tailored dietary education to decrease sodium intake and to increase vitamin C and carotene intakes either in the first year (intervention group) or in the second year (control group). Dietary changes were assessed using a validated self-administered diet history questionnaire, fasting blood samples, and 48-hour urine samples, which were obtained before and after the one year period.

*Results.* During the first year, changes differed significantly between the intervention and control group for both dietary sodium intake (−384 and +255 mg/day, intervention and control respectively, $p < 0.001$) and urinary sodium excretion (−1003 and −84 mg/day, $p < 0.001$). Although favorable net changes were also observed in dietary carotene (+418 and +220 μg/day, $p < 0.05$) and vitamin C (+13 and +2 mg/day, $p < 0.05$), the serum level differences were modest (+13 and −25 mg/L, $p = 0.09$ for carotene, +0.1 and −0.5 mg/L, $p = 0.07$ for ascorbic acid).

*Conclusion.* The present dietary intervention strategy effectively decreased sodium and increased carotene and vitamin C intakes, although the former was more distinct.



*Keywords:* Intervention studies; Randomized controlled trials; Sodium; Carotenoid; Ascorbic acid; Dietary; Biological marker

## Introduction

Although stomach cancer and stroke have been decreasing, they are still major causes of death in Japanese populations [1–3]. Primary prevention through lifestyle modification has been regarded as an important strategy for reducing these diseases at the population level in Japan. These two diseases share common etiologic dietary factors. High sodium/salted food intake is an established risk factor of both stomach cancer and cardiovascular disease, especially stroke of which the most important risk factor is high blood pressure [4–6]. Reducing salt intake may be also beneficial for blood pressure [7]. Average salt intake in Japan still exceeds the upper limit of the governmental recommendation, 10 g/day [8]. High vitamin C and carotene intakes are possible preventive factors for these two diseases [4,5,9,10]. Therefore, an intervention method designed to modify these nutrient intakes is urgently needed in Japan.

Many intervention methods have been developed and evaluated in western countries [11–23]. Several dietary intervention studies have targeted individuals at high risk of disease, such as survivors of cancer at several sites [18,20]

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and ischemic heart disease [19]. In general, these interventions, which are based on intensive individual or group counseling, can yield substantial changes in dietary behavior. However, such intensive interventions are not practical for broad-scale dissemination due to their high cost and low participation rates.

Recently, several dietary intervention approaches, as part of a public health program, have been designed both to be accessible to a broad population of healthy individuals and to encourage small dietary changes in everyone, regardless of disease risk [13]. Moderate-intensity dietary intervention programs, involving only one or two individualized tailored dietary counseling sessions, and a follow-up mailing or phone call, have shown encouraging results [14,17]. Several studies provided subjects either individually tailored dietary advice or generic advice. The individualized advice resulted in significantly greater effects compared to the generic advice [21–23]. The use of computers allows individualized materials available for larger-scale groups in a cost-effective intervention method.

We therefore developed a moderate-intensity dietary intervention method aimed at reducing salt and increasing carotene and vitamin C intakes for Japanese populations in whom the target diseases and dietary habits were markedly different from those in western populations, and examined their effectiveness in the Hiraka dietary intervention study, a community-based randomized cross-over trial. This article describes the design of the study, use of materials, and main intervention effects.

## Methods

The study was conducted in 1998–2000 in two rural villages in Akita Prefecture which high incidences of stomach cancer and stroke [24] and in which nutrient intakes were undesirable from the preventive viewpoint, i.e., high salt and low carotene [25].

We used a community-based two-group randomized cross-over design of tailored-individual dietary intervention vs control with assessment only.

### *Study population and randomization*

Participants were recruited through public magazines and posters, in which potential respondents were asked to participate in a research project. Eligibility criteria for this study were: (1) 40–69 years of age, and (2) permission from the individual's doctor to participate if he/she was under medical treatment/dietary control. Five hundred and fifty volunteers (202 men and 348 women) were included. Signed informed consent was obtained from all participants before randomization. Participants were randomly assigned to two groups with dietary intervention in 1998 (phase 1) or in 1999 (phase 2). Subjects within one family were assigned to the same group. Two hundred and seventy-four and 276 subjects were randomized into the intervention and control groups in phase 1, respectively.

### *Outcome measures*

The principal outcome measures were changes in sodium, vitamin C, and carotene intakes, serum concentrations of vitamin C and carotene, and urinary sodium excretion.

### *Dietary assessment*

A validated self-administered diet history questionnaire (DHQ) was completed four times; just before the annual health check-up (April to August, in 1998, 1999, and 2000) for all subjects, and the mid-term point of each intervention period only for the intervention group. The DHQ surveyed dietary habits for the previous one-month. The questionnaire contained the following seven sections: (1) dietary behaviors, (2) semi-quantitative frequency and quantity for 127 selected food items, (3) main staples such as rice, bread, and noodles, (4) major cooking methods for vegetables, fish, and meats, (5) alcohol beverage, (6) nutrient supplements, and (7) open-ended questions. For the computations of nutrient and food intakes, an ad-hoc computer program was used after minor modification for some local foods. We also incorporated the food composition of alpha- and beta-carotenes into the food compositions of the calculation algorithm [26]. The DHQ has been validated using three different gold standards as follows. Firstly, we compared nutrient intakes assessed by DHQ with those assessed by 3-day dietary record among middle-aged women ($n = 47$). The Pearson correlation coefficients were 0.32, 0.38, and 0.45 for sodium, vitamin A, and vitamin C, respectively [27]. Secondly, we compared sodium intake assessed by DHQ with those 24-hour urinary excretions among university students. The Pearson correlation coefficient were 0.14 and 0.23 for men ($n = 154$) and women ($n = 69$), respectively [28]. Thirdly, we compared carotene intake assessed by DHQ with those of serum concentrations, which often been used as reliable biomarkers, among middle-aged men ($n = 42$) and women ($n = 42$). A significantly positive correlation was observed ($r = 0.44$ and 0.56 in men and women, respectively, $p < 0.001$ for both) [29]. The output of this computer program consisted individualized feedback sheets. Carotene and vitamin C intakes from fortified foods and vitamin supplements were not included in the analysis due to inadequate information on the composition and bioavailability. The questionnaires were checked by trained dietitians, and missing or illogical answers were obtained or corrected by telephone interview.

### *Biological measures; blood samplings and measurement*

Fasting blood samples (5 mL) were collected at annual health check-ups. Serum was immediately prepared by centrifugation, then transferred into stock tubes and stored at −80C°. All blood samples were analyzed after completion

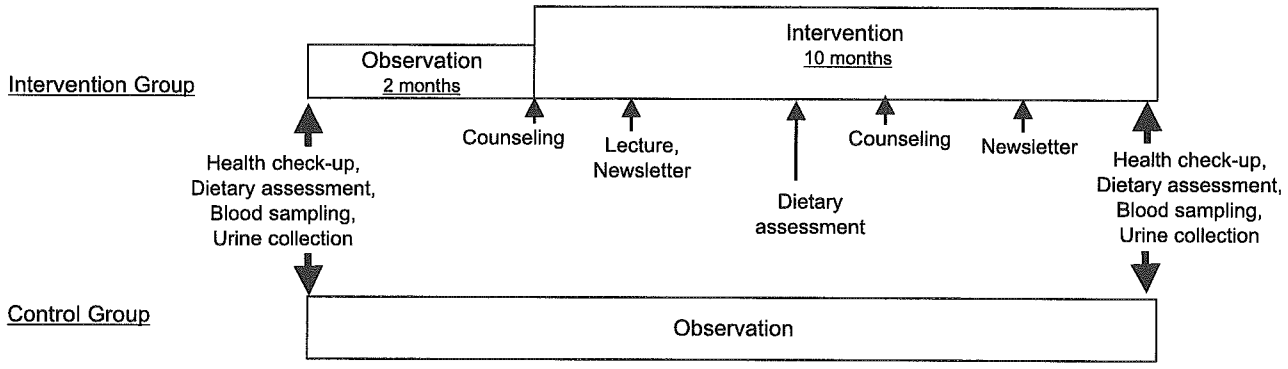


Fig. 1. Design of the first half of the 2 year cross-over study.

of the trial, at the same time. Serum for ascorbic acid measurement were stabilized by addition of meta-phosphoric acid (Wako Pure Chemical, Osaka, Japan). Serum carotenoids were analyzed by high-performance liquid chromatography (HPLC) using a modified method [30]. Serum ascorbic acid was measured by spectrophotometer using modified method originally described previously [31]. Persons blinded to the information on the intervention conducted the assays.

*Urine samplings and measurements*

Forty-eight hour urine samples were collected just after the annual health check-up. Subjects were requested to collect their urine into IL plastic containers. Urinary volume was measured, and a part of each samples was stored at −20C° until measurement. Whenever some urine had not been collected, the estimated volume was reported by the subjects and added to that of the collected urine to estimate the total urinary volume. The urinary concentrations of sodium and potassium were analyzed by flame photometry and creatinine by Jaffe's procedure using an autoanalyzer (Hitachi Clinical Analyzer 7070). The expected intakes were computed using observed urinary excretions and the urinary excretion/intake ratio reported by a carefully designed balance study, i.e., 0.86 for sodium and 0.77 for potassium [32].

*Other relevant data collected*

Blood pressure, past medical history, smoking status, and anthropometric data were collected at annual health check-ups. Body mass index (BMI) was computed as self-reported weight (kg) divided by height (m) squared.

*Intervention*

The intervention consisted of three components: (1) two dietary counseling sessions, (2) one group lecture, and (3) two newsletters. First, dietary counseling was provided after the annual health check-up. A trained dietitian provided individual 15-minute dietary counseling to each subject. Individualized education schemes were prepared based on the results of the dietary survey and health check-up. The individual feedback sheets consisted of a summary of dietary habits and nutrient intakes and a check-list of the dietary behavior. To increase carotene and vitamin C intakes, we recommended increasing intakes of fruits and vegetables. In particular, we emphasized the nutritional benefits of carotene- and vitamin C-rich vegetables, such as dark-green leafy vegetables and carrots. To decrease sodium intake, we mainly instructed subjects to decrease miso (fermented and salted soy-bean paste with 12% salt), salted vegetable pickles, salted fish, and seasonings with a high salt content. We recommended choosing carotene- and vitamin C-rich vegetables, if they ate salted pickles. Subjects were encouraged to set explicit and proximal subgoals from prelisted 50 items on check sheets. Furthermore, we prepared 40 different one-page leaflets that provided detailed nutrition information and helpful hints regarding cooking, and 4 to 5 leaflets were tailored to individual dietary intake levels and dietary patterns by computer program. Selected leaflets were checked by the trained dietitian and modified slightly when necessary. About 5 months later, a second dietary assessment using DHQ was performed and the same individual dietary counseling was provided to each subject. During the intervention period, we mailed two newsletters about recommended diet, to maintain motivation throughout the trial. Group lecture was performed at the mid-point of the intervention period. Study staff members were accessible to the subjects, allowing the occurrence of possible adverse effects, to be monitored.

A brief schematic diagram of the first half of the two year cross-over study is presented as Fig. 1.

*Dietary goals*

Dietary goals at the group level were to decrease salt intake and increase carotene and vitamin C intakes by approximately 80% considering the average intakes of this area, i.e., less than 8 and 10 g/day for salt in women and men, respectively, more than 5000 μg/day for carotene, and more than 200 mg/day for vitamin C.