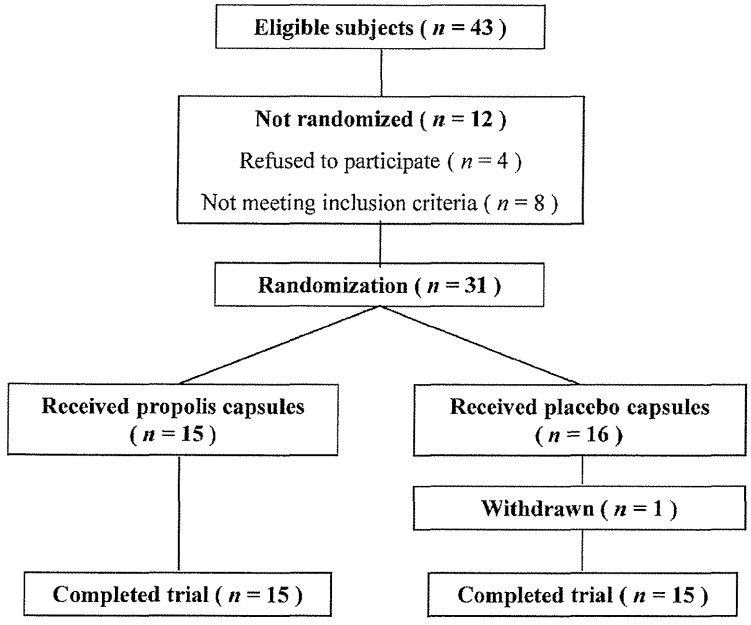

Fig. 1. Flow chart describing the progress of volunteers during the trial.

Table 1. Basal Characteristics of the Study Population[1]

Baseline Variables	Propolis Group (n = 15)	Placebo Group (n = 15)	*p* Value[2]
Male [*n* (%)]	11 (73)	11 (73)	—
Age (y)	61.1 ± 6.3	64.5 ± 8.7	0.230
Height (cm)	162.0 ± 7.3	164.3 ± 8.0	0.411
Body weight (kg)	60.5 ± 9.7	65.0 ± 12.1	0.269
Body mass index (kg/m^2)[3]	23.0 ± 3.3	23.9 ± 2.9	0.443
Tumors removed endoscopically			
>5 tumors [*n* (%)]	9 (60)	14 (93)	
>10 tumors [*n* (%)]	6 (40)	9 (60)	
Size of tumors removed endoscopically			
>8 mm [*n* (%)]	11 (73)	9 (60)	
>10 mm [*n* (%)]	8 (53)	6 (40)	
Cancer in adenoma [*n* (%)]	7 (47)	7 (47)	
Attenuated familial adenomatous polyposis [*n*(%)]	1 (7)	1 (7)	
Physical activity [*n* (%)]	8 (53)	6 (40)	—
NSAID user [*n* (%)][4]	4 (27)	3 (20)	—
L. casei preparation user [*n* (%)][4]	7 (47)	8 (53)	—
Smoking status [*n* (%)]			
Current	3 (20)	3 (20)	—
Former	6 (40)	6 (40)	—
Never	6 (40)	6 (40)	—
Drinking status [*n* (%)]			
Current	7 (47)	6 (40)	—
Former	5 (33)	6 (40)	—
Never	3 (20)	3 (20)	—

[1] Values are the mean ± SD.

[2] In a comparison between propolis and placebo groups, 2-sided Student *t* test was used for quantitative variables, and statistical significance was set at $p < 0.05$.

[3] Body mass index is equal to the weight in kilograms divided by the square of the height in meters.

[4] NSAID = nonsteroidal anti-inflammatory drugs, *L. casei* = *Lactobacillus casei*.

Table 2. Daily Intake of Nutrients of the Study Population[1]

Nutrients	Propolis Group (n = 15)	Placebo Group (n = 15)	*p* Value[2]
Energy (kcal)	2073 ± 474	1746 ± 286	0.030
Protein (g)	62.6 ± 13.5	51.6 ± 9.5	0.015
Fat (g)	48.2 ± 11.0	43.4 ± 11.0	0.236
Carbohydrate (g)	280.3 ± 78.1	240.7 ± 59.1	0.129
Sodium (mg)	1741 ± 479	1380 ± 265	0.016
Potassium (mg)	2315 ± 347	1997 ± 550	0.069
Calcium (mg)	572 ± 163	502 ± 164	0.249
Iron (mg)	7.1 ± 1.2	6.2 ± 1.6	0.124
Carotenes (μg)	3466 ± 1214	2903 ± 1048	0.185
Vitamin A (μg)	1085 ± 638	726 ± 254	0.053
Vitamin D (μg)	9 ± 3	7 ± 3	0.196
Vitamin E (mg)	8.5 ± 1.7	8.1 ± 2.1	0.558
Vitamin B_1 (mg)	0.66 ± 0.08	0.62 ± 0.05	0.091
Vitamin B_2 (mg)	1.17 ± 0.23	1.01 ± 0.27	0.101
Folate (μg)	364 ± 75	316 ± 115	0.185
Vitamin C (mg)	111 ± 40	101 ± 55	0.558
Total dietary fiber (g)	11.4 ± 3.1	9.6 ± 2.8	0.092
Soluble dietary fiber (g)	2.1 ± 0.5	1.7 ± 0.6	0.077
Insoluble dietary fiber (g)	8.5 ± 2.3	7.4 ± 2.3	0.191
Cholesterol (mg)	268 ± 62	240 ± 57	0.198
Saturated fatty acids (g)	12.83 ± 3.06	11.03 ± 2.51	0.090
Monounsaturated fatty acids (g)	16.29 ± 2.77	16.02 ± 4.20	0.837
Polyunsaturated fatty acids (g)	12.61 ± 1.98	12.21 ± 2.98	0.665
n-3 Polyunsaturated fatty acids (g)	2.296 ± 0.394	2.282 ± 0.456	0.929
n-6 Polyunsaturated fatty acids (g)	10.56 ± 2.07	10.15 ± 2.71	0.645
n-3 Highly unsaturated fatty acids (g)	0.845 ± 0.237	0.723 ± 0.302	0.231

[1] Values are the mean ± SD.

[2] In a comparison between propolis and placebo groups, 2-sided Student *t* test was used for quantitative variables, and statistical significance was set at $p < 0.05$.

Statistical Analyses

Analysis was conducted using Microsoft Excel 2002. The results are expressed as the mean ± SD or as a percentage. For comparisons between groups or intragroup changes between the beginning and end of the intervention, 2-sided Student *t* test or paired *t* test was used for quantitative variables. The level of statistical significance was set at $p < 0.05$.

Stratified analyses by smoking status (current, former, none) was also conducted.

RESULTS

Subjects

Forty-three subjects were eligible, of which 12 were excluded before randomization for the reasons shown in Fig. 1. Eight participants were excluded because they were receiving treatment for hypertension (n = 1), were taking propolis supplements (n = 5), and were aged 75 or over (n = 2). One man withdrew because of mild brain infarction and was unblinded in the placebo group. Subsequent data refer to the 30 participants who completed the study.

Table 1 shows the baseline characteristics and lifestyle factors possibly related to colon cancer risk: use of nonsteroidal anti-inflammatory drugs [31], *Lactobacillus casei* supplements [32], smoking, and drinking. No significant differences were detected between the treatment groups. All participants had a recent history of colon tumors with atypia or cancer in situ (Table 1), which had been removed before the start of the study. In Table 2, the daily intake of nutrients, energy, protein, and sodium was significantly higher in the propolis group than in the placebo group.

Number of 8-OHdG at Baseline and End

A marker of DNA base oxidation, the 8-OHdG number, varied widely in colon tissue and ranged between 0.61 and 9.46 per 10^5 dG in the propolis group and between 0.73 and 5.27 per 10^5 dG in the placebo group at baseline (Fig. 2A). These values in both groups were relatively higher than the 8-OHdG numbers found in human colorectal carcinoma [33,34]. These higher values suggest that they might be attributed to the use of laxatives in endoscopic analysis, so we stopped the use of laxatives at the final examination. In both the propolis and placebo groups, the 8-OHdG levels at the final examination were significantly decreased compared to levels at the baseline: from 2.76 ± 2.71 to 0.92 ± 0.41 per 10^5 dG in the propolis

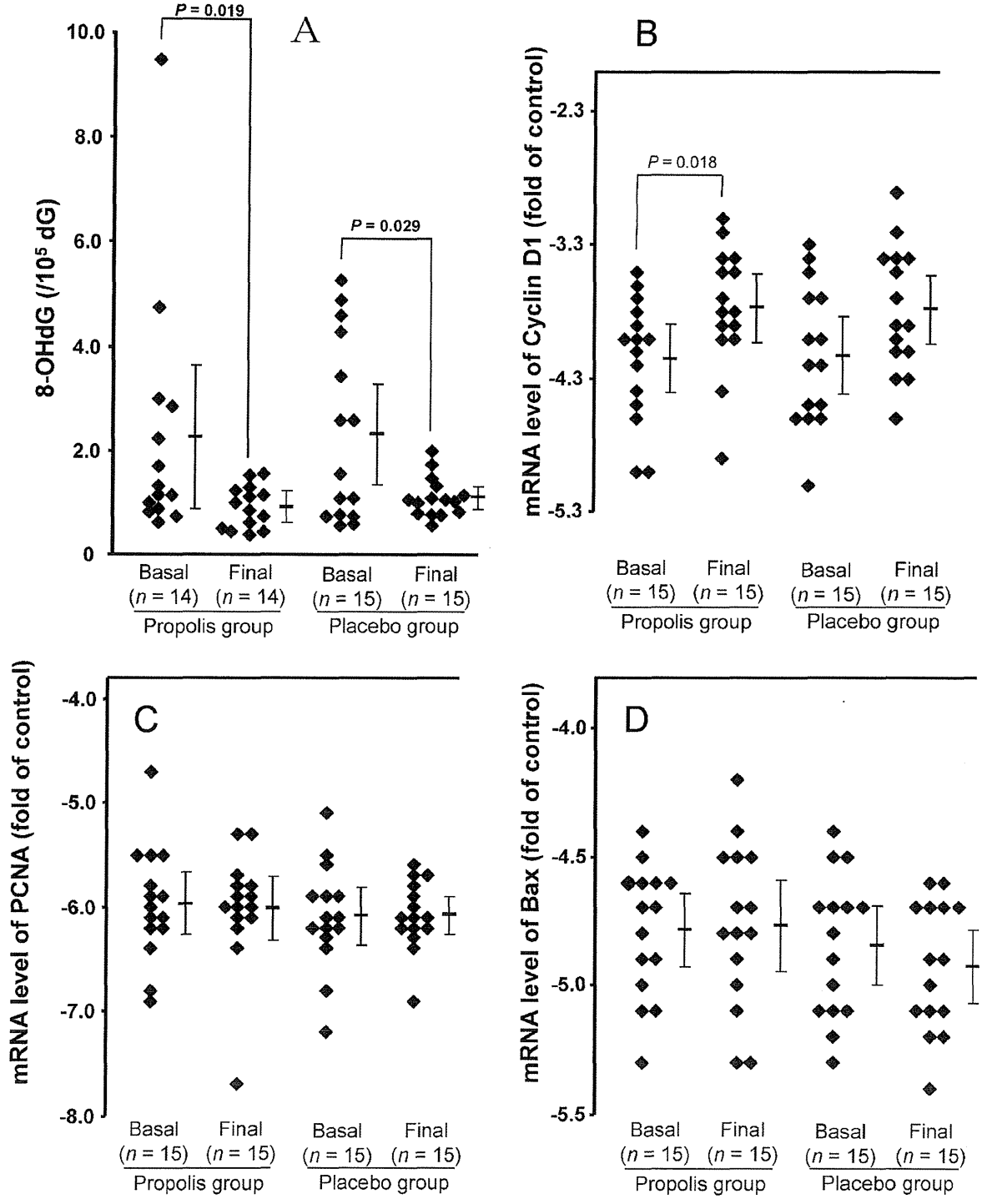

Fig. 2. Effects of propolis supplementation on levels of intermediate biomarkers for colon cancer: (A) level of 8-OHdG; (B) expression of cyclin D1; (C) expression of PCNA; (D) expression of Bax. Square symbol shows datum of each subject, and bar shows mean ± 95% confidence intervals. In (A), 1 result in the propolis groups was lost due to technical error during analysis (n =14). The proportions of final to basal in the propolis and the placebo groups were 0.6/0.5 and 1.0/1.0, respectively, ($p = 0.152$) in 8-OHdG level; 0.9/0.1 and 0.9/0.2 ($p = 0.763$) in expression of cyclin D1; 1.0/0.1 and 1.0/0.1 ($p = 0.752$) in expression of PCNA; 1.0/0.1 and 1.0/0.1 ($p = 0.564$) in expression of Bax.

Table 3. Changes in Ratio of CPK Isozymes of the Study Population[1]

Isozymes[2]	Basal	Final	*p* Value[3]
CPK-MM (%)			
Propolis group[4]	98.3 ± 0.3 (14)	98.2 ± 0.4 (14)	0.206
Placebo group	98.5 ± 0.3 (15)	98.4 ± 0.5 (15)	0.882
CPK-MB (%)			
Propolis group[4]	1.4 ± 0.3 (14)	1.6 ± 0.4 (14)	0.044
Placebo group	1.3 ± 0.3 (15)	1.4 ± 0.4 (15)	0.765
CPK-BB (%)			
Propolis group[4]	0.3 ± 0.1 (14)	0.2 ± 0.2 (14)	0.109
Placebo group	0.2 ± 0.2 (15)	0.2 ± 0.2 (15)	0.783

MM = skeletal muscle form, MB = myocardial band form, BB = brain form.

[1] Values are the means ± SD, and *n* is in parentheses. CPK activity in the propolis (n = 15) and the placebo (n = 15) groups were 12,747 and 11,737 units per milliliter at the beginning of study, respectively, ($p = 0.333$) and 14,352 and 10,438 units per milliliter at the end of study, respectively, ($p = 0.026$).

[2] Three isozyme forms were determined in the percentage of CPK.

[3] In intragroup changes between the beginning and end of the study, 2-sided paired *t* test was used for quantitative variables, with $p < 0.05$ significant. The proportion of final to basal in the propolis (n = 14) and the placebo (n = 15) groups were 1.2/0.4 and 1.0/0.4, respectively, ($p = 0.137$) in CPK activity; 1.0/0.0 and 1.0/0.0, respectively, ($p = 0.547$) in the ratio of CPK-MM; 1.2/0.4 and 1.1/0.4, respectively, ($p = 0.402$) in the ratio of CPK-MB; 0.7/0.6 and 1.1/1.0, respectively, ($p = 0.131$) in the ratio of CPK-BB.

[4] Plasma of 1 subject was not enough to analyze the isozymes, and the propolis group was determined using n = 14 at the final examination.

group ($p = 0.019$) and from 2.32 ± 1.75 to 1.10 ± 0.39 per 10^5 dG in the placebo group ($p = 0.029$). No significant difference was found between the propolis and placebo groups at the final analysis.

Effects of Brazilian Propolis Extract on Intermediate Biomarkers of Colon Cancer Risks

PCNA and cyclin D1 expressions are biomarkers of tumor cell proliferation [21,22], and Bax is a biomarker of apoptotic cell death [23,24]. The mRNA levels were not significantly different at the final examination between the propolis and placebo groups (Fig. 2B–D); however, in the propolis group (Fig. 2B), the expression of cyclin D1 mRNA increased from −4.2 ± 0.5 to −3.8 ± 0.5 ($p = 0.018$) with propolis intake, although no changes were observed in the placebo group.

Increased Activity of Myocardial Band (MB) form CPK

In blood analyses and blood biochemical tests, no significant differences were observed between the baseline and the final examination in both the propolis and placebo groups except for one result (data not shown). At the final examination after intervention, CPK activity in the propolis group was significantly higher than in the placebo group, 143 ± 52 and 104 ± 38 units/ml ($p = 0.026$), respectively. Determining the isozyme ratio in CPK, the MB forming CPK slightly but significantly increased in the propolis group (Table 3). None of the patients experienced side effects of supplementation except for 1 participant with evanescent persistent diarrhea and 1 participant with a transient rash in the propolis group, and 1 participant with a mild stroke in the placebo group.

Stratification Analysis in Smoking Status

Smoking status in this study was 3 each for current smokers, 5 and 6 for former smokers, and 3 each for never smokers in placebo and propolis groups, respectively (Table 1). Extracting the currently smoking subjects, the 8-OHdG level in the propolis group was significantly lower than in the placebo group at the end of the intervention (Fig. 3A). Also, in current smokers lactate dehydrogenase activity in blood biochemical analyses was 201 ± 22 IU/l in the propolis group and was significantly higher than 164 ± 6 IU/l in the placebo group ($p = 0.048$). The other biomarkers, mRNA expression levels of cyclin D1, PCNA, and Bax, did not show differences between placebo and propolis groups (data not shown). In both former and never smokers, propolis supplementation did not affect the 8-OHdG levels (Fig. 3B,C).

DISCUSSION

Brazilian propolis, which contains artepillin C and a mixture of other phenolic compounds and long-chain fatty acids, is a folk medicine used worldwide that shows cancer-preventive effects in animal models for colon cancer [17–19]. Our study was, to our knowledge, the first to evaluate in a small pilot study the efficacy and safety of Brazilian propolis in humans with higher risk for colon cancer and showed no beneficial effect on biomarkers associated with early stages of colorectal neoplasm.

The level of 8-OHdG is frequently used as a biomarker of oxidative DNA damage and is closely related to carcinogenesis [20]; 8-OHdG is known to lead to mutagenic G:C to T:A transversion on DNA replication [35], and the increased level has been recognized to be positively associated with the transition from colorectal adenoma to carcinoma [34]. PCNA is an auxiliary protein of DNA polymerase δ and starts to increase in the late G1 phase of the cell cycle, peaking during the S phase [36–38], and it has been employed as a marker of cell proliferation in normal-appearing colon mucosa [21]. Cyclin D1 is maximally expressed in the mid-G_1 to late-G_1 phase and regulates transition from the G1 to S phase [39]. Premature cell proliferation induced by highly expressed cyclin D1 facilitates the propagation of unrepaired DNA damage and genetic errors, resulting in a failure to delete the cells by apoptosis and leading to a selective advantage for abnormal cell proliferation. This is an important step in inducing adenoma development in colon carcinogenesis. The increased mRNA level of cyclin D1 is

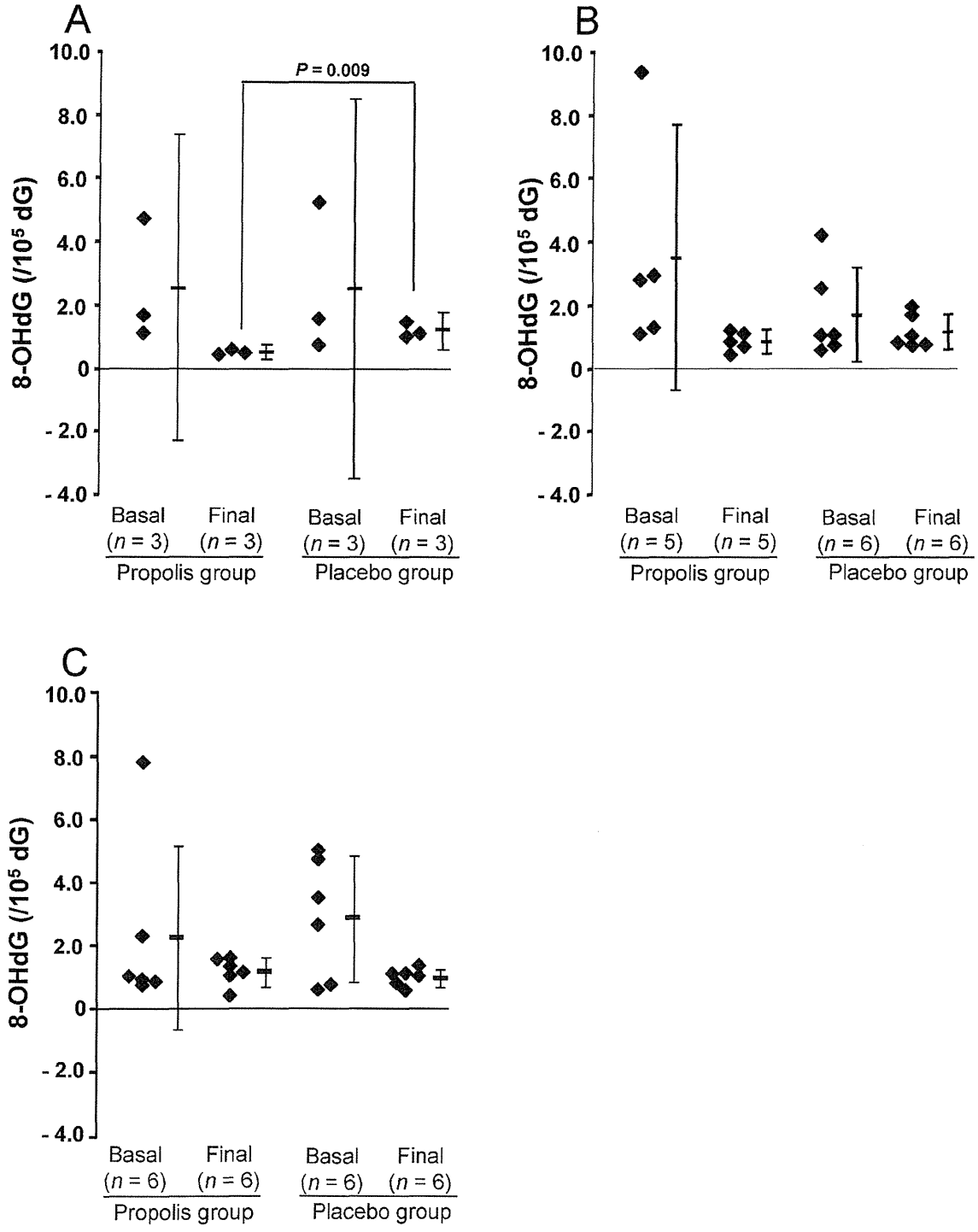

Fig. 3. Stratification analysis of effects of propolis supplementation on 8-OHdG level according to smoking status: (A) current smokers; (B) former smokers; (C) never smokers. Square symbol shows datum of each subject, and bar is mean ± 95% confidence intervals. In (B), 1 result in the propolis groups was lost due to technical error during analysis (n = 5). The proportions of final to basal in the propolis and the placebo groups were 0.3/0.2 and 0.8/0.7, respectively, ($p = 0.235$) in 8-OHdG level; 1.0/0.2 and 1.0/0.2 ($p = 0.793$) in expression of cyclin D1; 1.1/0.2 and 1.0/0.1 ($p = 0.248$) in expression of PCNA; 1.1/0.1 and 1.0/0.0 ($p = 0.111$) in expression of Bax.

recognized to be an early event in multistage colorectal carcinogenesis [22]. Bax is a regulating protein of apoptosis induction in mitochondria [40], and its expression in normal colon mucosa is considered to be highly correlated with increased colon cancer risk [23,24].

We supposed that the intake of artepillin C–rich extract would decrease levels of 8-OHdG and mRNA of PCNA without affecting cyclin D1 and Bax; however, 3-month supplementation with the artepillin C–rich extract did not decrease any of the biomarkers, the levels of 8-OHdG, or mRNAs of PCNA, cyclin D1, and Bax (Figs. 1 and 2). This inconsistency is considered to be due to (1) the difficulty of predicting the development of adenoma formation using these biomarkers, (2) the sensitivity of biomarkers being dependent on individuals, (3) the biopsy location causing methodological difficulty in evaluating overall changes of the large bowel, (4) the sample size (n = 15 in each group) being too small to evaluate changes in the biomarkers, and (5) the supplemented amount of artepillin C–rich extract of Brazilian propolis being unsuitable in the present study.

First, among the biomarkers, PCNA has been reported not to be a sensitive marker for evaluating neoplasia risk in intestinal mucosa [10,41], while 8-OHdG and Bax have been sensitive markers in several randomized clinical trials [42,43]. Recently, cyclin A as a marker of S-phase [44] and/or Ki-67 [45] has been recommended, and further studies using other cell proliferation markers instead of PCNA are needed.

Second, the use of laxatives prior to the basal endoscopic analysis varied the 8-OHdG formation, and direct use of an endoscope without laxative treatment at the final analysis showed little variety (Fig. 2A), indicating that laxative treatment injures mucosal surface cells in some subjects. Endoscopic biopsy may be preferable without laxative treatment.

Third, all biopsy samples were taken from the sigmoid colon in the present study. This limited location may make it difficult to evaluate changes in biomarkers that reflect the suppression of tumorigenesis after the supplementation of artepillin C–rich extract. In addition, HPLC determination of 8-OHdG and RT-PCR analysis of mRNA using whole biopsy cannot eliminate the effect of infiltrating lymphocytes and other cell types.

Fourth, the small sample size (n = 15 in each group) in this pilot study may have contributed to the absence of a significant treatment effect and made it difficult to conduct subgroup analyses, such as smoking status. The present study was the first randomized, double-blind, placebo-controlled trial investigating the effects of artepillin C-rich extract in high-risk patients with colon cancer using intermediate end points in normal-appearing colon mucosa, and the results support further studies to clarify the effects. This study gave limited results, but clearly showed that the artepillin C–rich extract could suppress the formation of 8-OHdG in the colon in current smokers (Fig. 3A). The artepillin C–rich extract may be effective in subjects with a lower antioxidant status, such as current smokers.

Finally, the supplementation amount of artepillin C–rich extract was too small. In the present study, we used 165 μmol (50 mg) artepillin C daily for 3 months. In the animal study of colon cancer prevention, the supplemented amount giving positive results was 10 mg artepillin C per kilogram of body weight daily for 4 weeks [24]. In the present intervention, we reduced the supplemented amount, as it was the first human trial. Imai et al. [46] reported that the effective level of an ethanol extract of propolis was 787.5 mg per day per capita to show antioxidant activity in humans. A higher amount might better suppress the biomarkers in normal-appearing colon mucosa; however, at the end of the intervention study, the expression of cyclin D1 mRNA had significantly increased in the propolis group (Fig. 2B), and there was a slight but significant leak of the MB form of CPK into blood (Table 3). The MB form mainly exists in cardiac muscle cells, and heavy leakage accompanies myocardial necrosis [47]. No subjects showed abnormalities, and we found no dysfunction in any subjects. The artepillin C–rich extract of Brazilian propolis is considered to stimulate cell proliferation in sigmoid colon mucosal polyps and to exhibit slight damage to cardiac muscle cells.

CONCLUSION

Brazilian propolis showed no beneficial effect on biomarkers associated with early stages of colorectal neoplasm, except for the specific effect in current smokers on 8-OHdG levels. Use of laxatives prior to endoscopic biopsy may increase 8-OHdG levels in some subjects.

ACKNOWLEDGMENTS

This study was funded partly by the Yamada Apiculture Center, Okayama, Japan. Propolis supplement and placebo were also prepared by Yamada Apiculture Center. The authors would like to thank Ayako Nasu for inputting the data and Kyoko Keuven-Uchiyama for contributing to this article. We are very grateful to Dr. Keiko Saito (Chairperson, Kyoto University), Dr. Kenji Watanabe (Osaka City University), and Mr. Yoshihiro Nunotani (patient) for their cooperation as Ethics Monitoring Committee members.

REFERENCES

1. Doll R, Peto R: The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 66:1191–1308, 1981.
2. Willett W: Diet and cancer. Oncologist 5:393–404, 2000.

3. Wakai K, Hirose K, Matsuo K, Ito H, Kuriki K, Suzuki T, Kato T, Hirai T, Kanemitsu Y, Tajima K: Dietary risk factors for colon and rectal cancers: a comparative case-control study. J Epidemiol 16:125–135, 2006.
4. Riboli E, Norat T: Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. Am J Clin Nutr 78:559S–569S, 2003.
5. Amin A, Kucuk O, Khuri F, Shin D: Perspectives for cancer prevention with natural compounds. J Clin Oncol 27:2712–2725, 2009.
6. Ministry of Health, Labor and Welfare: "Vital Statistics Japan." Tokyo: Ministry of Health, Labor and Welfare, 2010.
7. Matsuda T, Zhang M: Comparison of time trends in colorectal cancer mortality (1990–2006) in the world, from the WHO mortality database. Jpn J Clin Oncol 39:777–778, 2009.
8. Matsuda T, Marugame T, Kamo K, Katanoda K, Ajiki W, Sobue T: Cancer incidence and incidence rates in Japan in 2002: based on data from 11 population-based cancer registries. Jpn J Clin Oncol 38:641–648, 2008.
9. Center M, Jemal A, Smith R, Ward E: Worldwide variations in colorectal cancer. Cancer J Clin 59:366–378, 2009.
10. Sandler R, Baron J, Tosteson T, Mandel J, Haile R: Rectal mucosal proliferation and risk of colorectal adenomas: results from a randomized controlled trial. Cancer Epidemiol Biomarkers Prev 9:653–656, 2000.
11. Vainio H, Weiderpass E: Fruit and vegetables in cancer prevention. Nutr Cancer 54:111–142, 2006.
12. Rajamanickam S, Agarwal R: Natural products and colon cancer: current status and future prospects. Drug Dev Res 69:460–471, 2008.
13. Park Y, Paredes-Guzman J, Aguiar C, Alencar S, Fujiwara F: Chemical constituents in *Baccharis dracunculifolia* as the main botanical origin of southeastern Brazilian propolis. J Agric Food Chem 52:1100–1103, 2004.
14. Murota K, Terao J: Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. Arch Biochem Biophys 417:12–17, 2003.
15. Shimizu S, Ashida H, Matsuura Y, Kanazawa K: Antioxidative bioavailability of artepillin C in Brazilian propolis. Arch Biochem Biophys 424:181–188, 2004.
16. Konishi Y, Hitomi Y, Yoshida M, Yoshioka E: Absorption and bioavailability of artepillin C in rats after oral administration. J Agric Food Chem 53:9928–9933, 2005.
17. Shimizu K, Das S, Baba M, Matsuura Y, Kanazawa K: Dietary artepillin C suppresses the formation of aberrant crypt foci induced by azoxymethane in mouse colon. Cancer Lett 240:135–142, 2006.
18. Yasui Y, Miyamoto S, Kim M, Kohno H, Sugie S, Tanaka T: Aqueous and ethanolic extract fractions from the Brazilian propolis suppress azoxymethane-induced aberrant crypt foci in rats. Oncol Rep 20:493–499, 2008.
19. Shimizu K, Das S, Hashimoto T, Sowa Y, Yoshida T, Sakai T, Matsuura Y, Kanazawa K: Artepillin C in Brazilian propolis induces G0/G1 arrest via stimulation of Cip1/p21 expression in human colon cancer cells. Mol Carcinog 44:293–299, 2005.
20. Kasai H: Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 387:147–163, 1997.
21. Bostick R, Fosdick L, Lillemoe T, Overn P, Wood JR, Grambsch P, Elmer P, Potter JD: Methodological findings and considerations in measuring colorectal epithelial cell proliferation in humans. Cancer Epidemiol Biomarkers Prev 6:931–942, 1997.
22. Kristt D, Turner I, Koren R, Ramadan E, Gal R: Overexpression of cyclin D1 mRNA in colorectal carcinomas and relationship to clinicopathological features: an in situ hybridization analysis. Pathol Oncol Res 6:65–70, 2000.
23. Liu L, Holt P, Krivosheyev V, Moss S: Human right and left colon differ in epithelial cell apoptosis and in expression of Bak, a pro-apoptotic Bcl-2 homologue. Gut 45:45–50, 1999.
24. Anti M, Armuzzi A, Morini S, Iascone E, Pignataro G, Coco C, Lorenzetti R, Paolucci M, Covino M, Gasbarrini A, Vecchio F, Gasbarrini G: Severe imbalance of cell proliferation and apoptosis in the left colon and in the rectosigmoid tract in subjects with a history of large adenomas. Gut 48:238–246, 2001.
25. Sakakibara H, Honda Y, Nakagawa S, Ashida H, Kanazawa K: Simultaneous determination of all polyphenols in vegetables, fruits, and teas. J Agric Food Chem 51:571–581, 2003.
26. Goto C, Tokudome Y, Imaeda N, Takekuma K, Kuriki K, Igarashi F, Ikeda M, Tokudome S: Validation study of fatty acid consumption assessed with a short food frequency questionnaire against plasma concentration in middle-aged Japanese people. Scand J Food Nutr 50:77–82, 2006.
27. Kavsak P, MacRae A, Newman A, Lustig V, Palomaki GE, Ko DT, Tu JV, Jaffe AS: Effects of contemporary troponin assay sensitivity on the utility of the early markers myoglobin and CKMB isoforms in evaluating patients with possible acute myocardial infarction. Clin Chim Acta 380:213–216, 2007.
28. Takemasa I, Yamamoto H, Sekimoto M, Ohue M, Noura S, Miyake Y, Matsumoto T, Aihara T, Tomita N, Tamaki Y, Sakita I, Kikkawa N, Matsuura N, Shiozaki H, Monden M: Overexpression of CDC25B phosphatase as a novel marker of poor prognosis of human colorectal carcinoma. Cancer Res 60:3043–3050, 2000.
29. Tanaka K, Nishioka J, Kato K, Nakamura A, Mouri T, Miki C, Kusunoki M, Nobori T: Mitotic checkpoint protein hsMAD2 as a marker predicting liver metastasis of human gastric cancers. Jpn J Cancer Res 92:952–958, 2001.
30. Seki Y, Yamamoto H, Ngan C, Yasui M, Tomita N, Kitani K, Takemasa I, Ikeda M, Sekimoto M, Matsuura N, Albanese C, Kaneda Y, Pestell RG, Monden M: Construction of a novel DNA decoy that inhibits the oncogenic beta-catenin/T-cell factor pathway. Mol Cancer Ther 5:985–994, 2006.
31. Huls G, Koornstra J, Kleibeuker J: Non-steroidal anti-inflammatory drugs and molecular carcinogenesis of colorectal carcinomas. Lancet 362:230–232, 2003.
32. Ishikawa H, Akedo I, Otani T, Suzuki T, Nakamura T, Takeyama I, Ishiguro S, Miyaoka E, Sobue T, Kakizoe T: Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors. Int J Cancer 116:762–767, 2005.
33. Tsurudome Y, Hirano T, Hirata K, Higure A, Nagata N, Takahashi K, Itoh H, Kasai H: Age-associated increase of 8-hydroxydeoxyguanosine in human colorectal tissue DNA. J Gerontol A Biol Sci Med Sci 56:B483–B485, 2001.
34. Kondo S, Toyokuni S, Iwasa Y, Tanaka T, Onodera H, Hiai H, Imamura M: Persistent oxidative stress in human colorectal carcinoma, but not in adenoma. Free Radic Biol Med 27:401–410, 1999.
35. Shibutani S, Takeshita M, Grollman A: Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature 349:431–434, 1991.

36. Bravo R, Frank R, Blundell P, Macdonald-Bravo H: Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. Nature 326:515–517, 1987.
37. Celis J, Madsen P, Celis A, Nielsen H, Gesser B: Cyclin (PCNA, auxiliary protein of DNA polymerase delta) is a central component of the pathway(s) leading to DNA replication and cell division. FEBS Lett 220:1–7, 1987.
38. Kurki P, Vanderlaan M, Dolbeare F, Gray J, Tan E: Expression of proliferating cell nuclear antigen (PCNA)/cyclin during the cell cycle. Exp Cell Res 166:209–219, 1986.
39. Sherr C: Cancer cell cycles. Science 274:1672–1677, 1996.
40. Brady H, Gil-Gómez G: Bax. The pro-apoptotic Bcl-2 family member, Bax. Int J Biochem Cell Biol 30:647–650, 1998.
41. Risio M, Candelaresi G, Rossini F: Bromodeoxyuridine uptake and proliferating cell nuclear antigen expression throughout the colorectal tumor sequence. Cancer Epidemiol Biomarkers Prev 2:363–367, 1993.
42. Fedirko V, Bostick R, Long Q, Flanders WD, McCullough ML, Sidelnikov E, Daniel CR, Rutherford RE, Shaukat A: Effects of supplemental vitamin D and calcium on oxidative DNA damage marker in normal colorectal mucosa: a randomized clinical trial. Cancer Epidemiol Biomarkers Prev 19:280–291, 2010.
43. Fedirko V, Bostick R, Flanders W, Long Q, Shaukat A, Rutherford RE, Daniel CR, Cohen V, Dash C: Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: a randomized, double-blind, placebo-controlled clinical trial. Cancer Prev Res (Phila) 2:213–223, 2009.
44. Scott I, Morris L, Bird K, Davies RJ, Vowler SL, Rushbrook SM, Marshall AE, Laskey RA, Miller R, Arends MJ, Coleman N: A novel immunohistochemical method to estimate cell-cycle phase distribution in archival tissue: implications for the prediction of outcome in colorectal cancer. J Pathol 201:187–197, 2003.
45. Akedo I, Ishikawa H, Ioka T, Kaji I, Narahara H, Ishiguro S, Suzuki T, Otani T: Evaluation of epithelial cell proliferation rate in normal-appearing colonic mucosa as a high-risk marker for colorectal cancer. Cancer Epidemiol Biomarkers Prev 10:925–930, 2001.
46. Imai H, Era S, Hayashi T, Negawa T, Matsuyama Y, Okihara K, Nakatsuma A, Yamada H: Effect of propolis supplementation on the redox state of human serum albumin during high-intensity kendo training. Adv Exerc Sports Physiol 11:109–113, 2005.
47. Zaninotto M, Mion M, Novello E, Delprete E, Moretti M, Plebani M: Creatine-kinase MB mass: age and sex-associated reference limits in two different platforms that use the same method. Clin Chim Acta 401:162–164, 2009.

Received July 21, 2010; revision accepted April 10, 2012.

Surg Endosc
DOI 10.1007/s00464-013-2903-x

Current status of endoscopic resection strategy for large, early colorectal neoplasia in Japan

Takeshi Nakajima · Yutaka Saito · Shinji Tanaka · Hiroyasu Iishi · Shin-ei Kudo · Hiroaki Ikematsu · Masahiro Igarashi · Yuusuke Saitoh · Yuji Inoue · Kiyonori Kobayashi · Takashi Hisasbe · Takahisa Matsuda · Hideki Ishikawa · Ken-ichi Sugihara

Received: 1 October 2012 / Accepted: 15 February 2013

Abstract
Background Conventional endoscopic resection (CER) for early colorectal neoplasia (CRN) is widely accepted as a minimally invasive treatment. Endoscopic submucosal dissection (ESD) was developed in Japan to resect larger lesions, but ESD was not covered by the Japanese national health insurance until April 2012. In addition, treatment strategies vary considerably among medical facilities. To evaluate the current situation in Japan regarding endoscopic treatment of CRNs measuring ≥20 mm, we conducted a prospective multicenter study at 18 medium-volume and high-volume specialized facilities in cooperation with the Japan Society for Cancer of the Colon and Rectum (JSCCR).
Methods The JSCCR conducted a multicenter, observational study of all patients treated by CER and ESD of CRNs measuring ≥20 mm.
Results From October 2007 to December 2010, CERs and ESDs were performed on 1,845 CRNs (CERs 1,029; ESDs 816). Lesions diagnosed as protruded, flat, and depressed totaled 541, 1224, and 48, respectively. En bloc resection rates and mean procedure times for CER/ESD were 56.9 %/94.5 % ($P < 0.01$) and 18 ± 23 min/96 ± 69 min, respectively. The average ESD procedure time was 129 ± 83 min in the ≥40-mm group. As lesion size increased, the CER en bloc resection rate decreased significantly (trend $P < 0.01$), but the ESD en bloc resection rate remained over 93 %. Perforation and delayed bleeding rates of CER/ESD were 0.8 %/1.6 % ($P < 0.05$) and 2 %/2.2 % ($P = 0.3$), respectively.

This study was reported at the United European Gastroenterology Week held at Stockholm, Sweden, October 24, 2011.

This study was conducted on behalf of the Colorectal Endoscopic Resection Standardization Implementation Working Group, Japanese Society for Cancer of the Colon and Rectum, Tokyo, Japan. The working group that participated in this study are listed in "Appendix".

T. Nakajima (✉) · Y. Saito · T. Matsuda
Endoscopy Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: tnakajim@ncc.go.jp

S. Tanaka
Department of Endoscopy, Hiroshima University Hospital, Hiroshima, Japan

H. Iishi
Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

S. Kudo
Digestive Disease Center, Showa University Northern Yokohama Hospital, Yokohama, Kanagawa, Japan

H. Ikematsu
Department of Gastroenterology & Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan

M. Igarashi
Department of Endoscopy, Cancer Institute Ariake Hospital, Tokyo, Japan

Y. Saitoh
Digestive Disease Center, Asahikawa City Hospital, Hokkaido, Japan

Y. Inoue
Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo, Japan

K. Kobayashi
Department of Gastroenterology, Kitasato University East Hospital, Sagamihara, Kanagawa, Japan

T. Hisasbe
Department of Gastroenterology, Fukuoka University Chikushi Hospital, Chikushino, Fukuoka, Japan

Published online: 19 March 2013

Conclusions The en bloc resection rate for ESD was significantly higher than for CER, although complication rates were fairly low. Despite a longer procedure time, safety of colorectal ESD has improved in various facilities in Japan. However, ESD for lesions measuring ≥40 mm must be performed by experienced endoscopists due to the longer procedure time.

Keywords Endoscopic mucosal resection · Endoscopic submucosal dissection · Colorectal cancer · Colorectal neoplasia

The number of endoscopic submucosal dissections (ESDs) for colorectal neoplasms has been increasing in Japan, and the effectiveness of colorectal ESD has been reported not only in Japan but also in western countries. However, colorectal ESD still has a higher risk of perforation because the colonic wall is thinner and endoscope stabilization is more difficult than in gastric and esophageal ESD. Consequently, treatment strategies for CRN vary considerably among facilities even in Japan.

Colorectal cancer is a major cause of morbidity and mortality in the world [1]. According to the adenoma–carcinoma sequence theory, early detection and resection of colorectal neoplasm (CRN) is essential for improving cancer mortality [2, 3]. CRNs without risk of lymph node metastasis, including adenomas, are good candidates for endoscopic resection (ER) [4]. Conventional endoscopic resection (CER), including polypectomy and endoscopic mucosal resection (EMR), was developed as a minimally invasive treatment for CRN [5, 6] and is widely accepted. However, CER for lesions exceeding 20 mm in diameter sometimes results in piecemeal resection, decreasing the accuracy of pathological diagnosis and resulting in local recurrences [7–9].

ESD is an established therapeutic technique for the treatment of gastrointestinal neoplasms. Because it is typically completed as en bloc resection, this technique provides a complete specimen for precise histopathological evaluation [10–12]. Following widespread use in treatment of gastric ESDs, the number of medical facilities performing colorectal ESDs has been increasing not only in Japan, but also in western countries [13–21]. However, In the guidelines of the Japanese Society for Cancer of the Colon and Rectum (JSCCR), CRN diagnosed as clinical mucosal cancer or superficial submucosal cancer (invasion depth of <1,000 μm), a size of ≥20 mm was initially recommended for surgical resection [22] because of the greater technical difficulty involved and the risk of perforation and resultant peritonitis [19, 23, 24].

Consequently, treatment strategies (and payment arrangements) for CRN vary considerably among facilities. To evaluate the current situation in Japan regarding endoscopic treatment of CRNs measuring ≥20 mm, we conducted a cross-sectional multicenter study in cooperation with JSCCR. We seek to convey the effectiveness and safety of both CER and ESD treatments to the world.

Materials and methods

From October 2007 to December 2010, patients were prospectively and consecutively enrolled at the 18 institutions affiliated with the Colorectal Endoscopic Resection Standardization Implementation Working Group of JSCCR, and all obtained data were sent to a data center. JSCCR has proposed Japanese guidelines and this working group has a responsibility in ER section of the guideline [19, 20]. The study was conducted with the approval of each institution's ethical review board, and informed written consent was obtained from all patients for each specific colonoscopic treatment. The clinical trial number of this study is UMIN000001642.

We analyzed the following clinicopathological factors: ER method, patient age at the initial ER, sex, tumor size, location, macroscopic type, histological margin, histological grade, depth of submucosal invasion, and lymphatic/venous involvement, determined based on the Japanese classification of cancer of the colon and rectum (JCCCR) [22].

All procedures were performed by experienced colonoscopists, or under their supervision, with a standard videoendoscopic system (EVIS LUCERA system, Olympus Optical, Tokyo, Japan; or Advancia HD/Advancia, Fujifilm, Tokyo, Japan).

Inclusion criteria

ER is indicated to treat intramucosal CRNs and lesions with submucosal invasion limited to less than 1,000 μm, because the risk of lymph node metastasis is very low [4, 25]. Before treatment, only depth of invasion could be estimated endoscopically in combination with conventional endoscopic findings and, if possible, pit pattern analysis with magnifying chromoendoscopy (CF-H260AZI, CF-Q260AZI, or PCF-Q240ZI, Olympus, Tokyo, Japan; and EC590Z series, Fujifilm, Tokyo, Japan) [26–32]. We have indicated the use or nonuse of magnification.

The Colorectal ESD Standardization Implementation Working Group has attempted to standardize colorectal ESD, and guidelines have been proposed by this group

H. Ishikawa
Department of Molecular-Targeting Cancer Prevention, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Osaka, Japan

K. Sugihara
Department of Surgical Oncology, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan

[19, 20]. Based on extensive clinicopathological analyses, the indications for colorectal ESD in this study are the same as those recommended in the guidelines: a tumor for which the use of a snare EMR for en bloc resection is difficult, such as a laterally spreading tumor of the nongranular type (LST-NG) [7, 20, 33, 34], especially the pseudo-depressed type, a tumor with a type V_I pit pattern, a shallow infiltrating submucosal carcinoma, a large depressed tumor, and large elevated, probably malignant lesions (tall nodule-aggregating lesions such as a granular-type LST; LST-G), because these lesions have a high submucosal invasion rate and are difficult to treat even by piecemeal EMR [19, 33, 35]. Other lesions, such as intramucosal tumors accompanied by submucosal fibrosis, which are induced by a biopsy or peristalsis of the lesion, sporadic localized tumors in chronic inflammation, including ulcerative colitis, and local residual early carcinomas after EMR, also are indications for colorectal ESD [19].

Exclusion criteria for ER

Exclusion criteria included findings of submucosal cancer such as V_N pit pattern, an invasive pattern as determined by magnification chromoendoscopy [27, 29, 36], and presence of other invasive cancers and circumferential tumors that require surgical treatment because of the increased technical difficulty involved and the anticipated risk of stenosis.

Clinicopathological characteristics

The location of tumors was based on the Japanese classification of cancer of the colon and rectum [22, 37] and included the cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum.

For macroscopic typing, we divided the lesions into five macroscopic groups according to the Paris classification and LST features as follows: (1) protruded type, which is 0-Is(p); (2) flat type, which is 0-IIa and 0-IIc with LST features; (3) mixed type, 0-Is(p) + IIa, most of which have LST character; (4) depressed type, 0-IIc or IIa + IIc in Paris classification without LST features; and (5) recurrent cases.

CER procedures

In this study, CER was defined as snare technique, EMR, or snare polypectomy; endoscopists, including gastroenterologists and digestive surgeons, chose treatment methods according to the size and endoscopic features of the CRN. In EMR, after successful fluid injection of normal saline and/or glycerol [38] and/or 0.4 % hyaluronic acid solution into the submucosal layer, the endoscopist performed resection using the snare [6]. After resection, additional snare resection or coagulation using hot biopsy forceps also was performed if there was a suspicion of small residual tumors in the resection plane.

ESD procedures

Procedures were primarily performed using one or two ESD knives, including a bipolar needle knife (Xeon Medical Co, Tokyo, Japan) [9, 15, 39], flex knife [40], hook knife (Olympus Co, Tokyo, Japan) [41], flush knife (Fujinon Co, Tokyo, Japan) [42], and insulation-tipped knife (Olympus) [10, 13]. Hemostatic forceps (Coagrasper; Olympus) and Hemostat Y (PENTAX Co., Tokyo, Japan) were used for hemostasis. Lesion margins were delineated before ESD using 0.4 % indigo-carmine spray dye. Following injection of Glycerol and/or sodium hyaluronate into the submucosal layer, a circumferential incision was made using the ESD knife [14]. Both partial circumferential incision and subsequent submucosal dissection were performed alternately using ESD knives.

Definition of ESD and CER

Some lesions were treated by a combined CER/ESD technique, using a special ESD knife and resected by snaring. We defined those cases of resection by snaring with only circumferential incision [43] as CER and cases in which the physician performed any submucosal dissection after marginal resection as ESD.

Definition of complication

Perforation during an ESD procedure was defined as immediate, and delayed perforation was defined as any perforation occurring after completion of the procedure. Immediate perforation was defined as a full-thickness defect in the colonic wall. Closure with endoscopic clips was performed or surgical treatment was pursued. Postoperative bleeding was defined as bleeding that required repeat colonoscopy for hemostasis therapy, blood transfusion, or decreased level of hemoglobin >2 g/dl.

Histological assessment

All specimens were fixed in 10 % formalin, cut into 2-mm sections, and examined microscopically for histological type, depth of invasion, and lateral and vertical resection margins. Resections were considered tumor-free when the lateral and vertical margins of a specimen were both negative for tumor cells, independent of histological features. The submucosal depth was defined as the distance determined by microscopic observation of specimens using an optical micrometer [4]. A curative resection was achieved when both the lateral and vertical margins of the specimen were free of cancer, with none of the following features: submucosal invasion deeper than 1,000 μm, lymphatic invasion, vascular involvement, or poorly differentiated

components [4]. An adenoma with an unknown lateral margin also was considered to be resected curatively when the neoplasm met all other criteria. Lesions resected in a piecemeal fashion were reconstructed faithfully on the basis of the mirror endoscopic images obtained before treatment and fixed in formalin. Histological diagnoses were based on the Japanese classification of cancer of the colon and rectum [37] and the Vienna classification [44]. The former is a standard pathological classification in Japan, and these results were converted to the latter form for standardization with global classifications.

Statistical analysis

Statistical analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL). Data are presented as mean ± standard deviation, medians, ranges, and percentages. For analysis of clinicopathological characteristics, we used Student's t test and χ^2 and Fisher's exact tests, as appropriate. All tests were two-tailed, and $P < 0.05$ was considered significant.

Results

Patient and lesion characteristics

A total of 1,845 CRNs that were ≥20 mm in size were examined in this study. CER was used in 1,029 cases and ESD in 816. Mean lesion sizes in CER and ESD cases were 26.4 ± 8.6 (range 20–120) mm and 39.4 ± 18.2 (range 20–174) mm, respectively. Patient characteristics and distributions of lesions are detailed in Tables 1 and 2. Tumor distribution between the two groups was different ($P < 0.01$). The frequency of cecum and sigmoid colon lesions were higher in the CER group than in the ESD group. On the other hand, lesions of the rectum were less frequent in the CER group. Submucosal cancer, including both superficial submucosal cancer and deep submucosal invasion cancer, was more common in the ESD group. Thus, the distribution of tumor characteristics differed between CER and ESD. The frequency of use of magnification colonoscopy also differed in the two groups (71.9 % in CER vs. 85.7 % in ESD, $P < 0.01$).

Differences in endoscopic treatment choice according to tumor size and macroscopic type

We divided the cases into three groups according to lesion size: 20–29, 30–39, and ≥40 mm. Associations between tumor size, macroscopic type, and treatment choice are detailed in Table 3. In the 20–29-mm group, 77 % (729/948) of the lesions were treated by CER. In contrast, as the lesion size increased, lesions were more likely to be treated by ESD. Macroscopic type also influenced treatment choice. In the 20–29-mm group, 93.8 % of the protruded lesions were treated by CER, whereas 37 % of the flat lesions were treated by ESD. In the 30–39-mm group, approximately 70 % of mixed and flat lesions were treated by ESD. In the ≥40-mm group, approximately 80 % of mixed and flat lesions were treated by ESD. All five cases of recurrence were treated by ESD, regardless of lesion size.

Table 1 Patients and tumor location

	CER	ESD	Total	*P* value
No. of lesions	1,029	816	1,845	
Age, mean ± SD (range)	65.2 ± 11.7 (20–89)	66.6 ± 9.9 (23–91)	65.8 ± 10.9	<0.01
Sex, male/female (ratio)	637/392 (1.6)	468/348 (1.3)	1105/740 (1.5)	<0.05
Use of magnification (%)	740 (71.9)	700 (85.7)	1440 (78)	<0.01
Distribution				
Cecum (%)	137 (13.3)	71 (8.7)	208 (11.3)	
Ascending colon (%)	231 (22.4)	152 (18.6)	383 (20.8)	
Transverse colon (%)	161 (15.6)	144 (17.6)	305 (16.5)	
Descending colon (%)	38 (3.7)	32 (3.9)	70 (3.8)	
Sigmoid colon (%)	300 (29.2)	121 (14.8)	421 (22.8)	
Rectum (%)	162 (15.7)	296 (36.3)	458 (24.8)	<0.001
Total (%)	1,029 (100)	816 (100)	1,845 (100)	

Table 2 Pathological results, by procedure type and lesion size

Range of lesion sizes (mm)	20–29 CER/ ESD	30–39 CER/ ESD	≥40 CER/ ESD	Total CER/ ESD	Total
Pathological results					
Adenoma	380/72	86/95	36/95	502/262	764
(%)	(84/16)	(48/52)	(27/73)	(66/34)	
Intramucosal cancer	283/95	85/109	65/194	433/398	831
(%)	(75/25)	(44/56)	(25/75)	(52/48)	
Submucosal cancer	48/52	17/51	5/47	70/150	220
(%)	(48/52)	(25/75)	(10/90)	(32/68)	
<1,000 μm	18/34	8/31	3/23	29/88	117
(%)	(35/65)	(21/79)	(12/88)	(25/75)	
≥1,000 μm	30/18	9/20	2/24	41/62	103
(%)	(63/38)	(31/69)	(8/92)	(40/60)	
Unknown	0/0	0/1	0/2	0/3	3
(%)	(0/0)	(0/100)	(0/100)	(0/100)	
Others	18/0	6/2	0/1	24/3	27
(%)	(100/0)	(75/25)	(0/100)	(89/11)	
Total	729/219	194/258	106/339	1029/816	

Treatment results: comparison of CER and ESD

The treatment results for CER and ESD are detailed in Table 4. Operation time for ESD was much longer than for CER, although the en bloc resection rate was significantly higher in the ESD group. We found that procedure time and tumor size were associated, especially in ESD cases. Compared with the CER cases, as lesion size increased, the ESD procedure time increased.

Even in the 20–29-mm group, the en bloc resection rate for CER was only 66.5 %, which is significantly lower than that of the ESD group. As lesion size increased, the en bloc resection rate for CER decreased; the en bloc resection rate in the ≥40-mm group was only 12.3 %. In contrast, the en bloc resection rate for ESD was maintained at >93 %, even in the ≥40-mm group.

Complication rate

The number of delayed bleeding cases was 18 (1.7 %) in the CER group and 18 (2.2 %) in ESD ($P = 0.3$). The number of perforation cases in these groups was 8 (0.8 %) and 16 (2 %; $P < 0.05$), respectively. The ESD perforation rate was higher than the CER rate, but most ESD and CER perforation cases were successfully treated endoscopically; only three cases (1 CER, 2 ESD) required emergency surgery.

Table 3 Macroscopic type of lesion, by procedure type and lesion size

Range of lesion sizes (mm)	20–29 CER/ESD	30–39 CER/ESD	≥40 CER/ESD	Subtotal CER/ESD	Total
Lesion number	729/219	194/258	106/339	1,029/816	1,845
(%)	(23)	(57)	(76)	(44)	
Macroscopic type					
Protruded	363/24	87/27	25/17	475/68	543
(%)	(6)	(24)	(40)	(13)	
Mixed	88/28	39/86	56/220	183/334	517
(%)	(24)	(69)	(80)	(65)	
Flat	275/164	68/144	25/101	368/409	777
(%)	(37)	(68)	(80)	(53)	
Depressed	3/0	–/–	–/–	3/0	3
(%)	(0)			(0)	
Recurrence	0/3	0/1	0/1	0/5	5
(%)	(100)	(100)	(100)	(100)	

Table 4 Treatment results by procedure type and lesion size

Range of lesion sizes (mm)	CER			ESD			CER	ESD	Total
	20–29	30–39	≥40	20–29	30–39	≥40	Sub total		
Lesion number	729	194	106	219	258	339	1029	816	1,845
Procedure time (min, mean ± SD)	13 ± 13	43 ± 23	42 ± 46	66 ± 45	79 ± 42	129 ± 83	18 ± 23	96 ± 69	53 ± 63
Complication									
Delayed bleeding	12	4	2	3	7	8	18	18	36
(%)	(1.6)	(2.1)	(1.9)	(1.4)	(2.7)	(2.4)	(1.7)	(2.2)	(2)
Perforation	5	3	0	4	7	5	8	16	24
(%)	(0.7)	(1.5)	(0)	(1.8)	(2.7)	(1.5)	(0.8)	(2.0)	(1.3)
Emergency surgical operation	–	1	–	–	–	2	1	2	3
(%)		(0.5)				(0.6)	(0.1)	(0.2)	(0.2)
En bloc resection rate	485	88	13	206	248	317	586	771	1,357
(%)	(66.5)	(45.4)	(12.3)	(94.1)	(96.1)	(93.5)	(56.9)	(94.5)	(73.6)
Non-curative resection	33	9	2	23	24	31	44	77	122
(%)	(4.5)	(4.6)	(1.9)	(10.5)	(9.3)	(9.1)	(4.3)	(9.4)	(6.6)
Additional surgery	29	9	3	17	22	23	41	62	103
(%)	(4.0)	(4.6)	(2.8)	(7.8)	(8.5)	(6.8)	(4.0)	(7.6)	(5.6)

Pathological results and additional surgery

Histopathological assessment led to the diagnosis of 44 (4.3 %) CER cases and 77 (9.4 %) ESD cases as noncurative resections. Furthermore, 41 CER patients (4 %) and 62 ESD patients (7.6 %) underwent additional surgery.

Discussion

Key findings

In this prospective, multicenter study in Japan, we surveyed the current status of endoscopic treatment for relatively large CRNs (≥20 mm). As size increased, Japanese endoscopists were more likely to select ESD, especially for the treatment of flat- and mixed-type CRNs. As a result, the en bloc resection rate for ESD was significantly higher than for CER, although complication rates were very low in both groups. Despite longer procedure time, ESD is becoming safe and is considered a standard procedure in Japan for the treatment of large, superficial CRNs.

Before this study, Saito et al. reported the results of an initial, prospective, multicenter cohort study of ESD [18]. They analyzed the results of all colorectal ESD cases from the time of introduction of the procedure, performed at ten specialized facilities ($n = 1{,}111$). By contrast, the present study was planned after approval of ESD with advanced medical care systems, with a strict treatment indication; therefore, both highly advanced medical facilities and general facilities participated, enrolling the cases in a limited research registration period. In our study, the overall perforation rate was only 1.3 % ($n = 24$), and the rate of emergency surgery was extremely low (0.3 %, $n = 3$) compared with the previous study [18], suggesting improved safety of colorectal ESD in various facilities.

Japanese guidelines for ER for colorectal cancer and ESD indication

According to the guidelines of the JSCCR, CRNs diagnosed as clinical mucosal cancer or superficial submucosal cancer (<1,000 μm) are indicated for ER. However, 20 mm is the largest size of a tumor that can be easily resected en bloc by polypectomy or snare EMR. If the preoperative diagnosis is adenoma or carcinoma in adenoma, piecemeal resection can be performed. It should be noted, however, that piecemeal resection is associated with a high incomplete resection rate and a high local recurrence rate. Therefore, such lesions were a relative indication for surgical resection [22]. After introduction of the ESD technique for CRN treatment, it became possible to perform en bloc resections for lesions measuring > 20 mm. Our study shows that Japanese endoscopists selected ESD rather than CER, as tumor size increased, especially for mixed- and flat-type CRNs. Endoscopists make this choice, because they know the pathological character of large CRNs, which incur the risk of noncurative resection contrary to pre-ESD expectations and because they know the indication of colorectal ESD.

Treatment selection and outcome as related to tumor size

In this study, we performed endoscopic treatment according to the guidelines of JSCCR and indication of ESD. As size increased, selection of ESD became more common, perhaps because Japanese endoscopists understand the difficulty of performing en bloc resection for larger CRNs by CER.

However, ESD has a big limitation. As tumor size increased, procedure time increased, compared with CER. Our data showed that the average procedure time for colorectal ESD for lesions measuring ≥40 mm was more than 2 h. It should be noted that such lesions were treated by surgery before ESD became widespread; therefore, it may be more informative to compare ESD procedure time with that of surgical cases instead of EMR cases. ESD for CRNs measuring ≥40 mm is thought to be a difficult and time-consuming treatment, so we recommend that ESD for lesions <40 mm be considered a general procedure but that lesions measuring ≥40 mm should be treated in medical facilities with more experienced staff.

Treatment selection as related to tumor macroscopic type

In the 20–29-mm group, protruded-type CRNs were likely to be treated by CER. However, the proportion of CER for mixed and flat lesions was less than that for protruded type. As the lesion size increased, mixed and flat lesions were more likely to be treated by ESD. As the proportion of the flat component in the CRN groups increased, the proportion of ESD increased.

En bloc resection rate and complication rate

We found that the en bloc resection rate for ESD was significantly higher than for CER, although complication rates in both groups were quite low in these representative Japanese facilities. The ESD technique enabled complete resection even for the large-sized tumors. This may indicate that recent improvements in endoscopic devices and instruments have reduced complications (such as perforation) in ESD. Most perforation cases were managed

endoscopically; only two ESD cases required emergency surgical treatment. Due to these improvements, the ESD technique is now widely accepted for the management of large CRNs in Japan.

Considering the learning curve for colorectal ESD, in a previous study, we retrospectively reviewed clinical outcomes of colorectal ESD performed by trainees. Under the guidance of experienced specialists, trainee endoscopists are able to perform colorectal ESD without serious complications after preparatory training and experience with ≥30 cases [45]. Saito et al. [18] reported that the complication rate was 17.6 % at medical facilities in which the number of ESDs performed was less than 50. Univariate and multivariate analysis revealed that large tumor size (>50 mm) and less experience performing ESDs (<50 cases) were independent risk factors for complications [15]. Tanaka et al. [19] reported that the perforation rate in colorectal ESD decreased annually with experience.

Multicenter studies of ER have been conducted outside Japan. Moss et al. [46] reported outcomes of ER for lesions more than 20 mm, in an important Australian prospective, multicenter, observational study. They concluded that EMR is a safe and effective therapy for large sessile polyps. Some differences between their study and ours include the following: (1) their study reported only EMR results; (2) the percentage of submucosal cancers in their study was relatively low compared with our study (33 cases, 6.9 % and 220 cases, 11.9 %, respectively). Based on these differences, we assume that the Australian group referred some cases directly for surgical resection, whereas we may perform ESD as the first treatment. Long-term follow up evaluation was not extensive in both studies.

Saito et al. [9] reported the results of long-term follow-up after EMR and ESD and described one recurrence case as invasive cancer after 2.5 years; the case was histologically diagnosed in the first EPMR as a curative resection. In an evaluation of one leading Japanese hospital, Kobayashi et al. [47] reported that after introduction of colonic ESD, use of ER was widespread and reduced the incidence of repeat surgery for large-size intramucosal cancer.

Moss et al. also concluded that lesions having a high possibility of submucosal deep invasion, such as LST-NG or lesions with advanced pit pattern, should be treated en bloc to achieve accurate pathological assessment. In light of these findings, our study shows that ESD is becoming a standard procedure for en bloc resection in Japan.

Limitations

The major strength of our study is the large sample size; however, our study has some limitations. First, this was a prospectively enrolled, multicenter cohort study, not randomized; thus, eligibility criteria for performing colorectal ESDs were sometimes unclear at some institutions. Until the end of 2011, ESD was performed in more than 180 medical facilities in Japan as a generalized technique; therefore, randomization between CER and ESD was considered too difficult. Second, the recurrence rate of CER and ESD 1 year after initial endoscopic treatment is an important goal of this working group study; therefore, we will analyze those data and report them elsewhere. For the prescription of colorectal ESD by Japanese national health insurance, an additional nationwide survey to assess the clinical outcomes of colorectal ESD is recommended.

Conclusions

The en bloc resection rate for ESD was significantly higher than that for CER regardless of the tumor size; otherwise, submucosal cancer was more common in the ESD group. In addition, there was no significant difference in complications between the two groups. Our study proves that ESD is a feasible treatment for patients with mucosal CRN >20 mm, although long-term outcomes should be evaluated in the future. Such findings were presented at the annual meeting of United European Gastroenterology Week, 2012. For en bloc resection of lesions measuring ≥40 mm, ESD is an essential technique, but the procedure must be performed by experienced endoscopists in well-equipped medical facilities, because the procedure time is long.

Acknowledgments This study was performed within the framework of a project undertaken by the Colorectal Endoscopic Resection Standardization Implementation Working Group in Japanese Society for Cancer of the Colon and Rectum Grants.

Disclosures Takeshi Nakajima, Yutaka Saito, Shinji Tanaka, Hiroyasu Iishi, Shin-ei Kudo, Hiroaki Ikematsu, Masahiro Igarashi, Yuusuke Saitoh, Yuji Inoue, Kiyonori Kobayashi, Takashi Hisasbe, Takahisa Matsuda, Hideki Ishikawa, and Ken-ichi Sugihara have no conflict of interests or financial ties to disclose.

Appendix

Facilities that participated the study

The patients were enrolled at the 18 institutions affiliated with the Colorectal Endoscopic Resection Standardization Implementation Working Group of JSCCR as follows: (1) Endoscopy Division, National Cancer Center Hospital, Tokyo, Japan (Takeshi Nakajima, Yutaka Saito, Takahisa Matsuda); (2) Department of Endoscopy, Hiroshima University Hospital, Hiroshima, Japan (Shinji Tanaka); (3)

Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan (Hiroyasu Iishi); (4) Digestive Disease Center, Showa University Northern Yokohama Hospital, Kanagawa, Japan (Shin-ei Kudo); (5) Department of Gastroenterology & Gastrointestinal Oncology, National Cancer Center Hospital East, Chiba, Japan (Hiroaki Ikematsu); (6) Department of Endoscopy, Cancer Institute Ariake Hospital, Tokyo, Japan (Masahiro Igarashi); (7) Digestive Disease Center, Asahikawa City Hospital, Hokkaido, Japan (Yuusuke Saitoh); (8) Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo, Japan (Yuji Inoue); (9) Department of Gastroenterology, Kitasato University East Hospital, Kanagawa, Japan (Kiyonori Kobayashi); (10) Department of Gastroenterology, Fukuoka University Chikushi Hospital, Fukuoka, Japan (Takashi Hisasbe); (11) Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan (Osamu Tsuruta); (12) Gastrointestinal Center, Sano Hospital, Hyogo, Japan (Yasushi Sano); (13) Department of Gastroenterology, Akita Red Cross Hospital, Akita, Japan (Hiro-o Yamano); (14) Department of Gastroenterology, JR West Osaka Railway Hospital, Osaka, Japan (Seiji Shimizu); (15) Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan (Naohisa Yahagi); (16) Department of Surgery, Teikyo University Hospital, Tokyo, Japan (Toshiaki Watanabe); (17) Department of Gastroenterology, Chofu Surgical Clinic, Tokyo, Japan (Hisashi Nakamura); (18) Gastroenterology, Takahiro Fujii Clinic, Tokyo, Japan (Takahiro Fujii).

References

1. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, Jemal A, Schymura MJ, Lansdorp-Vogelaar I, Seeff LC, van Ballegooijen M, Goede SL, Ries LA (2010) Annual report to the nation on the status of cancer, 1975–2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer 116:544–573
2. Muto T, Bussey HJ, Morson BC (1975) The evolution of cancer of the colon and rectum. Cancer 36:2251–2270
3. Winawer SJ, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Sternberg SS, Waye JD, Schapiro M, Bond JH, Panish JF et al (1993) Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. N Engl J Med 329:1977–1981
4. Kitajima K, Fujimori T, Fujii S, Takeda J, Ohkura Y, Kawamata H, Kumamoto T, Ishiguro S, Kato Y, Shimoda T, Iwashita A, Ajioka Y, Watanabe H, Watanabe T, Muto T, Nagasako K (2004) Correlations between lymph node metastasis and depth of submucosal invasion in submucosal invasive colorectal carcinoma: a Japanese collaborative study. J Gastroenterol 39:534–543
5. Soetikno RM, Gotoda T, Nakanishi Y, Soehendra N (2003) Endoscopic mucosal resection. Gastrointest Endosc 57:567–579
6. Kudo S (1993) Endoscopic mucosal resection of flat and depressed types of early colorectal cancer. Endoscopy 25:455–461
7. Tanaka S, Haruma K, Oka S, Takahashi R, Kunihiro M, Kitadai Y, Yoshihara M, Shimamoto F, Chayama K (2001) Clinicopathologic features and endoscopic treatment of superficially spreading colorectal neoplasms larger than 20 mm. Gastrointest Endosc 54:62–66
8. Hotta K, Fujii T, Saito Y, Matsuda T (2009) Local recurrence after endoscopic resection of colorectal tumors. Int J Colorectal Dis 24:225–230
9. Saito Y, Fukuzawa M, Matsuda T, Fukunaga S, Sakamoto T, Uraoka T, Nakajima T, Ikehara H, Fu KI, Itoi T, Fujii T (2010) Clinical outcome of endoscopic submucosal dissection versus endoscopic mucosal resection of large colorectal tumors as determined by curative resection. Surg Endosc 24:343–352
10. Ono H, Kondo H, Gotoda T, Shirao K, Yamaguchi H, Saito D, Hosokawa K, Shimoda T, Yoshida S (2001) Endoscopic mucosal resection for treatment of early gastric cancer. Gut 48:225–229
11. Oka S, Tanaka S, Kaneko I, Mouri R, Hirata M, Kawamura T, Yoshihara M, Chayama K (2006) Advantage of endoscopic submucosal dissection compared with EMR for early gastric cancer. Gastrointest Endosc 64:877–883
12. Gotoda T, Kaltenbach T, Soetikno R (2008) Is en bloc resection essential for endoscopic resection of GI neoplasia? Gastrointest Endosc 67:805–807
13. Gotoda T, Kondo H, Ono H, Saito Y, Yamaguchi H, Saito D, Yokota T (1999) A new endoscopic mucosal resection procedure using an insulation-tipped electrosurgical knife for rectal flat lesions: report of two cases. Gastrointest Endosc 50:560–563
14. Yamamoto H, Kawata H, Sunada K, Satoh K, Kaneko Y, Ido K, Sugano K (2002) Success rate of curative endoscopic mucosal resection with circumferential mucosal incision assisted by submucosal injection of sodium hyaluronate. Gastrointest Endosc 56:507–512
15. Saito Y, Uraoka T, Matsuda T, Emura F, Ikehara H, Mashimo Y, Kikuchi T, Fu KI, Sano Y, Saito D (2007) Endoscopic treatment of large superficial colorectal tumors: a case series of 200 endoscopic submucosal dissections (with video). Gastrointest Endosc 66:966–973
16. Antillon MR, Bartalos CR, Miller ML, Diaz-Arias AA, Ibdah JA, Marshall JB (2008) En bloc endoscopic submucosal dissection of a 14-cm laterally spreading adenoma of the rectum with involvement to the anal canal: expanding the frontiers of endoscopic surgery (with video). Gastrointest Endosc 67:332–337
17. Soetikno R, Gotoda T (2009) Con: colonoscopic resection of large neoplastic lesions is appropriate and safe. Am J Gastroenterol 104:272–275
18. Saito Y, Uraoka T, Yamaguchi Y, Hotta K, Sakamoto N, Ikematsu H, Fukuzawa M, Kobayashi N, Nasu J, Michida T, Yoshida S, Ikehara H, Otake Y, Nakajima T, Matsuda T, Saito D (2010) A prospective, multicenter study of 1111 colorectal endoscopic submucosal dissections (with video). Gastrointest Endosc 72:1217–1225
19. Tanaka S, Oka S, Chayama K (2008) Colorectal endoscopic submucosal dissection: present status and future perspective, including its differentiation from endoscopic mucosal resection. J Gastroenterol 43:641–651
20. Tanaka S, Terasaki M, Kanao H, Oka S, Chayama K (2012) Current status and future perspectives of endoscopic submucosal dissection for colorectal tumors. Dig Endosc 24(Suppl 1):73–79
21. Niimi K, Fujishiro M, Kodashima S, Goto O, Ono S, Hirano K, Minatsuki C, Yamamichi N, Koike K (2010) Long-term outcomes of endoscopic submucosal dissection for colorectal epithelial neoplasms. Endoscopy 42:723–729
22. Watanabe T, Itabashi M, Shimada Y, Tanaka S, Ito Y, Ajioka Y, Hamaguchi T, Hyodo I, Igarashi M, Ishida H, Ishiguro M,

Kanemitsu Y, Kokudo N, Muro K, Ochiai A, Oguchi M, Ohkura Y, Saito Y, Sakai Y, Ueno H, Yoshino T, Fujimori T, Koinuma N, Morita T, Nishimura G, Sakata Y, Takahashi K, Takiuchi H, Tsuruta O, Yamaguchi T, Yoshida M, Yamaguchi N, Kotake K, Sugihara K, Japanese Society for Cancer of the Colon and Rectum (2012) Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2010 for the treatment of colorectal cancer. Int J Clin Oncol 17(1):1–29

23. Taku K, Sano Y, Fu KI, Saito Y, Matsuda T, Uraoka T, Yoshino T, Yamaguchi Y, Fujita M, Hattori S, Ishikawa T, Saito D, Fujii T, Kaneko E, Yoshida S (2007) Iatrogenic perforation associated with therapeutic colonoscopy: a multicenter study in Japan. J Gastroenterol Hepatol 22:1409–1414
24. Uraoka T, Kawahara Y, Kato J, Saito Y, Yamamoto K (2009) Endoscopic submucosal dissection in the colorectum: present status and future prospects. Dig Endosc 21(Suppl 1):S13–S16
25. Ueno H, Mochizuki H, Hashiguchi Y, Shimazaki H, Aida S, Hase K, Matsukuma S, Kanai T, Kurihara H, Ozawa K, Yoshimura K, Bekku S (2004) Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology 127:385–394
26. Kudo S, Kashida H, Tamura T, Kogure E, Imai Y, Yamano H, Hart AR (2000) Colonoscopic diagnosis and management of nonpolypoid early colorectal cancer. World J Surg 24:1081–1090
27. Fujii T, Hasegawa RT, Saitoh Y, Fleischer D, Saito Y, Sano Y, Kato S (2001) Chromoscopy during colonoscopy. Endoscopy 33:1036–1041
28. Matsuda T, Saito Y, Fujii T, Uraoka T, Nakajima T, Kobayashi N, Emura F, Ono A, Shimoda T, Ikematsu H, Fu KI, Sano Y, Fujimori T (2009) Size does not determine the grade of malignancy of early invasive colorectal cancer. World J Gastroenterol 15:2708–2713
29. Matsuda T, Fujii T, Saito Y, Nakajima T, Uraoka T, Kobayashi N, Ikehara H, Ikematsu H, Fu KI, Emura F, Ono A, Sano Y, Shimoda T, Fujimori T (2008) Efficacy of the invasive/non-invasive pattern by magnifying chromoendoscopy to estimate the depth of invasion of early colorectal neoplasms. Am J Gastroenterol 103:2700–2706
30. Sano Y, Ikematsu H, Fu KI, Emura F, Katagiri A, Horimatsu T, Kaneko K, Soetikno R, Yoshida S (2009) Meshed capillary vessels by use of narrow-band imaging for differential diagnosis of small colorectal polyps. Gastrointest Endosc 69:278–283
31. Ikematsu H, Matsuda T, Emura F, Saito Y, Uraoka T, Fu KI, Kaneko K, Ochiai A, Fujimori T, Sano Y (2010) Efficacy of capillary pattern type IIIA/IIIB by magnifying narrow band imaging for estimating depth of invasion of early colorectal neoplasms. BMC Gastroenterol 10:33
32. Wada Y, Kudo SE, Kashida H, Ikehara N, Inoue H, Yamamura F, Ohtsuka K, Hamatani S (2009) Diagnosis of colorectal lesions with the magnifying narrow-band imaging system. Gastrointest Endosc 70:522–531
33. Uraoka T, Saito Y, Matsuda T, Ikehara H, Gotoda T, Saito D, Fujii T (2006) Endoscopic indications for endoscopic mucosal resection of laterally spreading tumours in the colorectum. Gut 55:1592–1597
34. Tanaka S, Oka S, Chayama K (2008) Strategy of endoscopic treatment for colorectal tumor; recent progress and perspective. Springer, Tokyo
35. Saito Y, Fujii T, Kondo H, Mukai H, Yokota T, Kozu T, Saito D (2001) Endoscopic treatment for laterally spreading tumors in the colon. Endoscopy 33:682–686
36. Matsuda T, Parra-Blanco A, Saito Y, Sakamoto T, Nakajima T (2010) Assessment of likelihood of submucosal invasion in non-polypoid colorectal neoplasms. Gastrointest Endosc Clin N Am 20:487–496
37. (1983) General rules for clinical and pathological studies on cancer of the colon, rectum and anus. Part I. Clinical classification. Japanese Research Society for Cancer of the Colon and Rectum. Jpn J Surg 13:557–573
38. Uraoka T, Fujii T, Saito Y, Sumiyoshi T, Emura F, Bhandari P, Matsuda T, Fu KI, Saito D (2005) Effectiveness of glycerol as a submucosal injection for EMR. Gastrointest Endosc 61:736–740
39. Sano Y, Fu KI, Saito Y, Doi T, Hanafusa M, Fujii S, Fujimori T, Ohtsu A (2006) A newly developed bipolar-current needle-knife for endoscopic submucosal dissection of large colorectal tumors. Endoscopy 38(Suppl 2):E95
40. Fujishiro M, Yahagi N, Kakushima N, Kodashima S, Muraki Y, Ono S, Yamamichi N, Tateishi A, Oka M, Ogura K, Kawabe T, Ichinose M, Omata M (2007) Outcomes of endoscopic submucosal dissection for colorectal epithelial neoplasms in 200 consecutive cases. Clin Gastroenterol Hepatol 5:678–683 quiz 645
41. Tamegai Y, Saito Y, Masaki N, Hinohara C, Oshima T, Kogure E, Liu Y, Uemura N, Saito K (2007) Endoscopic submucosal dissection: a safe technique for colorectal tumors. Endoscopy 39:418–422
42. Toyonaga T, Man IM, Morita Y, Sanuki T, Yoshida M, Kutsumi H, Inokuchi H, Azuma T (2009) The new resources of treatment for early stage colorectal tumors: EMR with small incision and simplified endoscopic submucosal dissection. Dig Endosc 21(Suppl 1):S31–S37
43. Sakamoto T, Matsuda T, Nakajima T, Saito Y (2012) Efficacy of endoscopic mucosal resection with circumferential incision for patients with large colorectal tumors. Clin Gastroenterol Hepatol 10:22–26
44. Schlemper RJ, Riddell RH, Kato Y, Borchard F, Cooper HS, Dawsey SM, Dixon MF, Fenoglio-Preiser CM, Flejou JF, Geboes K, Hattori T, Hirota T, Itabashi M, Iwafuchi M, Iwashita A, Kim YI, Kirchner T, Klimpfinger M, Koike M, Lauwers GY, Lewin KJ, Oberhuber G, Offner F, Price AB, Rubio CA, Shimizu M, Shimoda T, Sipponen P, Solcia E, Stolte M, Watanabe H, Yamabe H (2000) The Vienna classification of gastrointestinal epithelial neoplasia. Gut 47:251–255
45. Sakamoto T, Saito Y, Fukunaga S, Nakajima T, Matsuda T (2011) Learning curve associated with colorectal endoscopic submucosal dissection for endoscopists experienced in gastric endoscopic submucosal dissection. Dis Colon Rectum 54:1307–1312
46. Moss A, Bourke MJ, Williams SJ, Hourigan LF, Brown G, Tam W, Singh R, Zanati S, Chen RY, Byth K (2011) Endoscopic mucosal resection outcomes and prediction of submucosal cancer from advanced colonic mucosal neoplasia. Gastroenterology 140:1909–1918
47. Kobayashi N, Saito Y, Sano Y, Uragami N, Michita T, Nasu J, Matsuda T, Fu KI, Fujii T, Fujimori T, Ishikawa T, Saito D (2007) Determining the treatment strategy for colorectal neoplastic lesions: endoscopic assessment or the non-lifting sign for diagnosing invasion depth? Endoscopy 39:701–705

J Epidemiol 2013;23(2):139-145
doi:10.2188/jea.JE20120102

Original Article

Obesity/Weight Gain and Breast Cancer Risk: Findings From the Japan Collaborative Cohort Study for the Evaluation of Cancer Risk

Sadao Suzuki[1], Masayo Kojima[1], Shinkan Tokudome[2], Mitsuru Mori[3], Fumio Sakauchi[3], Kenji Wakai[4], Yoshihisa Fujino[5], Yingsong Lin[6], Shogo Kikuchi[6], Koji Tamakoshi[7], and Akiko Tamakoshi[8], for the Japan Collaborative Cohort Study Group

[1]Department of Public Health, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
[2]National Institute of Health and Nutrition, Tokyo, Japan
[3]Department of Public Health, Sapporo Medical University School of Medicine, Sapporo, Japan
[4]Department of Preventive Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan
[5]Department of Preventive Medicine and Community Health, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan
[6]Department of Public Health, Aichi Medical University School of Medicine, Nagakute, Aichi, Japan
[7]Department of Nursing, Nagoya University School of Health Sciences, Nagoya, Japan
[8]Department of Public Health, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Received May 18, 2012; accepted October 30, 2012; released online February 23, 2013

ABSTRACT

Background: We analyzed data from the Japan Collaborative Cohort Study (36 164 women aged 40–79 years at baseline in 1988–1990 with no previous diagnosis of breast cancer and available information on weight and height) to examine the association between baseline body mass index (BMI)/weight gain from age 20 years and breast cancer risk in a non-Western population.

Methods: The participants were followed prospectively from enrollment until 1999–2003 (median follow-up: 12.3 years). During follow-up, breast cancer incidence was mainly confirmed through record linkage to population-based cancer registries. A Cox proportional hazards model was used to calculate hazard ratios (HRs) and 95% CIs for the association between breast cancer risk and body size.

Results: In 397 644.1 person-years of follow-up, we identified 234 breast cancer cases. Among postmenopausal women, the adjusted HR increased with BMI, with a significant linear trend ($P < 0.0001$). Risk was significantly increased among women with a BMI of 24 or higher (HR: 1.50, 95% CI: 1.09–2.08 for BMI of 24–28.9, and 2.13, 1.09–4.16 for BMI ≥ 29) as compared with women with a BMI of 20 to 23.9. Weight gain after age 20 years and consequent overweight/obesity were combined risk factors for postmenopausal breast cancer risk. This combined effect was stronger among women aged 60 years or older. However, the HRs were not significant in premenopausal women.

Conclusions: Our findings support the hypothesis that weight gain and consequent overweight/obesity are combined risk factors for breast cancer among postmenopausal women, particularly those aged 60 years or older.

Key words: breast cancer; obesity; weight gain; cohort study

INTRODUCTION

Since the early 1990s, breast cancer has been the most frequently diagnosed cancer in Japanese women.[1] Among women, the mortality rate of breast cancer is second only to that of stomach cancer. The recent continuous increase in breast cancer incidence has been an important public health concern in Japan, and the attention devoted to obesity/weight gain as a risk factor for breast cancer has also increased.

Obesity is a well-known risk factor for postmenopausal breast cancer.[2–4] Numerous epidemiologic studies have reported positive associations between obesity and breast cancer risk among white,[5–10] African-American,[11–13] and East Asian women.[14–17] Furthermore, weight gain has been reported as an independent risk factor.[8,9,11,17–21] Several studies have reported an inverse association between body weight in early adulthood and breast cancer incidence.[17,19,20] However, the association has been somewhat inconsistent among

Address for correspondence. Sadao Suzuki, Department of Public Health, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan (e-mail: ssuzuki@med.nagoya-cu.ac.jp).

premenopausal women. Obesity is associated with a decreased risk of breast cancer among white women,[4,10,22–24] although accumulating evidence suggests that the inverse association is limited to women with estrogen receptor- and progesterone receptor-positive tumors.[25–28] Studies of non-white racial/ethnic groups are more limited, and the results are mixed.

To assist in cancer prevention, we analyzed data from a large cohort study—the Japan Collaborative Cohort (JACC) Study—which included 64 327 Japanese women, to examine the association of baseline body mass index (BMI)/weight gain with breast cancer risk, considering menopausal status at baseline. We also investigated the interaction of age on this association.

METHODS

Study population

We analyzed data from the JACC Study, a prospective cohort study that evaluated cancer risk associated with lifestyle factors among the Japanese population. The study has been described in detail previously.[29,30] In brief, the JACC Study was initiated in 1988–1990 and included 110 792 individuals (46 465 men and 64 327 women) aged 40 to 79 years from 45 areas throughout Japan. All participants were subsequently followed for all-cause mortality. In addition, study participants living in 24 areas with cancer registry systems were followed for cancer incidence.

Of the 64 327 women in the baseline cohort, 38 720 lived in the 24 areas where data on cancer incidence were available. The present study excluded 248 women who reported a previous diagnosis of breast cancer and 2308 women who did not provide information on height or weight at baseline. Thus, 36 164 women were included in the present analysis.

Informed consent was obtained from the participants in the form of signatures on the cover pages of the questionnaires, with the exception of those in a few study areas where informed consent was provided at the group level after the aims and data confidentiality had been explained to community leaders. The Ethics Board of Sapporo Medical University approved our study.

Exposure assessment

As a relative indicator of body weight, BMI was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Information regarding weight and height was obtained from the self-reported questionnaire. Change in weight from age 20 years to the baseline measurement was calculated as the difference in the reported values at baseline among 20 418 women whose information on weight at age 20 years was available. We did not use BMI for age 20 years because we did not have access to height information at that age.

Information on other potential breast cancer risk factors such as family history of breast cancer, tobacco and alcohol use, age at menarche, marital status, parity, age at first birth, menopausal status, hormone use, and physical activity was collected in the baseline questionnaire. We have no information after baseline, including information on body size or menopausal status.

Follow-up and identification of breast cancer cases

We followed the study participants from enrollment until 1999–2003. During this period, a population registry was used in each municipality to ascertain the residential status and vital status of the participants. In Japan, the Family Registration Law requires registration of all deaths, which theoretically provides complete mortality data. Breast cancer incidence was confirmed mainly through record linkage to population-based cancer registries in each area. To complete the incidence data, we also conducted a systematic review of death certificates and medical records at major local hospitals in some areas.

During the study period, 1799 (5.0%) participants were lost to follow-up due to moving out of their designated study areas. Among the 234 breast cancer cases, no information on diagnosis was available for 13 (5.6%), ie, they were identified with death certification only (DCO). The world standard for DCO in cancer registration is less than 10%. The mortality-to-incidence ratio for breast cancer was 0.262 (58/221) in the cohort covered by cancer registries, which was within the range calculated using available data from population-based cancer registries in Japan (0.20–0.30). We estimated that 36.5 cases of incident breast cancer were not included in the cancer registries.

Statistical analysis

For each cohort subject, person-years of follow-up were counted as time from enrollment to diagnosis of breast cancer, death from any cause, or end of follow-up (1999–2003), whichever occurred first. For breast cancer cases ascertained only by death certificates, person-years of follow-up were calculated from enrollment to death from breast cancer. Those who died from causes other than breast cancer or who moved out of the study areas were treated as censored cases. We used a Cox proportional hazards model to estimate hazard ratios (HRs) and 95% CIs for the association of breast cancer risk with baseline BMI/weight change. Women were divided into 5 categories, using baseline BMI (in accordance with the World Health Organization classification)[31]: less than 18.5, 18.5–19.9, 20–23.9, 24–28.9, and 29 kg/m^2 or higher. Furthermore, BMI was entered directly to evaluate the linear trend of relative weight. The effect of age on the association between BMI and breast cancer risk was examined by analyzing the relationship between age and BMI. Finally, to investigate the combined effect of baseline BMI and weight change from age 20 years, we recategorized the participants into 4 groups using the following cutoff points: baseline BMI less than 24 kg/m^2 and weight gain of less than 10 kg from age 20 years to the baseline measurement.

Table 1. Baseline characteristics associated with BMI in the JACC Study

Characteristics	BMI at baseline				
	<18.5	18.5–19.9	20–23.9	24–28.9	≥29
Number, *n* (row%)	2373 (6.6%)	3654 (10.1%)	18231 (50.4%)	10737 (29.7%)	1169 (3.2%)
Height (cm)	152.0 ± 7.0	151.0 ± 5.8	151.3 ± 5.5	150.7 ± 5.6	149.3 ± 6.4
BMI	17.4 ± 1.0	19.3 ± 0.4	22.0 ± 1.1	25.8 ± 1.3	31.0 ± 2.0
Weight at age 20 years (kg)	46.5 ± 6.1	47.8 ± 5.7	49.6 ± 6.2	51.0 ± 6.6	52.2 ± 6.8
Weight change[a] (kg)	−6.3 ± 5.9	−3.7 ± 5.4	1.1 ± 6.3	7.8 ± 7.0	17.1 ± 8.3
Age at inclusion (years)	61.3 ± 10.8	58.5 ± 10.7	57.1 ± 10.0	57.9 ± 9.3	58.3 ± 9.3
Age at menarche (years)	15.2 ± 1.8	15.0 ± 1.8	14.9 ± 1.8	14.8 ± 1.8	14.9 ± 1.9
Age at first birth (years)	25.4 ± 3.5	25.2 ± 3.3	25.0 ± 3.2	24.9 ± 3.2	25.0 ± 3.5
Age at menopause (years)	48.2 ± 4.9	48.5 ± 4.5	48.8 ± 4.6	48.7 ± 4.8	48.5 ± 5.1
Years of education	16.5 ± 2.2	16.6 ± 2.1	16.7 ± 2.1	16.3 ± 2.0	16.0 ± 2.1
Nulliparous, *n* (%)	144 (6.6%)	175 (5.2%)	700 (4.1%)	404 (4.0%)	53 (4.9%)
Not married, *n* (%)	69 (3.4%)	61 (1.9%)	227 (1.4%)	111 (1.2%)	20 (2.0%)
Exogenous female hormone use, *n* (%)	124 (6.2%)	160 (5.2%)	792 (5.1%)	471 (5.2%)	61 (6.1%)
Family history of breast cancer, *n* (%)	30 (1.3%)	42 (1.2%)	269 (1.5%)	167 (1.6%)	13 (1.1%)
Current smoker, *n* (%)	162 (7.6%)	201 (6.2%)	779 (4.7%)	470 (4.8%)	81 (7.7%)
Current drinker, *n* (%)	453 (20.4%)	790 (23.1%)	4250 (24.8%)	2444 (24.2%)	223 (20.5%)

BMI, body mass index.
Mean (SD) or %, calculated from subjects with no missing data for any variable.
[a]Difference in body weight at age 20 years and baseline.

We evaluated the association using age-adjusted and multivariable models with adjustment for age (using 10-year age groups), tobacco smoking (never, past, current, or unknown), alcohol consumption (never, past, current, or unknown), age at menarche (<15, 15–16, ≥17 years, or unknown), education level (attended school until age <16, 16–18, ≥19 years, or unknown), parity (nulliparous, 1, 2–3, ≥4 births, or unknown), age at first birth (<22, 22–23, 24–25, ≥26 years, or unknown), menopausal status (premenopausal at baseline, <45, 45–49, or ≥50 years), use of exogenous female hormone (yes, no, or unknown), first-degree family history of breast cancer (yes, no, or unknown), and physical activity categories[32] (4 groups using the following cutoff points of physical activity: daily walking <1 h and exercise time <1 h a week, or unknown). All analyses were performed with regard to menopausal status and stratified by 6 study areas (Hokkaido and Tohoku, Kanto, Chubu, Kinki, Chugoku, and Kyushu).

We repeated the analysis after excluding the first 2 years of follow-up, during which 38 cases of breast cancer were diagnosed. All *P* values were 2-sided, and a *P* value less than 0.05 was considered to indicate statistical significance. All regression analyses were performed using the PROC PHREG procedure of SAS Version 9.1 (SAS Institute, Cary, NC, USA). Study areas were not incorporated in the Cox model with other potential confounders but were adjusted for using the strata option in the PHREG procedure.

RESULTS

Average age and BMI (SD) at baseline of the 36164 women were 57.8 (10.0) years and 22.9 (3.1) kg/m^2, respectively. In 397644.1 person-years of follow-up (median follow-up time, 12.3 years), we identified 234 breast cancer cases. Table 1 shows the distribution of risk factors for breast cancer in association with BMI. Women with a BMI less than 18.5 were older and more likely to be nulliparous and unmarried. The 2 extreme BMI groups had higher percentages of smokers and lower percentages of drinkers. Groups with higher BMI at baseline had increased weights at age 20 years and greater weight gain from age 20 years to baseline. However, the difference in weight at age 20 years between the 2 extreme BMI groups was relatively small (46.5 kg vs 52.2 kg), and weight change from age 20 years (−6.3 kg vs 17.1 kg) was a stronger contributor to body size at baseline. The average (SD) overall change in weight during the period was 2.7 (8.2) kg.

Table 2 shows breast cancer risk associated with baseline BMI in relation to menopausal status. After adjustment for potential confounding factors, neither a significant HR nor a linear trend was observed among the 8131 premenopausal women. In contrast, among 28033 postmenopausal women, the adjusted HR increased with BMI and showed a significant linear trend ($P < 0.0001$). Furthermore, significantly increased risk was observed among women with a BMI of 24 or higher (HR: 1.50, 95% CI: 1.09–2.08 for BMI of 24–28.9; 2.13, 1.09–4.16 for BMI ≥29) as compared with those with a BMI of 20 to 23.9. The adjusted HRs per 5-kg/m^2 increment in BMI among pre- and postmenopausal women were 0.95 (95% CI: 0.60–1.50) and 1.68 (95% CI: 1.34–2.01), respectively.

To observe the effect of age on the association between BMI and breast cancer risk among postmenopausal women, we calculated the HR for a 5-kg/m^2 increment in BMI in younger (40–59 years) and older (60–79 years) age groups. The older group had a higher HR (2.00, 95% CI: 1.48–2.70) than the younger group (1.37, 95% CI: 0.96–1.96) for a