Table 2. Serum levels of oxLDL, oLAB, total cholesterol, and α-tocopherol and BMI for colorectal cancer cases and controls

	Cases		Controls		*P*
	n		*n*		
oxLDL (units/L), median (25th-75th percentiles)	119	39.2 (31.6-47.8)	316	36.2 (29.2-44.7)	0.045
oLAB (units/L), median (25th-75th percentiles)	153	201.0 (142.0-312.0)	376	192.0 (135.5-272.5)	0.120
Total cholesterol (mmol/L), mean ± SD	159	5.22 ± 0.96	382	5.17 ± 0.98	0.225
α-Tocopherol (μmol/L), median (25th-75th percentiles)	155	21.87 (15.67-27.35)	377	21.69 (17.30-26.75)	0.834
BMI (kg/m^2), mean ± SD	158	23.1 ± 3.4	380	23.2 ± 2.8	0.779

oLAB levels displayed no correlation with serum levels of oxLDL, total cholesterol, or α-tocopherol in either gender.

Table 4 shows ORs and 95% CIs for colorectal cancer by serum levels of oxLDL and oLAB after adjusting for confounding factors. ORs (95% CIs) across quartiles for serum oxLDL adjusted for gender, age, and study area (OR1) were 1.21 (0.57-2.55), 1.49 (0.71-3.14), and 2.34 (1.03-5.30; P_{trend} = 0.030). OR (95% CI) for serum oxLDL adjusted for gender, age, study area, smoking and drinking habits, intake frequency of green leafy vegetables, time spent in sports or physical exercise, family history of colorectal cancer, and BMI (OR2) was significantly higher in the highest quartile compared with the lowest quartile [3.65 (1.50-8.92); P_{trend} = 0.004]. OR1 and OR2 for oLAB tended to be higher in the highest quartile of serum oLAB but not significantly (OR1, 1.66; 95% CI, 0.91-3.01; P_{trend} = 0.148; OR2, 1.68; 95% CI, 0.90-3.13; P_{trend} = 0.140).

When the analysis was limited to incident cases and corresponding controls, the higher risk was still found in relation to higher serum levels of oxLDL. OR2s (95% CIs) for colorectal cancer across quartiles of serum oxLDL were 3.11 (1.09-8.87), 2.25 (0.79-6.39), and 4.77 (1.50-15.10; P_{trend} = 0.027). OR2s (95% CIs) for colorectal cancer across quartiles of serum oLAB were 0.67 (0.32-1.41), 0.89 (0.41-1.92), and 1.22 (0.51-2.62; P_{trend} = 0.412).

Associations of serum oxLDL and oLAB with risk of colorectal cancer were also evaluated after further adjustment for quintiles of total cholesterol and α-tocopherol (OR3). However, no substantial change in results was observed. When evaluated by gender, no apparent difference between males and females was noted.

The same analyses were attempted using only colon cancer cases (n = 80 for oxLDL and n = 106 for oLAB) and corresponding controls (n = 215 for oxLDL and n = 261 for oLAB). OR3s (95% CIs) for colon cancer across quartiles of serum oxLDL were 2.97 (0.97-9.06), 1.90 (0.55-6.59), and 4.68 (1.19-18.38; P_{trend} = 0.062). A similar trend was observed for serum oLAB levels: OR3s (95% CIs) across quartiles were 1.75 (0.73-4.20), 1.69 (0.68-4.15), and 2.20 (0.90-5.37; P_{trend} = 0.119).

Furthermore, modified data sets excluding cases diagnosed within 2 years from baseline were also analyzed. Results of these analyses were consistent with those of analyses without exclusion (data not shown).

Table 3. Spearman correlation coefficients (no. subjects) among serum levels of oxLDL, oLAB, total cholesterol, and α-tocopherol among control subjects

	oxLDL	oLAB
Males		
oLAB	0.066 (136)	
Total cholesterol	0.525* (138)	-0.023 (172)
α-Tocopherol	0.397* (140)	0.011 (169)
Females		
oLAB	0.021 (161)	
Total cholesterol	0.429* (166)	-0.093 (191)
α-Tocopherol	0.227* (168)	0.006 (192)

*P < 0.001.

Discussion

The present investigation represents the first prospective study to examine associations between serum oxLDL and risk of colorectal cancer. Significant positive associations were observed between serum oxLDL levels and risk of colorectal cancer. There was no association between serum oLAB levels and risk of colorectal cancer. Risk of colorectal cancer was higher in the presence of higher levels of serum oxLDL, independent of confounders. The mechanisms involved in this association between oxLDL and colorectal cancer remain unclear.

The adjustment for lifestyle factors, family history, and BMI somewhat strengthened the positive association between oxLDL and risk of colorectal cancer. This may not be in line with our initial hypothesis that serum oxLDL levels represent a marker reflecting lifestyles related to the cancer. Serum oxLDL may be a predictor of the risk independently of other risk factors.

There are some reports that studied the association between serum or plasma oxLDL levels and coronary heart disease (7, 28). It is well known that oxLDL is found in monocyte-derived macrophages in atherosclerosis lesions and that plasma oxLDL levels were significantly higher in patients with coronary artery disease (28). Several studies have been carried out on the modified forms of oxLDL, which are prepared by oxidizing LDL under various conditions *in vitro* (28). However, there is little information about oxLDL present *in vivo* (28).

We have also studied associations between serum carotenoids levels and risk of colorectal cancer in this prospective epidemiologic study. We found inverse associations of some carotenoids with colorectal cancer risk in men.[9] Crohn disease is a chronic inflammatory disorder and is associated with increased risk of colon cancer (29). Although the etiology of Crohn disease is unknown, patients with Crohn disease have increased

[9] Unpublished data.

Table 4. ORs and 95% CIs for colorectal cancer risk by serum levels of oxLDL and oLAB

	Range	Cases	Controls	OR1	95% CI	P_{trend}	OR2	95% CI	P_{trend}	OR3	95% CI	P_{trend}
oxLDL (units/L)												
Q1	≤29.1	22	79	1.00	—	0.030	1.00	—	0.004	1.00	—	0.038
Q2	29.2-36.1	26	79	1.21	0.57-2.55		1.55	0.70-3.46		1.15	0.49-2.72	
Q3	36.2-44.6	31	79	1.49	0.71-3.14		1.90	0.84-4.28		1.38	0.54-3.51	
Q4	≥44.7	40	79	2.34	1.03-5.30		3.65	1.50-8.92		3.10	1.04-9.23	
oLAB (units/L)												
Q1	≤135.4	34	94	1.00	—	0.148	1.00	—	0.140	1.00	—	0.212
Q2	135.5-191.9	41	96	1.14	0.64-2.01		0.98	0.54-1.80		1.11	0.58-2.11	
Q3	192.0-272.4	28	92	0.87	0.46-1.64		0.75	0.39-1.48		0.74	0.36-1.52	
Q4	≥272.5	50	94	1.66	0.91-3.01		1.68	0.90-3.13		1.69	0.85-3.35	

NOTE: OR1: OR adjusted for gender, age, and study area; OR2: OR adjusted for gender, age, study area, smoking and drinking habits, intake frequency of green leafy vegetables, time spent in sport or physical exercise, family history of colorectal cancer, and BMI; OR3: OR adjusted for gender, age, study area, smoking and drinking habits, intake frequency of green leafy vegetables, time spent in sport or physical exercise, family history of colorectal cancer, BMI, and serum levels of total cholesterol and α-tocopherol.

production of ROS (29). It was reported that lipid peroxidation and F_2 isoprostane was significantly higher in patients with Crohn disease than in healthy control subjects (30).

Various potentially toxic oxidized lipids are contained in oxLDL such as lipid peroxides, oxysterol, and aldehydes (31). These oxidized lipids elicit oxidative stress and lipid peroxidation (31). As oxLDL reduces antioxidant enzymes such as Cu/Zn superoxide dismutase (32) and glutathione peroxidase (33) and ROS degradation is decreased following increases in oxLDL (31), ROS levels are elevated. Lipid peroxidation is initiated by ROS attacks, generating large amounts of reactive products that have been implicated in tumor initiation and promotion (34). Increased levels of malondialdehyde, a major genotoxic carbonyl compound generated by lipid peroxidation (34), have been reported in tumor tissue from colorectal cancer patients compared with normal mucosa from the same individuals (35).

In another experimental study (8), oxLDL-induced oxidative stress enhanced p53 DNA binding activity and p53 protein synthesis. As a tumor suppressor, p53 is induced by various kinds of cell stress (36) to protect the cell. Genetic information is protected by the functions of p53, including induction of cell cycle arrest or apoptosis after DNA damage and maintenance of genomic stability (37). Given the above, high levels of oxLDL might induce excess stress against the cell. This stress may induce DNA damage and mutation, because oxidative stress is known to cause such damage (38). Mutation of *p53* gene is found in >50% of all human cancers and >75% of colorectal adenocarcinomas (39). Mutation of the *p53* gene is known to play crucial roles in tumor development and progression (37).

Cyclooxygenase-2 (COX-2) expression is reportedly induced by oxLDL in a murine macrophage-like cell line (40) and human monocytes (41). COX is an enzyme that initiates the conversion of arachidonic acid into all of the prostaglandins and thromboxanes (42). Lipid peroxidation is necessary for initiation of COX activity (43), and reactive oxygen intermediates (ROI) induce COX-2 (44). Levels of arachidonic acid (45) and prostaglandin E_2 (46) are higher in colon tumor than in normal colonic mucosa. Prostaglandin E_2, a major product of COX, stimulates proliferation and growth of human colorectal cancer cells (47).

Analysis of COX-2 expression (induced by cytokines, growth factors, and mitogens) has revealed elevated levels in up to 90% of sporadic colon carcinomas and 40% of colonic adenomas but no elevation in normal colonic epithelium (48). Recent clinical epidemiologic studies have shown that COX inhibitors such as aspirin and other nonsteroidal anti-inflammatory agents exert preventive effects on colorectal cancer (49, 50). Such inhibition of COX-2 is considered to lead to decreased incidence of colorectal cancer, although the mechanisms are not fully understood.

Functions of oxLDL such as increasing oxidative stress and inducing COX-2 expression might play an important role in colorectal carcinogenesis. At the very least, oxidative stress is increased in subjects with high levels of serum oxLDL, and oxidative stress should be related to colorectal carcinogenesis.

Epidemiologic studies showed the close association between insulin resistance and colon cancer risk (51). The consumption of excess dietary energy results in the development of insulin resistance with increased circulating levels of insulin, triglycerides, and nonesterified fatty acids. These circulating factors subject colonic epithelial cells to a proliferative stimulus and also expose them to reactive oxygen intermediates. Other study reported that LDL oxidizability is increased in insulin resistance subjects compared with healthy subjects (52). These long-term exposures are expected to result in the promotion of colon cancer.

Serum oLAB levels were not significantly associated with risk of colorectal cancer. Serum oLAB is generated from immunoresponses against oxLDL. Serum oLAB levels, in addition to serum oxLDL levels, may therefore also depend on various lifestyle factors such as dietary intake of antioxidants and smoking habits. Plasma oLAB levels are reported to show a negative correlation with plasma oxLDL levels in healthy subjects (53), and oLAB may play a role in maintaining low levels of blood oxLDL. Wide ranges of serum oLAB levels might reflect interindividual differences in immune responses rather than in oxLDL generation. The immune system is also affected by various lifestyle factors such as smoking habits. We considered that almost no relationship between serum oLAB and oxLDL in controls was derived from interindividual differences in immune responses. Interindividual difference in immune responses may

have also attenuated the association between serum oLAB and risk of colorectal cancer.

Although oxLDL is an oxidant and α-tocopherol is an antioxidant, our results show positive association between serum oxLDL and α-tocopherol levels. We suggest that this association was observed because serum LDL binds to α-tocopherol (54). Similarly, serum oxLDL is positively associated with serum cholesterol levels.

Cases included both colon and rectal cancers. Risk for colon cancer only was increased with high serum oxLDL levels after adjusting for gender, age, study area, and potential confounders. The sample population for rectum cancer cases was too small to analyze associations between serum levels of oxLDL and oLAB and risk of rectum cancer. These associations warrant further study.

In conclusion, the present study showed that increased levels of serum oxLDL represent a risk factor for colorectal cancer among Japanese. Although further investigations are needed to clarify the role of oxLDL in tumorigenesis for colorectal cancer, serum oxLDL levels may be one biomarker for predicting risk of colorectal cancer.

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LETTER TO THE EDITOR

Re: *Helicobacter pylori* Infection and Gastric Cancer: Facing the Enigmas

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Dear Sir,

Lunet and Barros[1] reported the results of an international ecologic study on *Helicobacter pylori* infection and incidence and mortality rates of gastric cancer after adjustment for intake of vegetables and fruit, cigarette smoking and alcohol drinking. They found positive correlations between *H. pylori* seroprevalence and the incidence of and mortality from gastric cancer for most countries and concluded that *H. pylori* infection is a definite factor of stomach cancer. However, they found negative correlations in African and Asian countries, for which they proposed the term "enigmas."

Stomach cancer may be caused by chronic inflammation related to *H. pylori* infection after initiation by exogenous and endogenous carcinogens.[2–4] The former include pyrolysate chemicals and components of tobacco smoke, and the latter include nitrosamines generated in the stomach from nitrite and amine precursors. Vegetables and fruit are classified as convincing preventive factors associated with intake of vitamin C and other antioxidants. However, intake of salt and salted foods is another important factor,[3–5] regarded as having promoting or progressing effects. Thus, the authors are advised to examine salt consumption or salting as a confounding factor in future studies.

Lunet and Barros intensively collected *H. pylori* seroprevalence data based on several assay systems and amalgamated them. Commercial IgG kits react with specific *H. pylori* strains and thus may yield false-negative results. The urea breath test detects bacteria that possess urease activity and may provide false-positive data. Analysis procedures should be standardized or one method, *e.g.*, the urea breath test, needs to be chosen for international comparisons. In addition, prevalence rates of *H. pylori* infection appear to differ across age groups,[2] *e.g.*, of the EUROGAST study,[6] and age-adjusted rates should be adopted for comparison.

There are some different genotypes of *H. pylori* with discrepant pathogenicity and different outcomes with regard to persistent inflammation: indeed, there are Western, African and East Asian types.[7–9] Of these, the East Asian type appears to be particularly pathogenic for chronic inflammation. Atrophic gastritis markers in the blood, like pepsinogen I, pepsinogen II and the ratio of the two,[10] which correlate well with the Sydney System for diagnosing gastritis,[11] need to be analyzed. Host genetic factors related to cellular immunity against bacterial infection and persistent inflammation may differ with ethnicity and should be examined.[12]

It thus appears premature to label the phenomenon described by Lunet and Barros as "enigmas". Variation in diagnostic techniques may to some extent exist in African and Asian countries. We need to accumulate data for standardized *H. pylori* seropositivity with adjustment for age and salt consumption along with vegetable and fruit intake and smoking. Furthermore, we should add information on chronic atrophic gastritis, *H. pylori* strain/genetic type and host genetic polymorphisms for cellular immunity for bacterial infection and chronic inflammation.

Yours sincerely,

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LETTER TO THE EDITOR

Condom Use Promotes Regression of Cervical Intraepithelial Neoplasia and Clearance of Human Papillomavirus: A Randomized Clinical Trial

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Dear Sir,

Hogewoning *et al.*[1] reported recently that condom use promotes regression of cervical intraepithelial neoplasia (CIN) lesions and clearance of human papillomavirus (HPV), according to their randomized clinical trial.

CIN/cervical cancer is increasingly defined as an infectious disease associated with HPV,[2] but under the influence of exogenous/endogenous carcinogenic agents, including tobacco-related carcinogens/mutagens (TRCM).[3–9]

Women are exposed to TRCM *via* their own smoking and inhaling sex partners' passive/environmental tobacco smoke.[10,11] Moreover, exposure of the cervical membrane to TRCM via semen/seminal fluid from smoking sex partners should not be overlooked.[12] In the data of Hogewoning *et al.*, smoking rates were lower in condom users than in the control group, and the odds ratio (95% CI) was estimated to be 0.35 (0.11–1.09). This was not statistically significant, however, which seems partly due to a low response rate (46%) and insufficient statistical power. Thus, the authors are requested to examine effects of own and sex partners' smoking, or execute multivariate analysis with adjustment for these parameters. They should at least discuss possible effects of smoking, environmental tobacco smoke and exposure via semen/seminal fluid.

Finally, as the authors concluded, it seems plausible that condom use is beneficial not only for prevention of HPV and other infectious agents, including HIV and STD-associated pathogens, but also for blockade of TRCM exposure.[5,8,12] A diaphragm-type contraceptive therefore may also favor CIN regression, HPV clearance and prevention of cervical cancer.

Yours sincerely,

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LETTER

The Mediterranean *vs* the Japanese diet

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Both the Mediterranean and Japanese diets are known to be healthy (Tokudome *et al*, 2000; Trichopoulou & Vasilopoulou, 2000; Ferro-Luzzi *et al*, 2002; Serra-Majem *et al*, 2003). People of the Mediterranean countries enjoy a low risk of cardiovascular disease, while Japanese are famous for their longevity/healthy life expectancy (UN, 1998). However, there are both similarities and discrepancies in intake of foods and beverages between the two cases. The Mediterranean diet is characterized by high consumption of cereals (wheat), vegetables and fruit, fish and olive oil (Trichopoulou & Vasilopoulou, 2000; Ferro-Luzzi *et al*, 2002; Serra-Majem *et al*, 2003). Japanese also consume large amounts of cereals (rice), vegetables and fruit, and fish, but there is much lower intake of energy and oils/fats (Tokudome *et al*, 2000; Health Promotion and Nutrition Division, 2003).

In a recent issue of *EJCN*, Dr Serra-Majem *et al* (2003) reported an interesting ecological finding that typical Mediterranean individuals consume high amounts of total lipids (approximately 100 g/day in males and 80 g in females) and also polyunsaturated fatty acids (PUFAs) in males, and lipids (more than 40% energy) and PUFAs in both genders along with high concentrations and proportions of monounsaturated fatty acids (MUFAs), largely from olive oil.

In contrast, the traditional Japanese diet has been characterized by low intake of total lipids, including saturated fatty acids, MUFAs and PUFAs, particularly of n-6 PUFAs, not only absolute concentrations as well as proportions (Okuyama *et al*, 1997; Tokudome *et al*, 2000). However, the recent past has seen a change from 20% energy from lipids to 30%, whereas the ratio of n-6 PUFAs/n-3 PUFAs has shifted from 2–3 to 4–5. We assume that these changes will enhance the risk of fat-related cancers, cardiovascular disease and cerebrovascular embolisms.

Therefore, we wonder if Dr Serra-Majem *et al* could provide information that the risk of cardiovascular disease is explained with reference to concentrations and/or percentage energy from total lipids, n-6 PUFAs and n-3 PUFAs together with its ratio. Furthermore, comments on whether the risk is modulated when the intake of vegetables and fruit is adjusted would be welcomed because they contain antioxidant nutrients, including α-tocopherol, carotenoids, vitamin C and folic acid.

There is evidence that not only absolute concentrations of total lipids but also the balance of fatty acids of n-6 PUFAs/n-3 PUFAs, in particular, are crucial to our health (Lands, 1995; Okuyama *et al*, 1997; Rose & Connolly, 1999). We propose that, even if olive oil comprises antioxidant nutrients, intake at high levels may be unhealthy. According to values for macronutrients set for the Japanese diet (Health Promotion and Nutrition Division, 2003), intake of 20–25% energy from lipids on average, with more than 50% from carbohydrates and 15–20% from proteins may be recommended for adults.

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Original Article

Anthropometric, Lifestyle and Biomarker Assessment of Japanese Non-professional Ultra-marathon Runners

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BACKGROUND: Anthropometric characteristics, lifestyle, and baseline biological markers of Japanese non-professional ultra-marathon runners have not been fully assessed.
METHODS: We evaluated anthropometric characteristics, lifestyle, and baseline biological markers of 180 Japanese amateur ultra-marathon runners (144 males [mean age: 50.5±9.4 (standard deviation) years] and 36 females [48.9±6.9]), and compared them with those of participants in a community heath check-up program and with the figures in the literature. We furthermore evaluated baseline blood indices according to monthly running distance with analysis of variance adjusted for age, body mass index, smoking and alcohol drinking habits.
RESULTS: The ultra-marathon runners demonstrated more favorable values for body mass index and bone density, and the proportion of smoking, and undertaking physical activity (for both sexes), eating breakfast (for males), and having daily bowel movements (for females), while greater proportion of alcohol drinking habit (for both sexes), than the comparison group. Average monthly running distances and standard deviations (km) were 257.2±128.9 for males and 209.0±86.2 for females. Male runners possessed beneficial markers, including lowered triglyceride and elevated high-density lipoprotein cholesterol, and their values showed hockey-stick (or inverse hockey-stick) patterns depending on their monthly running distance. Some subjects running more than 300 km/month exhibited signs of an over-reaching/training syndrome, including somewhat lowered hemoglobin, ferritin and white blood cell count, and elevated creatine kinase and lactate dehydrogenase.
CONCLUSIONS: Together with a desirable lifestyle, Japanese non-professional ultra-marathon runners with vigorous exercise habit demonstrated a preferable health status according to biological indices.
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Key words: biomarker measures, health indices, lifestyle-related diseases, physical activity, non-professional ultra-marathon runners.

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There are advantages and disadvantages to physical activity, exercise, and sports. Advantages include elevated bowel motility,[1,2] modification of lipid metabolism and amelioration of insulin resistance and glucose intolerance,[3-5] improvement of cardiovascular parameters, and prevention of obesity.[6,7] Decreased serum concentrations of arachidonic acid and prostaglandin E_2, reduced generation of radical oxygen species,[8-15] enhancing oxygen radicals absorbance capacity and immune surveillance,[16-19] including an increased natural killer cell activity, and diminishing cancer risk[1,2,20] may all be achieved. Thus, appropriate physical activity in the long-term may decrease mortality from lifestyle-related diseases, prolong active life expectancy, alleviate mental stress, support mental health and self-efficacy, and finally, enhance quality of life.[6,7,21-23]

On the other hand, disadvantages include damage in the hematopoietic system, skeletal or muscular injuries, oxidative stress/damage, cardiac arrest, arrhythmia, and sudden death.[2,6,7,9,11,13-18] As is well known, moreover, there exists an overreaching/training syndrome, and research is needed to clarify the type, intensity, duration, and frequency of physical activity/exercise/sports favorable to our health.

Here, we studied anthropometric characteristics, lifestyle, and baseline biomarker measures among non-professional but vigorously-trained runners entering an ultra-distance race, and compared them with those of people receiving an annual health check-up program and with reference values in the literature. We also assessed baseline blood indices according to their monthly running distance.

METHODS

Ultra-marathon race

The ultra-marathon race is not a competitive one. It is nicknamed "*Maranic*" (*mara*thon and *pic*nic), and the tenth race was held in Gifu Prefecture, Japan, during July 27-28, 2002. The midsummer weather was partly cloudy, very hot and sultry. According to the meteorological authority, the temperature was approximately 35℃, and the relative humidity was about 55% at noon on both days. The race covered 130 km of distance running and mountaineering over two days. On the first day, at 11 a.m., the participants started a full-length marathon race to be completed within 6 hr and 30 min. On the second morning, at 3:30 a.m., they resumed the race to run approximately 90 km, including climbing up to a mountain lake approximately 1,100 m high, then returning to the starting point within 15 hr and 30 min.

Subjects and methods

Six weeks prior to the race, we asked 325 ultra-marathon runners entering the *Maranic* race to enroll in our study. Of these, 202 runners agreed to participate in the project. We received written informed consent from them for completing a questionnaire survey, measuring anthropometric characteristics and bone density, sampling of blood, urine, and saliva, and analyzing genetic polymorphisms, including human 8-oxoguanine DNA glycosylase 1 (*h*-OGG1), aldehyde dehydrogenase 2 (ALDH2), peroxisome proliferators-activated receptor gamma (PPAR γ), leptin, angiotensin converting enzyme (ACE), β-adrenergic receptor, and CD36 genes. The protocol was approved by the institutional review board of the Nagoya City University Graduate School of Medical Sciences and by the chairman and organizing committee of the race.

We administered our questionnaire to 202 runners by mail and obtained information on anthropometric characteristics and lifestyle, including sex, age (date of birth), height, and dietary, smoking and alcohol drinking habits. We checked unfilled items on the race day along with securing information on smoking, alcohol drinking and supplements taken during the race.

For external comparison, the values of anthropometric characteristics and lifestyle of the participants in a community health check-up program in 2002, except for calcaneal bone density, body temperature, and resting pulse rate, were utilized. We received written informed consent from these participants, and the protocol was approved by the institutional review board of the Nagoya City University Graduate School of Medical Sciences. For calcaneal bone density, body temperature, and resting pulse rate, we used the figures reported in the literature for comparison.[24-26]

Energy intake was assessed by the short food frequency questionnaire (FFQ).[27] A regression equation was applied, adopting intake frequency of foods/food groups, average portion size and nutrient concentrations/100 g of foods[28] as independent variables and energy intake as a dependent variable. Anthropometric measurements and sampling of blood, urine and saliva were performed at the pre- (baseline), mid-, and post-race stages. We measured body weight, body temperature at the tympanum (*Nipro* 43-130, Morishita Jintan, *K.K.*) and blood lactate (*Lactate Pro, LT-1710*, Kyoto Daiichi Kagaku Co., Ltd.). Calcaneal bone density (Speed of Sound) (Stiffness [%]) was gauged ultrasonographically once on three measurement occasions (*A-1000 Express*, GE LUNAR).

We analyzed urine for protein, glucose, occult blood, urobilin, urobilinogen, and pH at the site (*Urisys 2400*, Sysmex K.K.). Baseline serum parameters, including total protein, blood urea nitrogen (BUN), uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GTT), lactate dehydrogenase (LDH), creatine kinase (CK), creatinine, total cholesterol, high-density lipoprotein cholesterol (HDL-C), total bilirubin, triglyceride, free fatty acid (*Hitachi 7600*, Hitachi K.K.), myoglobin (radioimmunoassay), lipid peroxide (enzyme method), white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets (*XE2100*, Sysmex K.K.), ferritin (chemical immunoluminiscence), HbA_{1c} (HPLC analysis), and serum electrolytes, including sodium (Na), potassium (K), and chlorine (Cl) (*Hitachi 7600*, Hitachi K.K.), were assayed.[29] Resting pulse in bed

in the morning was surveyed by mail after the race.

Statistical analysis

Anthropometric characteristics and lifestyle values, including bone density,[24] body temperature at the tympanum,[25] and resting pulse,[26] were age-adjusted, adopting the reference population or the study subjects in the literature as standard. The means ±95% confidence interval were computed, and contrasted with those of the participants in a community health check-up program in 2002 or with reference values in the literature. Baseline blood and urine biomarkers were compared with reference values.[29] Full-length and ultra-marathon completion rates, time and blood indices among males were collated according to monthly running distance (km/month) (≤100, 101-200, 201-300, and 301+) with analysis of variance adjusted for age, body mass index (BMI [kg/m²]), smoking, and alcohol drinking.[30] Tukey's post hoc multiple t-test was performed to examine differences in the least square means, and the linear trends were statistically verified. The p values smaller than 0.05 were considered statistically significant.

RESULTS

Anthropometric measures, and major lifestyle characteristics

Of our participants, 187 runners actually attended the race, and anthropometric measures were taken together with sampling of biomaterials. Seven participants were excluded from the analysis: three with uncompleted questionnaires, and two who were late for the pre-race examination. One finally declined pre- and mid-race blood sampling, and one was excluded due to abnormal liver function. The remaining 180 subjects (144 males and 36 females) were included in this study.

Mean ages were 50.5 ± 9.4 (± standard deviation) years for males and 48.9 ± 6.9 for females, respectively. The differences of means between the groups were obvious: that is, the values for BMI, body temperature (℃), and resting pulse rate were smaller, while those for calcaneal bone density (Stiffness [%]) (for both sexes) were greater (Table 1). The percentages of smoking and enjoying physical activity (for both sexes), eating breakfast (for males), and having daily bowel movements (for females) were more favorable than for the comparison group. However, the proportion of alcohol drinking habit (for both sexes) was greater in runners than the general public.

Blood analysis

Average figures for all blood measures were located within the ranges of the reference values (Table 2). They were mostly favorable readings in both sexes. However, hematological markers, such as Hb, ferritin, and WBCs, shifted to be lower than the standard values. On the other hand, damage/repair markers of the musculo-skeletal system, including CK and LDH, tended to be greater than the reference values.

Urine analysis

Positive rates for urine glucose of 9.9% for males and 14.6% for females were greater than those in the general people partly because urine was collected on a spot sampling basis (data not shown).

Table 1. Comparison of anthropometric characteristics and lifestyle between Japanese non-professional ultra-marathon runners and people receiving an annual community health check-up program and other reference people.

Item	males		females	
	ultra-marathon runners* (n=144)	reference values†	ultra-marathon runners (n=36)	reference values
Body mass index (kg/m²)	22.2 (21.7-22.8)	23.3 (23.1-23.4)	21.2 (20.3-22.1)	22.5 (22.4-22.7)
Eating breakfast (%)	96.5 (94.2-98.9)	90.0 (88.5-91.6)	93.9 (89.5-98.4)	92.4 (91.2-93.6)
Energy intake (kcal)	2,302 (1,936-2,668)	2,117 (2,095-2,139)	1,732 (1,428-2,037)	1,956 (1,942-1,970)
Smoking habit (%)	7.3 (1.7-12.9)	31.1 (28.6-33.6)	1.0 (0-2.4)	6.6 (5.5-7.7)
Alcohol drinking habit (%)	78.6 (70.4-86.7)	58.5 (55.9-61.2)	48.3 (24.1-72.5)	18.7 (16.9-20.4)
Undergoing physical activity (%)	100	40.2 (37.7-42.8)	100	42.1 (39.9-44.3)
Sleep duration (hours)	6.8 (6.6-7.1)	7.0 (6.9-7.0)	6.4 (5.9-6.8)	6.6 (6.6-6.7)
Having daily bowel movements (%)	91.4 (86.0-96.8)	88.9 (87.3-90.6)	96.5 (92.0-100)	70.5 (68.4-72.5)
Calcaneal bone density (Stiffness [%])‡	101.2 (97.6-104.7)	85.9 (83.9-87.9)	93.7 (84.6-102.8)	73.5 (72.4-74.6)
Body temperature at the tympanum (℃)§	36.2 (36.1-36.4)	36.9 (36.9-36.9)	36.3 (35.9-36.6)	36.9 (36.9-36.9)
Resting pulse (bpm)‖	53.6 (52.5-54.8)	65.9 (64.2-67.5)	54.7 (52.5-56.9)	66.5 (64.7-68.3)

* : Age-adjusted means ± 95% confidence intervals adopting corresponding reference populations or study subjects in the literature as standard.

† : Values of people receiving annual health check-up program among 1,346 males and 2,043 females, except for calcaneal bone density, body temperature, and resting pulse rate.

‡ : For comparison, values of calcaneal bone density were cited from the reference No. 24.

§ : For comparison, values of body temperature were cited from the reference No. 25.

‖ : For comparison, values of resting pulse were cited from the reference No. 26.

Table 2. Comparison of blood indices between Japanese non-professional ultra-marathon runners and reference values.

	males		females	
	Ultra-marathon runners (n=144) mean ± standard deviation	Reference values*	Ultra-marathon runners (n=36) mean ± standard deviation	Reference values
Total protein (g/dL)	7.2 ± 0.4	6.7 - 8.3	7.1 ± 0.4	6.7 - 8.3
Blood urine nitrogen (BUN, mg/dL)	18 ± 4	6 - 20	17 ± 4	6 - 20
Uric acid (mg/dL)	5.8 ± 1.3	3.7 - 7.6	4.1 ± 0.8	2.5 - 5.4
Aspartate aminotransferase (AST, IU/L)	25 ± 10	10 - 40	22 ± 9	10 - 40
Alanine aminotransferase (ALT, IU/L)	27 ± 13	5 - 40	21 ± 13	5 - 40
Gamma-glutamyltransferase (GTT, IU/L)	44 ± 36	≤ 70	21 ± 8	≤ 30
Lactate dehydrogenase (LDH, IU/L)	198 ± 35	115 - 245	197 ± 29	115 - 245
Creatine kinase (CK, IU/L)	183 ± 139	57 - 197	150 ± 91	32 - 180
Creatinine (mg/dL)	0.64 ± 0.14	0.61 - 1.04	0.48 ± 0.09	0.47 - 0.79
Myoglobin (ng/mL)	43 ± 15	≤ 60	30 ± 9	≤ 60
Total cholesterol (mg/dL)	206 ± 34	150 - 219	218 ± 34	150 - 219
High-density lipoprotein cholesterol (HDL-C, mg/dL)	62 ± 15	41 - 86	69 ± 14	41 - 96
Total bilirubin (mg/dL)	0.4 ± 0.2	0.2 - 1.0	0.4 ± 0.2	0.2 - 1.0
Triglyceride (mg/dL)	121 ± 66	50 - 149	93 ± 35	50 - 149
Free fatty acid (mEq/L)	0.36 ± 0.16	0.14 - 0.85	0.35 ± 0.17	0.14 - 0.85
Lipid peroxide (nmol/mL)	2.9 ± 0.7	1.8 - 4.7	2.6 ± 0.6	1.8 - 4.7
White blood cell count (WBCs, /μL)	5,553 ± 1,188	3,900 - 9,800	5,197 ± 1,281	3,500 - 9,100
Red blood cell count (RBCs, 10^4/μL)	457 ± 38	427 - 570	412 ± 30	376 - 500
Hemoglobin (Hb, g/dL)	14.3 ± 1.1	13.5 - 17.6	12.7 ± 1.1	11.3 - 15.2
Hematocrit (%)	42.9 ± 3.2	39.8 - 51.8	39.1 ± 2.6	33.4 - 44.9
Mean corpuscular volume (fl)	94.0 ± 4.9	82.7 - 101.6	94.8 ± 4.2	79.0 - 100.0
Mean corpuscular hemoglobin (pg)	31.2 ± 1.7	28.0 - 34.6	30.7 ± 1.6	26.3 - 34.3
Mean corpuscular hemoglobin concentration (%)	33.2 ± 0.9	31.6 - 36.6	32.4 ± 1.0	30.7 - 36.6
Ferritin (ng/mL)	55.4 ± 40.3	27 - 320	20.4 ± 14.5	3.4 - 89
Platelet count (10^4/μL)	22.6 ± 4.6	13.1 - 36.2	22.2 ± 4.0	13.0 - 36.9
Hemoglobin A_{1c} (HbA_{1c}, %)	5.1 ± 0.4	4.3 - 5.8	4.8 ± 0.3	4.3 - 5.8
Sodium (Na, mEq/L)	142 ± 2	136 - 147	141 ± 2	136 - 147
Potassium (K, mEq/L)	4.1 ± 0.6	3.6 - 5.0	4.1 ± 0.4	3.6 - 5.0
Chlorine (Cl, mEq/L)	105 ± 2	98 - 109	105 ± 2	98 - 109

*: Reference values are from the Test Directory 2002.[29]

Table 3. Blood indices according to average monthly running distance adjusted for age, body mass index, smoking and alcohol drinking in Japanese male non-professional ultra-marathon runners.

	Average monthly running distance (km/month)				
	-100 (n=20)	101-200 (n=44)	201-300 (n=46)	301+ (n=34)	linear trend
Total protein (g/dL)	7.2	7.2	7.2	7.2	
Blood urine nitrogen (BUN, mg/dL)	19	18	19	19	
Uric acid (mg/dL)	5.9	5.9	5.8	5.7	
Aspartate aminotransferase (AST, IU/L)	22	26	25	27	
Alanine aminotransferase (ALT, IU/L)	26	25	30	29	
Gamma-glutamyltransferase (GTT, IU/L)	37	47	42	46	
			*		
			*		
			*		
Lactate dehydrogenase (LDH, IU/L)	190	196	194	213	*
			*		
			*		
			*		
Creatine kinase (CK, IU/L)	143	169	168	246	**
Creatinine (mg/dL)	0.66	0.61	0.67	0.63	
Myoglobin (ng/mL)	43.0	41.8	44.0	44.7	
Total cholesterol (mg/dL)	200	203	204	216	
			*		
High-density lipoprotein cholesterol (HDL-C, mg/dL)	58	61	62	67	*
Total bilirubin (mg/dL)	0.5	0.4	0.5	0.4	
		#			
Triglyceride (mg/dL)	145	127	110	114	
Free fatty acid (mEq/L)	0.35	0.34	0.35	0.38	
Lipid peroxide (nmol/L)	2.9	2.9	3.0	2.8	
			*		
White blood cell count (WBCs, /μL)	5656	5735	5601	5191	#
Red blood cell count (RBCs, 10^4/μL)	456	459	462	449	
			*		
			*		
Hemoglobin (Hb, g/dL)	14.2	14.4	14.4	13.9	
Hematocrit (%)	42.4	43.2	43.5	42.1	
Mean corpuscular volume (fl)	93.2	94.1	94.3	94.1	
Mean corpuscular hemoglobin (pg)	31.2	31.4	31.3	31.1	
Mean corpuscular hemoglobin concentration (%)	33.5	33.3	33.2	33.0	
			#		
Ferritin (ng/mL)	60.8	59.7	58.5	42.6	#
Platelet count (10^4/μL)	22.5	23.3	22.7	21.7	
Hemoglobin A_{1c} (HbA_{1c}, %)	5.1	5.0	5.2	5.1	
Sodium (Na, mEq/L)	142	142	142	141	
Potassium (K, mEq/L)	4.1	4.1	4.2	4.2	
Chlorine (Cl, mEq/L)	105	105	105	105	

#: Marginally significant, * $p<0.05$, ** $p<0.01$.

Completion rates and time according to monthly running distance

Average monthly running distances (km) were 257.2 ± 128.9 (98 - 444) (minimum - maximum) for males and 209.0 ± 86.2 (23 - 222) for females. They mainly selected running, although some concurrently chose swimming, bicycling and other aerobic exercises and occasionally took part in weight/resistance training. Ninety-three percent (167 out of 180 of the study subjects) completed the first day full-length marathon, and 60% (108 out of 180) the 2-day ultra-marathon race. Full-length and ultra-marathon completion rates were positively, while completion time was inversely dependent on their monthly running distance for either sex (data not shown).

Differences in blood indices according to monthly running distance

The number of male runners by monthly running distance (km/month) of ≤100, 101-200, 201-300, 301+ were 20, 44, 46, and 34, respectively. As a whole, there were no significant discrepancies in anthropometric characteristics, including BMI, body temperature, resting pulse rate and bone density, according to monthly running distance (data not shown). Most blood measurements also showed no remarkable differences in proportion to monthly running distance after adjustment for age, BMI, smoking, and alcohol drinking, either (Table 3). Triglyceride was decreased according to monthly running distance, while HDL-cholesterol was steadily elevated. Some readings, however, revealed hockey-stick (or inverse hockey-stick) patterns, including lowered Hb, ferritin and WBCs, and elevated CK and LDH. The linear trends were statistically/marginally significant except for Hb and triglyceride.

DISCUSSION

Most of the present subjects followed the recommended Seven Heath Practices, including (1) hours of sleep, (2) smoking, (3) body weight, (4) alcohol drinking, (5) physical exercise, (6) eating breakfast, and (7) eating between meals, proposed by Breslow et al.[31] They showed preferable demographic characteristics and lifestyle, even when compared with health-conscious people receiving an annual health check-up program. Above all, they engaged in vigorous exercise regularly. Most maintained a desirable body weight and bone density,[24] and smoked less;[32] however, they experienced greater energy from alcoholic beverages than the general population, at least partly because they regularly expended energy by running. Alcohol consumption, however, up to 30 g net ethanol per day, may not be harmful, provided the subject is not a carrier of hepatitis B/C viruses, or has not hetero/mutant type genetic polymorphisms of ALDH-2.

Most blood measures showed no remarkable variation according to monthly running distance after adjustment for age, BMI, smoking, and alcohol drinking, in line with homeostasis and adaptation.[9,13,14,18] No significant differences were observed in typical antioxidant molecules of uric acid and bilirubin.[8,12] Ferritin levels, however, decreased in proportion to monthly running distance, along with lower body temperature[25] and resting pulse rates[26,33] were noted among the subjects. Taking into account these findings, we are now planning to make pre-, mid- and post-race comparisons of blood, urine and saliva bio-parameters, including serum d-ROM and Mn-SOD, and urinary 8-OHdG and biopyrrins, as markers of reactive oxygen species and oxygen radical absorbance capacity.[8-15]

Participants running more than 300 km/month exhibited signs of an over-reaching/training syndrome, including lowered Hb, ferritin and WBCs suggesting damage in the hematopoietic system to some degree, and elevated CK and LDH indicating injuries in musculo-skeletal organs. Vigorous exercisers running around 200 km/month, even those running less than 100 km/month, who were insufficiently trained to run an ultra-marathon race, had preferable biomarker indices, implying that such exercises are favorable to health. Namely, triglyceride was decreased and HDL-cholesterol was elevated according to their monthly running distance. Thus, people committed to vigorous exercise probably do not suffer from obesity, high lipidemia/cholesterol,[6,7] high blood pressure or high insulin resistance.[3-5] Furthermore, they undoubtedly enjoy a low risk of coronary heart disease, cerebrovascular diseases and fat-related cancers, including colon, prostate and breast cancer.[1,2,20]

In conclusion, the study subjects were admittedly rather self-selected as being non-professional marathon runners and possessed desirable demographic characteristics and lifestyle, even when compared with health-conscious people receiving an annual health check-up program. Runners committing to vigorous running up to around 200 km/month, but not over-reaching/training, appear to have preferable biomarker indices, suggesting that vigorous aerobic exercise is favorable to health, particularly for sedentary or physically-inactive workers. Further research is warranted to elucidate the type, intensity, duration, and frequency of physical activity/exercise/sports beneficial to promote health, to reduce the risk of lifestyle-related diseases and to enhance the quality of life.

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A prospective study of reproductive and menstrual factors and colon cancer risk in Japanese women: Findings from the JACC study

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The effects of reproductive factors on the etiology of colon cancer in Asian populations remain unexplored. So we examined 38,420 Japanese women aged 40–79 years who responded to a questionnaire on reproductive and other lifestyle factors from 1988 to 1990 in the Japan Collaborative Cohort Study for Evaluation of Cancer Risk. During an average 7.6 years of follow-up, we documented 207 incident colon cancers. Multivariate analysis indicated that colon cancer risk was likely to be lower among parous women than among nulliparous. Women who had two abortions or more had a 72% higher risk of developing colon cancer [relative risk (RR) 1.72; 95% confidence interval (CI) 1.16–2.55; trend $P<0.01$] compared with women who never had an abortion. The RR of colon cancer among postmenopausal women significantly decreased with increasing age at menarche (trend $P=0.01$). No apparent association between colon cancer and gravida, age at first birth, age at menopause, or duration of menstruation was seen. These prospective data support the hypothesis that female reproductive events modify colon cancer risk, and suggest that reproductive factors, particularly age at menarche and having an abortion, may be of importance in the etiology of colon cancer among Japanese women. (Cancer Sci 2004; 95: 602–607)

In 1980, McMichael and Potter[1)] reviewed the results of two earlier case-control studies of colorectal cancer together with other epidemiologic, metabolic, and animal studies, and suggested that reproductive events and endogenous and exogenous sex hormones may affect carcinogenesis in the large bowel via their effects on hepatic function and bile acid formation. Briefly, endogenous estrogens increase bile acid production. Progestin, pregnancy, and high-dose oral contraceptives reduce bile acid production; therefore, they decrease the risk of colon cancer. Since then, the results of several further studies,[2–26)] most of which were conducted in high-risk Western countries using a case-control design,[2–19)] dealing with this issue have been published. These studies suggested that some aspects of reproductive history including parity, age at first birth, age at menarche, and estrogen use after menopause may affect the development of colon cancer. However, these studies are inconsistent as to whether such reproductive factors are surrogates for hormonal effects or for some other lifestyle characteristics, such as diet or physical activity. Furthermore, the effects of reproductive factors on the etiology of colon cancer in Asian populations remain unexplored. Here, we report the findings from a large prospective study conducted among Japanese. Our study offers further evidence in support of the hypothesis proposed by McMichael and Potter.[1)]

Materials and Methods

The JACC study. The Japan Collaborative Cohort Study for Evaluation of Cancer Risk, the JACC Study (sponsored by the Ministry of Education, Culture, Sports, Science and Technology of Japan) is a nationwide multicenter collaborative study to prospectively evaluate the various risks and/or protective factors influencing cancer mortality and incidence. Study methods and ethical issues have been described in detail elsewhere.[27)] Briefly, our study was initiated in 1988, and enrollment continued until the end of 1990. Forty-five areas were selected from 7 out of 8 districts in Japan, thus covering almost the entire country. We enrolled 127,477 apparently healthy inhabitants in these areas with completion of the questionnaire. Two strategies were applied to obtain informed consent for participation, i.e., requesting individuals to sign the cover page of the questionnaire, or at the group level, by explaining the aim of the study and the confidentiality of the data to community leaders. Of 127,477 enrolled, 110,792 (46,465 men and 64,327 women), aged 40–79 years, were followed. Of 64,327 women, 38,720 lived in 24 study areas covered by cancer registries. Among those, we excluded from analysis 14 with a history of colon cancers at baseline, and 287 subjects with less than one year of follow-up time, leaving 38,420 women were enrolled in the present study (one woman had both exclusion criteria).

The present study protocol was approved by the Ethics Committee of Fujita Health University, Toyoake, Japan.

Data collection. A self-administered questionnaire was used to assess the baseline characteristics of participants. It covered medical history and included lifestyle-related items such as diet, physical activity, drinking and smoking, and family history of several medical conditions including cancer. For women, information was obtained on menstrual factors (age at menarche and age at menopause), reproductive variables (number of pregnancies, number of parity, and age at first birth). The number of abortions was calculated as the number of pregnancies minus the parity number. Induced and spontaneous abortions as well as stillbirths were included in our criteria without regard for gestational week. Among menopausal women, the duration of menstruation was defined as age at menopause minus age at menarche.

Follow-up and identification of colon cancer cases. We used population registries in local municipalities to determine the vital and residential status of the subjects. Registration of death is re-

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quired by the Family Registration Law in Japan and is enforced throughout the country. For logistical reasons, we discontinued the follow-up of subjects who moved out of their study areas.

We ascertained the incidence of cancer by consulting the records of population-based cancer registries, supplemented by a systematic review of death certificates. In some areas, medical records were also reviewed in local major hospitals. The follow-up was conducted from the time of the baseline survey through the end of 1997 except for three areas, where it ended in 1994, 1995, and 1996. During the study period, only 3.0% (1164) of the participants were lost to follow-up due to moving.

The incidence to death ratio for female colon cancer was 2.33 in the cohort covered by cancer registries. This figure is comparable with those in acceptably accurate population-based cancer registries in Japan[28] (1.69 to 3.03) and indicates that a high proportion of colon cancer cases was identified. The proportion of Death Certificate Only (DCO) registrations was 7.7% (16 of 207 cases).

Finally, the 7.6-year follow-up analyses verified 207 incident cases of colon cancer among 37,420 women. Because of missing values for certain reproductive variables, the total number of cases and person-years of follow-up varied somewhat among analyses (gravida: 192 cases during 272,684 person-years of follow-up; parity: 190 cases during 268,750 person-years; age at menarche: 180 cases during 270,253 person-years of follow-up; age at menopause: 154 cases during 190,989 person-years of follow-up).

Statistical analysis. In the present study, variables of interest were gravida, parity, age at first birth, number of abortions, age at menarche, age at menopause, and duration of menstruation. For each participant, the person-years of follow-up were calculated from the date of filling out the baseline questionnaire to development of colon cancer, death from any cause, moving out of the study area, or the end of follow-up period, whichever occurred first. Sixteen of 207 colon cancer cases were discovered only by death certificate and their person-years of follow-up were calculated from the date of enrollment to death from colon cancer. We used Cox proportional hazards modeling to compute relative risks (RRs), adjusting for age at enrollment. In another multivariate analysis, study area (Hokkaido and Tohoku, Kanto, Chubu, Kinki, Chugoku, Kyushu), smoking status (never, past, current), alcohol consumption (none, past, present), exercise: "How long do you take exercise or sports in a week?" (≥5, 3–4, 1–2 h per week, seldom), green leafy vegetable intake (3–7, 1–2 days per week, seldom), meat intake (3–7, 1–2 days per week, seldom), family history of colon cancer, and body mass index (weight in kilograms/[height in meters]^2) were further adjusted. Other reproductive and menstrual factor were also adjusted. These variables were assessed by the baseline questionnaire and were selected as covariates because they were known or suspected to modify the risk of colon cancer. In the analysis, all variables were entered as dummy variables except for age at enrollment. Missing values for each covariate were treated as an additional category and were included in the model. A linear trend of association was assessed by the regression model assigning score (0, 1, 2,...) to the levels of the independent variable.

Estrogens appear to influence colon cancer risk. In premenopausal women, ovarian sources of estrogen are of prime important but in postmenopausal women, conversion of androgens to estrogen in adipose tissue is the major source of estrogen, and the serum level of endogenous estrogen in postmenopausal women is lower than that in premenopausal women. We hypothesized that menopausal status may modify the association between reproductive factors and colon cancer risk, and to test this, we performed the same analysis as above only among menopausal women at baseline.

All data were analyzed using SAS software. The 95% confidence intervals (CIs) were presented for all RRs. All P values were based on 2-sided tests, in which $P<0.05$ was considered statistically significant.

Results

Association of age at menarche, gravida, parity, age at first birth, and the number of abortions with the risk of colon cancer. Table 1 presents the age-adjusted and multivariate RRs for colon cancer by age at menarche, gravida, parity, age at first birth, and the number of abortions among all subjects. With reference to age at menarche, compared to women whose menarche occurred at age 12 or less, the point estimates tended to be below unity, but no consistent trend in risk emerged. There was no association of gravida with colon cancer risk, whereas the multivariate RR of colon cancer for parous women compared with nulliparous women was 0.65 (95% CI: 0.35–1.20). The multivariate RRs by the number of deliveries were 0.63 for one birth, 0.80 for two, 0.57 for three, 0.60 for four and more. The point estimates were below unity, but there was no consistent pattern of trends. Among parous women, no consistent trend in risk was observed. There was also no association between age at first birth and colon cancer risk among parous women. The subjects who had had 2 or more abortions had a significantly increased risk compared to those with no abortions (RR: 1.72, 95% CI: 1.16–2.55) among the women with any pregnancy. There was a significantly increasing trend for an association between the number of abortions and the risk of colon cancer (P value for trend <0.01)

Association of reproductive and menstrual factors with the risk of colon cancer among menopausal women at baseline. Table 2 shows the age-adjusted and multivariate RRs for colon cancer by age at menarche, parity, age at menopause, and duration of menstruation only among menopausal women. The RRs of colon cancer was 0.64 (95% CI: 0.32–1.28) for women who had experienced menarche at age 13–15 and 0.49 (95% CI: 0.24–1.01) for those who did so at age 16 or more compared with those who did so at age 12 and under. There was a significantly decreasing pattern for a relation between age at menarche and the risk of colon cancer. We observed no significant association between parity and risk of colon cancer. In relation to age at menopause, the point estimates of colon cancer risk were below unity for subsequent age groups above 45, but there was no consistent pattern of trends. There were no apparent association between duration of menstruation and colon cancer risk.

Discussion

Most of the previous studies conducted in Western populations at high risk for colon cancer suggested that reproductive factors might be important in the etiology.[2–14, 16–18, 20–26] If reproductive and hormonal factors are determinants of colon cancer risk, it would be extremely valuable to determine whether their effects are similar in different populations. Although one case-control study[19] analyzed this issue among Japanese women, we attempted to resolve this question by means of a prospective study in order to avoid some of the problems inherent in a case-control study, such as selection and recall bias, which may distort the results.

Our prospective study found that parity was likely to have a protective effect against colon cancer risk, whereas, to our knowledge, only two[20, 23] of the seven published cohort studies[20–26] have shown a weak and statistically insignificant inverse effect of parity on colon cancer, and less than half[4, 6, 7, 11, 12, 14, 16] of the 18 case-control studies[2–19] found evidence of parity's protective effect. This protective effect was consistent in our study up to high parity number, though one case-control study has reported a U-shaped association between

Table 1. Adjusted relative risk (RR) for colon cancer by age at menarche, gravida, parity, age at first birth, and the number of abortions, JACC study, 1988–1997

	Person-years[1]	No. of cases[1]	RR (95% CI)[2]	RR (95% CI)[3]
Age at menarche				
≤12	17,649	12	1.00	1.00 [4]
13–15	159,100	101	0.67 (0.37–1.22)	0.74 (0.39–1.38)
≥16	93,352	67	0.53 (0.28–0.99)	0.62 (0.32–1.20)
P value for trend			<0.05	0.13
Gravida				
No pregnancy	13,357	11	1.00	1.00 [5]
Any pregnancy	259,326	181	1.00 (0.54–1.83)	0.82 (0.42–1.62)
1	13,631	11	1.00	1.00
2	57,649	33	0.94 (0.47–1.87)	1.39 (0.61–3.16)
3	75,319	45	0.87 (0.45–1.68)	1.27 (0.57–2.83)
≥4	112,727	92	0.89 (0.48–1.67)	1.28 (0.59–2.79)
P value for trend			0.70	0.87
Parity				
Nulliparous	13,438	14	1.00	1.00 [5]
Parous	255,311	176	0.77 (0.45–1.33)	0.65 (0.35–1.20)
1	19,199	15	1.00	1.00
2	97,564	60	1.04 (0.59–1.84)	1.24 (0.66–2.32)
3	87,065	48	0.75 (0.42–1.35)	0.88 (0.46–1.68)
≥4	51,483	53	0.75 (0.42–1.35)	0.90 (0.47–1.74)
P value for trend			0.10	0.24
Age at first birth (only parous women)				
<25	106,081	74	1.00	1.00 [6]
25– <30	105,979	77	1.09 (0.79–1.05)	1.04 (0.73–1.49)
30– <35	15,620	10	0.84 (0.43–1.63)	0.65 (0.31–1.40)
35–	3279	3	1.21 (0.38–3.83)	1.16 (0.35–3.83)
P value for trend			0.92	0.68
Number of abortions (only women with any pregnancy)				
0	158,765	105	1.00	1.00 [7]
1	57,820	40	1.29 (0.89–1.87)	1.29 (0.87–1.92)
≥2	36,467	31	1.69 (1.17–2.43)	1.72 (1.16–2.55)
P value for trend			<0.01	<0.01

1) Number of cases and person-years do not always add up to the total due to missing information for the risk factors.
2) Adjusted for age at baseline.
3) Adjusted for age at baseline, study area (Hokkaido and Tohoku, Kanto, Chubu, Kinki, Chugoku, Kyushu), smoking status (never, past, current), alcohol drinking habit (none, past, regular), exercise (≥5, 3–4, 1–2 days/week, seldom), meat intake (3–7, 1–2 days/week, seldom), green leafy vegetable intake (3–7, 1–2 days/week, seldom), family history of colon cancer (Y/N), and BMI at baseline (<18.5, 18.5– <22, 22– <25, 25– kg/m^2).
4) Additionally adjusted for menopausal status and parity.
5) Additionally adjusted for menopausal status and age at menarche.
6) Additionally adjusted for menopausal status, age at menarche, and parity.
7) Additionally adjusted for menopausal status and age at menarche.

the parity number and colon cancer risk.[14] Furthermore, the protective effect of parity became clear after adjustment for some confounding factors, such as smoking, alcohol consumption, intake of vegetables, physical activity, and body mass index. Several mechanisms have been suggested to explain the protective effect of parity on colon cancer, including modifications of estrogen profiles secondary to pregnancies and their effects on bile acid metabolism,[1, 29] immunological influences of ABO-incompatible fetal antigens,[30] increased physical activity associated with large families,[20] and unidentified lifestyle factors associated with having children.

In our study, there was an inverse association between age at menarche and colon cancer risk and the association was significant among menopausal women. Of five cohort studies[20–22, 24, 25] which examined the effect of age at menarche, only one[27] found this inverse association. It is unclear whether the effect of age at menarche on colon cancer risk is affected by some hormonal mechanism. Age at menarche may be a surrogate for some unidentified risk factors around puberty, such as body size, nutrition, or physical activity. Abdominal obesity, which is associated with hyperinsulinemia resulting from insulin resistance,[31] has been noted in girls even before puberty[32] and has been shown to be associated with an earlier onset of menarche.[33] Since insulin is a growth factor for colon epithelial cells, it has been suggested that hyperinsulinemia plays an important role as a colon cancer promoter.[34] In our study, the association between age at menarche and colon cancer risk still remained after multivariate adjustment; this is consistent with the above-mentioned reasoning and may suggest a role of childhood nutrition in the subsequent development of colon cancer. However, the reason why this association between age at menarche and colon cancer risk was observed more clearly among menopausal women also remains unclear. The predominant premenopausal profile of endogenous female sex hormones derived from the ovaries modifies the risk of female colon cancer through increased excretion of bile acids. The effect of hyperinsulinemia on the risk of colon cancer may predominate in postmenopausal women with low levels of female sex hormone. Adult women who had early menarche may have hyperinsulinemia, since tracking of serum insulin is observed especially in females.[35]

There are only a few studies on the effect of abortion or in-

Table 2. Adjusted relative risk (RR) for colon cancer by age at menarche, parity, age at menopause, and duration of menstruation among menopausal women at baseline, JACC study, 1988–1997

	Person-years[1]	No. of cases[1]	RR (95% CI)[2]	RR (95% CI)[3]
Age at menarche				
≤12	7653	9	1.00	1.00 [4]
13–15	99,600	82	0.61 (0.31–1.22)	0.64 (0.32–1.28)
≥16	80,038	58	0.47 (0.23–0.95)	0.49 (0.24–1.01)
P value for trend			<0.05	<0.05
Parity				
Nulliparous	7374	9	1.00	1.00 [5]
Parous	172,638	137	0.67 (0.34–1.32)	0.70 (0.36–1.39)
1	12,944	14	1.00	1.00
2	59,154	45	0.85 (0.46–1.55)	0.99 (0.52–1.89)
3	57,811	35	0.57 (0.31–1.07)	0.70 (0.36–1.35)
≥4	42,729	43	0.64 (0.34–1.18)	0.79 (0.41–1.53)
P value for trend			0.07	0.24
Age at menopause				
≤45	37,430	35	1.00	1.00 [6]
46– <50	51,011	44	0.94 (0.60–1.46)	0.87 (0.54–1.38)
50– <55	92,453	66	0.76 (0.50–1.15)	0.74 (0.49–1.14)
≥55	9956	9	0.83 (0.40–1.74)	0.73 (0.34–1.58)
P value for trend			0.19	0.16
Duration of menstruation (years)				
<30	31,264	27	1.00	1.00 [7]
30– <35	70,183	59	0.97 (0.61–1.52)	0.93 (0.59–1.49)
35– <40	74,796	53	0.86 (0.54–1.37)	0.83 (0.51–1.33)
≥40	11,085	10	1.00 (0.48–2.07)	0.94 (0.45–1.95)
P value for trend			0.63	0.51

1) Number of cases and person-years do not always add up to the total due to missing information for the risk factors.
2) Adjusted for age at baseline.
3) Adjusted for age at baseline, study area (Hokkaido and Tohoku, Kanto, Chubu, Kinki, Chugoku, Kyushu), smoking status (never, past, current), alcohol drinking habit (none, past, regular), exercise (≥5, 3–4, 1–2 days/week, seldom), meat intake (3–7, 1–2 days/week, seldom), green leafy vegetable intake (3–7, 1–2 days/week, seldom), family history of breast cancer (Y/N), and BMI at baseline (<18.5, 18.5– <22, 22– <25, 25– kg/m^2).
4) Additionally adjusted for parity and age at menopause.
5) Additionally adjusted for age at menarche and at menopause.
6) Additionally adjusted for age at menarche and parity.
7) Additionally adjusted for parity.

complete pregnancy on the risk of colon cancer. Negri *et al.*[13] found no consistent relation with the number of abortions, whereas Howe *et al.*[10] found a high number of non-live births among colorectal cases, and Peters *et al.*[14] reported a U-shaped association. The definitions of a failed pregnancy differed among the various studies. We found a consistent and positive association between the number of abortions and colon cancer risk. The adverse effect of abortion is similar to what has been observed in some studies on breast cancer,[36] but the underlying mechanisms remain unclear.

The fact that a first delivery early in life is a protective factor against colon cancer is supported by four cohort studies,[21–23, 25] though none of their results proved statistically significant. Evidence of a long-term shift in hormone profiles that would affect bile acid production after the first complete pregnancy[37, 38] may explain the protective effect not only of having been parous, but also of having given birth at an early age. However, we observed no association between age at first birth and colon cancer risk.

We found no effect of age at menopause or duration of menstruation. Previous results on these variables have been inconsistent.[13–16, 19–21] However, our study has an advantage over previous studies with respect to age at menopause and duration of menstruation, because few Japanese women had used estrogens after menopause. Hormone use may induce artificial menstruation, thus affecting accurate recall of when their periods had stopped naturally.

The study by Yoo *et al.*,[19] which is the first study on the association between reproductive factors and colorectal cancer in Japan, reported that menstrual regularity, late age at menopause, late age at first pregnancy, and late age at first full-term pregnancy were significantly associated with the risk of colorectal cancer. Yoo *et al.* also found a positive association between age at menarche and risk of distal colon cancer. Our study yielded a negative association, especially among postmenopausal women. The study by Yoo *et al.* gave no consideration to menopausal status and our study had no information on subsite. The effect of menarche on colon cancer needs further investigation.

There are both strengths and limitations in our study. The strengths include its prospective design and large sample size. Data on exposure were collected before diagnosis and prior to any colon cancer deaths, which could preclude recall bias. Moreover, since data on many kinds of exposure known or suspected to modify the risk of colon cancer were collected in the present study, we could elucidate the independent effects of reproductive factors by multivariate adjustment. One limitation of this study lies in the absence of information on the specific subsites of origin in the large bowel, since it has been suggested that the influence of sex hormones should be either greater at, or restricted to, the right side of the large bowel.[6] Another limitation arises from the validity of self-reported reproductive histories. Some reproductive exposures occurred long before enrollment or any diagnosis of disease. However, previous

studies on the validity of self-reported reproductive histories have shown good agreement between the respondents recall of reproductive events and their medical records.[39,40] Finally, our results may be chance findings, because the participants were not drawn uniformly from throughout Japan. Also, the number of incident cancer cases of the colon was small compared to those in Western studies.

In summary, the present study provides additional support for earlier suggestions by McMichael and Potter as well as other researchers that there is a hormonal component in the risk profile for female colon cancer. In Japan, the westernization of lifestyles has progressed rapidly since the early 1960s, suggesting that it will be necessary to examine the change of reproductive factors and their effect on colon cancer among Japanese women who have experienced largely westernized lifestyles since childhood.

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